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NEWSLETTER

to the
National Reference Laboratories for Antimicrobial Resistance

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European Union Reference Laboratory Antimicrobial Resistance

Content

Establishing a subgroup of the EU AMR One Health Network on National Action Plans (NAPs)

By Rene Hendriksen, EURL-AR

The Commission launched the sub-group of the AMR One Health Network following the network meeting on the 25th of March, 2021. Comments from Member States, in relation to establishing this sub-group, were taken into account. This subgroup, chaired by DG SANTE F5, will focus on the development of the NAPs and in particular on the review to be carried out by the Commission services. The sub-group met on the 7th of July, 2021 where the methodology for the review was presented. In early 2022, the sub-group will discuss the main findings of the review process. It is a key to ensure Member States' participation from a One Health perspective, either by being represented by national coordinators and/or by representatives of the main areas: human health, veterinary/food safety and environment. Read more information about this interesting initiative here: https://ec.europa.eu/health/amr/events/ev_20210707_en

Experiences from the EURL-AR online training course, 2021

By Jette Sejer Kjeldgaard and Troels Ronco, EURL-AR

Between 26th to the 29th of April, 2021 EURL-AR organized a virtual training course for 170 participants (37 different labs) with the main goal of ensuring harmonization on the monitoring of antimicrobial resistance in zoonotic and commensal bacteria across EU. The course focused on knowledge and tools for generating and using whole-genome sequence data in the future AMR monitoring according to the proposed new EU Decision (2020/1729/EU). Three exercises were included; 1) *Sequence quality control evaluation*, 2) *Pheno- and genotype interpretation* and 3) *Phylogenetic SNP analysis* and in addition, exercise 2 was divided into three sections; 2A) *Phenotype assessment*, 2B) *Genotype assessment* and 2C) *Overall assessment*. In contrast to previous years, a one-hour lecture related to each exercise was provided. On average, the percentage of correct answers from the NRLs, for exercise 1 and 2A was 95.6% and 81%, respectively whereas exercise 2B, 2C and 3 were not evaluated using percentages of correct answers. In exercise 2B, participants had not taken into account that specific genes mediate specific phenotypic resistance and in 2C the organizers had not clearly formulated in the evaluation scheme, that the ESBL phenotypes should also have been evaluated based on the results from 2A and 2B. In addition, the evaluation of exercise 3 was meant as a written discussion and thus, exercises 2B, 2C and 3 were not evaluated using percentages of correct answers. It is the EURL-AR organizers general impression that the participants had a good and beneficial understanding of the course content.

Discussion of tigecycline results for the EFSA/EURL confirmatory testing for 2018-data

By Anne Mette Seyfarth, EURL-AR

When EFSA observed a number of tigecycline (TGC) resistant isolates in the AMR surveillance database for the year 2018, these isolates (6 *E. coli* and 32 *Salmonella*) were selected for confirmatory testing at the EURL-AR. The MIC-distribution obtained by the EURL-AR was as follows: MIC ≤0.25 mg/L (15 % of the isolates), MIC 0.5 mg/L (15 % of the isolates), MIC 1 mg/L (35 % of the isolates) and MIC 2 mg/L (20 % of the isolates). According to the applied ECOFF at 1 mg/L back in 2018, only 20 % of the isolates were confirmed TGC resistant by the EURL-AR. A closer look at the data revealed that in most cases the discrepancy was a result of MIC-values differing only one dilution step between the EURL and the MSs, which is within the acceptable deviation of the method.

At the time of conducting the confirmatory testing, the EUCAST ECOFF values for *E. coli* was in revision and finally changed from 1 mg/L to 0.5 mg/L. For *Salmonella*, the ECOFF values applied by EFSA followed the same values as for *E. coli*. If we apply the new ECOFF values for these 40 isolates, almost no TGC discrepancies would be observed anymore, since all of the isolates at MIC 1 and 2 mg/L would be interpreted the same way (i.e. as resistant), but consequently the level of reported cases of TGC resistance would increase.

It has to be considered if any of these isolates with MIC-values at 1 and 2 mg/L are truly resistant to TGC. This theory is supported by two facts: 1) We have not detected any apparent genetic background for TGC resistance by sequencing of the isolates, and 2) A significant decrease in the number of reported TGC resistant isolates has been observed since 2018, despite the fact that a lower ECOFF value is expected to increase the level of resistance.

It is likely that the decrease in TGC resistance reported since 2018 is related to an increased awareness of the light sensitive nature of this antimicrobial. Our colleagues at the NRL of the Netherlands (Wageningen Food Safety Research, WFSR) observed that the activity of TGC quickly reduces, when exposed to light. This finding was disseminated to the NRL-AR network and this might have led to changes in lab procedures to minimize the exposure to light and thereby ensure more valid MIC-results reported for TGC.

Finally, since 2018, resistance to TGC was no longer included as a selection criterion, when EFSA selects the isolates for the annual confirmatory testing.

Notes on media for selective isolation of carbapenem resistant *E. coli* and *Salmonella*

By Jette Sejer Kjeldgaard, EURL-AR

Since the implementation of the new decision (EU) 2020/1729 by the beginning of this year, it has been mandatory for the NRL's to report carbapenemase producing *E. coli* and *Salmonella* (CPE's) as part of the harmonized monitoring. The challenge remains to find solid procedures that specifically and efficiently detect the various types of CPE's in a single protocol. In the current EURL-AR protocol, non-selective enrichment followed by plating on suitable, validated, selective agar plates is suggested, leaving it up to the NRL to find suitable, available agar plates for the purpose. The performance of the commercial agars is variable for the different types of carbapenemases (CPs), and there are different types of selective agar for detection of OXA-48(-like) and non-OXA-48 CPs. There have been made important efforts to compare carbapenem agars in several recent studies, like in the OH EJP IMPART project and in the study by Pauly et al., which also include promising results of in-house prepared media (presented at the EURL-AR Workshop 2021; doi: 10.3390/microorganisms9051105).
From the EURL-AR we do not recommend any certain media, but highlight that it is still needed to include specific selective media for detecting OXA-genotypes and that meropenem is only stable for a very short time in selective media. As such, the EURL-AR is hesitant to recommend non-commercial media without validated stability and shelf life. Thus, it is an area we will have to continue to discuss and evaluate in the years to come, and we appreciate all efforts to elucidate this challenge.

A brief report on antimicrobial resistance in *Salmonella* spp. isolates from poultry flocks

By Maja Velhner, *Scientific Veterinary Institute, Novi Sad, Serbia*

Five veterinary institutes from Serbia participated in a short survey with a goal to detect resistance to colistin in *Salmonella* isolates from poultry in 2018. We obtained 174 single *Salmonella* isolates from poultry farms in various regions of the country from the south to the north. Susceptibility testing was done according to the recommendation of the Clinical Laboratory Standards Institute (CLSI, M07-A10 and M100-S25). Examination of resistance to colistin was conducted using in-house MIC analysis and agar diffusion test on plates supplemented with 2mg/L colistin sulfate. The MIC analysis for colistin of *Salmonella* spp. (except *S. Enteritidis*) was interpreted according to Commission Implementing Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria 2013/652/EU, which recommends the following clinical breakpoint R >2mg/L. All isolates, presented with the MIC >2mg/L were additionally inoculated on Mueller Hinton agar supplemented with 2mg/L of colistin sulfate for confirmation of growth or the opposite.

Out of 174 *Salmonella* isolates, unrelated mechanisms of resistance to three or more classes of antibiotics were detected in nine *Salmonella* spp. Only seven *S. Infantis* isolates conferred resistance to colistin and four of them obtained multidrug resistant phenotype. However, the plasmid mediated resistance was not found in those isolates. Seven *S. Infantis* isolates resistant to colistin, were sent for the whole genome sequencing. One isolate from the Province of Vojvodina and two isolates from Jagodina (Central Serbia) were sequence type ST413 and ST11, respectively. Four isolates from Kraljevo (South-West Serbia) were ST32. We detected fosfomycin resistance gene *fosA7* in one isolate and *vgaA* conferring resistance to pleuromutilins in another isolate. At the time when this study was conducted the Sensititre™ plates were not available in our laboratories and AMR monitoring has not started yet in Serbia.

Resistance genes and mutations detected in isolates from the EFSA/EURL-AR confirmatory testing for 2019

By Anne Mette Seyfarth, EURL-AR

Antimicrobial resistance genes and mutations were detected by whole genome sequencing of the *E. coli* and *Salmonella* isolates selected for confirmatory testing at the EURL-AR by EFSA, with regard to the EU surveillance of AMR for 2019. The isolates were derived from 30 European countries and 332 *E. coli* and 4 *Salmonella* isolates were included. A list of the antimicrobial resistance genes and mutations detected by use of ResFinder 4.1 in relation to the antimicrobial panels used for the phenotypic AST surveillance, is presented in the appendix of this Newsletter.

Antimicrobial resistance monitoring in *Escherichia coli* from livestock

Evaluation & interpretation

The following is an abstract of Ayla Hesp's PhD thesis (public defense took place 11th of Nov 2021). (https://dspace.library.uu.nl/handle/1874/406770)

Ayla Hesp is a veterinarian by training and worked on her thesis in the AMR research team at Wageningen Bioveterinary Research (WBVR), The Netherlands from October 2016 till October 2021. In November 2021 she started working as project manager within antibiotic resistance at ZonMW in den Hague.
If you are interested in the details of Ayla's research you can download her thesis:
https://www.globalacademicpress.com/ebooks/ayla_hesp/12716_complete.pdf

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Effective antimicrobials are essential for adequate healthcare, but unfortunately, worldwide antimicrobial resistance (AMR) threatens this effectiveness, caused by antimicrobial use (AMU). The possibilities for development of antimicrobials are limited, and new antimicrobials will not become widely available. This leaves prudent AMU and other interventions to limit existing AMR as an important strategy and therefore, AMR must be monitored. Production animals are a relevant reservoir to monitor, because AMR may be transmitted to humans directly, or indirectly via food or the environment. This thesis is about monitoring of AMR in livestock as public health hazard in indicator organism *Escherichia coli*. In the European Union, monitoring of AMR in animals as public health hazard is performed by European legislation in commensal *E. coli* and food-borne pathogens *Salmonella* and *Campylobacter*. The international legislation has led to harmonisation and standardisation of the sampling and the microbiological methods. Elements not prescribed create room for improvement. The evaluation and interpretation by statistical analysis of AMR monitoring results is not prescribed, but is challenging and will be more complex when the amount of data increases. The updated EU legislation in 2020 has allowed whole-genome sequencing (WGS) as alternative method to culture-based antimicrobial susceptibility testing in AMR monitoring. So far, no statistical approaches were described to evaluate WGS versus culture-based methods. Analyses can be improved for optimal evaluation and interpretation of AMR monitoring data. Therefore, the first aim of this thesis is to evaluate AMR monitoring results with statistical methods. The second aim is to improve the interpretation of AMR monitoring in commensal *E. coli*. The third aim is to assess WGS versus culture-based methods to monitor AMR. The conclusions from this thesis are that *E. coli* is a useful indicator to monitor AMR in livestock, provided that bias in the sampling is prevented, and that proper statistical methods are used for the evaluation and interpretation. As we show, the randomized sample from healthy animals is well suited to analyse AMR trends over time. Other types of samples such as risk-based sampling (for example from diseased animals) are useful to detect rare or emerging resistance, but should be used next to a random sample to ensure representativeness for the whole animal population. To improve interpretation of AMR monitoring data, quantitative analyses should be incorporated in routine monitoring, because in the future the amount and complexity of data will further increase. The validity of the statistical approaches in this thesis should be further investigated in data with more variation, from different countries. We promote further evaluation of AMR surveillance systems, and the analysis of AMR monitoring outcomes should be harmonized, next to already existing harmonization of laboratory methods.

NARMS bioinformatics capacity building

By Gordon Martin and Errol Strain, US Food and Drug Administration, USA

The US National Antimicrobial Resistance Monitoring System (NARMS) program has leveraged rapid advancements in Whole-Genome Sequencing (WGS) and bioinformatic data analysis technologies to increase its ability to quickly recognize and identify emerging and potential antimicrobial resistance threats to public health. The adoption of WGS combined with growth of the NARMS program has led to the need for building bioinformatic analysis capacity for NARMS partner laboratories to perform local AMR analysis. This increase in laboratory efficiency allows for earlier recognition of potential emerging antimicrobial resistance. To achieve this, NARMS requires a bioinformatics platform that is accessible, easy to use, flexible, and composed of validated tools that are widely available.

GalaxyTrakr (https://galaxytrakr.org/) an instance of the popular Galaxy (https://usegalaxy.org/) platform, was created to support surveillance of bacterial foodborne pathogens. The platform is easily accessible, only needs an internet connection to work, and anyone who participates in NARMS testing can set up an account. There is a wide range of commonly used and free open-source bioinformatics tools available. The platform is flexible and easy to use. It was important for NARMS to be able to build AMR workflows that laboratory staff with little or no specialized data science, programming, or bioinformatics training could run since many sites lack these dedicated resources. In GalaxyTrakr, multiple tools and even multiple workflows can be easily "chained" together and the outputs configured to build automated "click and forget" workflows as simple or complex as needed. NARMS bioinformaticians built automated workflows that produce easy to read output reports specific for each of the pathogens monitored in the NARMS Retail Meat Program. For example, the NARMS *Salmonella* AMR workflow will trim and assemble the raw sequencer fastq output files and give quality assessments, serotype (SeqSero2), and AMR genes and mutations (AMRFinderPlus). For analyzing WGS sequence data from multiple or unknown pathogens, NARMS bioinformaticians built a workflow that will trim and assemble the fastq files and give quality assessments, identity (Genus and Species) information from tools using 7-gene MLST and KRAKEN2, and the core resistance genes (AMRFinderPlus). These workflows can be "shared" so others can use the exact same workflow resulting in a level of standardization. The accessibility, ease of use, flexibility, and availability of tools allowed FDA NARMS to build and share workflows specific to the needs of NARMS and its partners. NARMS also has the capacity to build WGS AMR workflows not only for other specific projects such as our newer seafood project, but also to support collaborators such as Veterinary Laboratory Investigation and Response Network (Vet-LIRN).

The GalaxyTrakr platform significantly increases the bioinformatics capabilities of the NARMS program to monitor AMR resistance not only at the local lab level, but also at the Program level. Its flexibility and ability to leverage bioinformatic tools and technologies has the potential to open new avenues of research and analysis. Future goals include having GalaxyTrakr incorporating AI/ML for MIC predictions, build in notifications for novel AMR genes, and incorporating new tools and methods for source attribution and to model and predict AMR spread, for example using Geographic Information Systems (GIS). GS-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.

Study finds drug-resistant *Campylobacter jejuni* connection to pet store puppies

By News Desk on October 6, 2021. Summary by Troels Ronco, EURL-AR

Originally, this story was published my News Desk (https://www.foodsafetynews.com/2021/10/study-finds-drug-resistant-campylobacter-jejuni-connection-to-pet-store-puppies/) and is based on a recent scientific paper by Louise K. Francois Watkins and co-authors entitled; "Ongoing Outbreak of Extensively Drug-Resistant *Campylobacter jejuni* Infections Associated With US Pet Store Puppies, 2016-2020" (JAMA Netw Open. 2021;4(9):e2125203. doi:10.1001/jamanetworkopen.2021.25203).

According to Centers for Disease Control and Prevention (CDC), *Campylobacter* is in the US, the most common bacterial cause of diarrhea and it is estimated to result in ~1.5 million cases of illness per year. Extensively drug-resistant *Campylobacter jejuni* infections constitute a worrisome threat to the public health since they cannot be treated with any commonly recommended antimicrobials.

Since 2011, drug-resistant *Campylobacter jejuni* strains have been associated with illness among pet store customers, employees, and others who have been in contact with pet store puppies. *Campylobacter* infections may arise by eating raw or undercooked poultry or other food products (seafood and meat), by being in contact with animals or drinking untreated water.

A survey study identified 168 cases from public health reports of *Campylobacter* infections with an epidemiologic or molecular link to pet store puppies from 2011 to 2020. Isolates were resistant to seven antibiotic classes, including all recommended treatment agents. Patients were interviewed regarding demographic characteristics, health outcomes and exposure to dogs during the seven days before illness. Laboratories used core genome multilocus sequence typing to assess isolate relatedness, and genomes were screened for resistance determinants to predict antibiotic resistance. Isolates resistant to fluoroquinolones, macrolides and three or more additional antibiotic classes were considered to be extensively drug resistant. Overall, the survey results are as follows:

- A total of 168 patients (median [interquartile range] age, 37 [19.5-51.0] years
- 105 of 163 patients were female, 64 %, with an epidemiologic/molecular association with pet store puppies studied
- A total of 137 cases occurred from Jan, 2016, to Feb, 2020, with 31 additional cases dating back to 2011
- A total of 97 % of patients reported contact with a dog, and 88 % of those reported contact with a pet store puppy
- Of the 168 isolates, 88 %, were extensively drug resistant
- A Traceback investigation did not find any particular breeder, transporter, distributor, store or chain

Suggestions:

1) Health care providers should ask about puppy exposure when treating patients with *Campylobacter* infections.

2) The commercial dog industry should take action to help prevent the spread of extensively drug-resistant *C. jejuni*.

Large diversity of linezolid-resistant isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019

*By Michael Timmermans[1,2], Bert Bogaerts[3], Kevin Vanneste[3], Sigrid C. J. De Keersmaecker[3], Nancy H. C. Roosens[3], Carole Kowalewicz[1], Guillaume Simon[1], Maria A. Argudín[4]†, Ariane Deplano[4,5], Marie Hallin[4,5,6], Pierre Wattiau[1], David Fretin[1], Olivier Denis[6,7] and Cécile Boland[1]**

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Background: Linezolid is a critically important antibiotic used to treat human infections caused by MRSA and VRE. While linezolid is not licensed for food-producing animals, linezolid-resistant (LR) isolates have been reported in European countries, including Belgium.
Objectives: To: (i) assess LR occurrence in staphylococci and enterococci isolated from different Belgian food-producing animals in 2019 through selective monitoring; and (ii) investigate the genomes and relatedness of these isolates.
Methods: Faecal samples (n=1325) and nasal swab samples (n=148) were analysed with a protocol designed to select LR bacteria, including a 44–48 h incubation period. The presence of LR chromosomal mutations, transferable LR genes and their genetic organizations and other resistance genes, as well as LR isolate relatedness (from this study and the NCBI database) were assessed through WGS.
Results: The LR rate differed widely between animal host species, with the highest rates occurring in nasal samples from pigs and sows (25.7% and 20.5%, respectively) and faecal samples from veal calves (16.4%). WGS results showed that LR determinants are present in a large diversity of isolates circulating in the agricultural sector, with some isolates closely related to human isolates, posing a human health risk.
Conclusions: LR dedicated monitoring with WGS analysis could help to better understand the spread of LR. Cross-selection of LR transferable genes through other antibiotic use should be considered in future action plans aimed at combatting antimicrobial resistance and in future objectives for the rational use of antibiotics in a One Health perspective.

The full paper has been published in Journal of Antimicrobial Chemotherapy, Oct 2021 (https://doi.org/10.1093/jac/dkab376).

Occurrence of linezolid transferable resistance mechanisms in LA-MRSA, *Enterococcus faecalis* and *Enterococcus faecium* from pigs

By Joana Gião, Célia Leão, Lurdes Clemente, Ana Amaro, INIAV, Portugal

Linezolid is a member of the oxazolidinone antibiotic class, approved to treat hospital-acquired pneumonia caused by *S. aureus*, including methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA), complicated skin and skin structure infections caused by MSSA, vancomycin-resistant *Enterococcus faecium* (VRE) infections, among others. Therefore, the emergence of linezolid-resistance determinants in gram-positive bacteria from non-human sources like animals and food products poses a significant public health concern.

Two *Enterococcus* strains (one *E. faecium* and one *E. faecalis*) and three methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated among 134 pig caecal samples and 171 nasal swabs samples, respectively, showing decreased susceptibility to linezolid. These isolates were sequenced using Illumina technology and CGE webtools, BLAST, PLACNETw, Artemis, and EasyFig were used for bioinformatics analysis.

E. faecium ST22 and *E. faecalis* ST474 strains harboured the *optrA* gene and *E. faecium* also carried *poxtA*, both genes encoding resistance to oxazolidinones and phenicols. The *optrA* gene was carried by rep33 plasmid in *E. faecium*, whereas in *E. faecalis* it is predicted to be located in the chromosome. Additional resistance determinants to other antibiotics were identified in accordance with the multidrug-resistant (MDR) phenotype.

MRSA ST398 strains belonged to *spa*-type t011 and SCCmec_type_Vc. Besides *mecA*, several other resistance genes were found in accordance with the MDR profile and we highlight the identification of *cfr* genes encoding resistance to oxazolidinones. The *cfr* gene was located at the pSAM13-0401 plasmid in all strains, flanked by the *ISSau9* and the transposon TnpR.

To our knowledge, this is the first time that oxazolidinones encoding resistance mechanisms such as *cfr* and *optrA* genes were detected in MRSA, *E. faecium*, and *E. faecalis* strains from healthy pigs in Portugal.

Co-localization of carbapenem (*bla*$_{OXA-162}$) and colistin (*mcr-1*) resistance genes on a transferable IncHI2 plasmid in *Escherichia coli* of chicken origin

By Valeria Bortolaia[1]†, Troels Ronco[1]†, Luminita Romascu[2,3], Isabela Nicorescu[4], Nicoleta M. Milita[2], Angela M. Vaduva[4], Pimlapas Leekitcharoenphon[1], Jette S. Kjeldgaard[1], Inge M. Hansen[1], Christina A. Svendsen[1], Hanne Mordhorst[1], Beatriz Guerra[5], Pierre-Alexandre Beloeil[5], Maria Hoffmann[6] and Rene´ S. Hendriksen[1]*

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Carbapenems are critically important antimicrobials for the treatment of clinical infections caused by MDR Enterobacteriaceae, with colistin reserved for those cases in which even carbapenems are ineffective. Therefore, co-localization of carbapenem and colistin resistance genes on the same plasmid is worrisome and, to date, has been described only in China in an *Escherichia coli* from a diseased chicken (*bla*$_{NDM-4}$ and *mcr-1* on an IncHI2/ST3 plasmid) and in an *E. coli* from a diseased pet cat (*bla*$_{NDM-5}$ and *mcr-1* on an IncX3-X4 hybrid plasmid). Here, we report a novel case of co-localization of *bla*$_{OXA-162}$ carbapenemase and *mcr-1* on a transferable IncHI2 plasmid in *E. coli* from a healthy broiler chicken in Europe.

The full letter has been published in Journal of Antimicrobial Chemotherapy, Nov 2021 (https://doi.org/10.1093/jac/dkab285).

From the EURL-AR we thank you for the fruitful collaboration in the year that passed and look forward to continuing this in 2022!

Merry Christmas!

Appendix

List of resistance genes and mutations per antimicrobial agent or antimicrobial class, in alphabetic order:

The prevalence of the resistance gene or mutation is presented as a percentage of the total number of genes/mutations detected for the specific antimicrobial, unless other is specified:

AMPICILLIN (cephalosporin resistance genes/mutations not included). 174 isolates harbored a total of 210 resistance genes conferring resistance to only ampicillin: TEM-genes (91.9 %), OXA-genes (5.7 %), LAP-2 (1.9 %) and CARB-2 (0.5 %). The TEM-genes were dominated by TEM-1B (70 %), TEM-1A (8 %) and TEM-30 (5 %), and the OXA-genes were OXA-1 (75 %) and OXA-10 (25 %).

AZITHROMYCIN. 63 isolates harbored a total of 64 azithromycin resistance genes: *mphA* (78.1 %), *mefB* (10.9 %), *mefC-mphG* (9.4 %) and *msrE* (1.6 %).

CARBAPENEMS. No resistance genes or mutations were detected for ertapenem, imipenem or meropenem.

CEPHALOSPORINS. 286 isolates were detected with cephalosporin resistance genes or mutations. For isolates with the so called ampC-profiles (i.e. resistant to beta-lactamase inhibitors), 65 % of the isolates had ampC promotor mutations, while 35 % carried the CMY-2 genes. For isolates with other profiles, like true ESBLs, the resistance was explained by CTX-M genes (93.7 % of the isolates), SHV-12 (5.3 % of the isolates) and TEM-genes (1.0 % of the isolates). The detected CTX-M genes were dominated by CTX-M-1 (48 %), CTX-M-15 (15 %), CTX-M-32 (15 %), CTX-M-14 (8 %) and CTX-M-55 (5 %).

CHLORAMPHENICOL. 110 isolates harbored a total of 152 chloramphenicol resistance genes: *floR* (54.6 %), *catA1* (33.6 %), *cmlA1* (8.6 %), *catB3* (2.6 %) and *catA2* (0.7 %).

CIPROFLOXACIN. 154 isolates were detected with fluoroquinolon resistance genes and/or mutations. For 11 % of the isolates a combination of genes and mutations were detected, whereas mutations alone were detected for 45 % of the isolates, and genes alone were detected for 44 % of the isolates. The genes observed were *qnr*-genes (94 %) and the *aac(6')-Ib-cr* gene (6 %). The *qnr*-genes observed were *qnrS1* (84 %), *qnrB19* (10 %), *qnrS2* (5 %) and *qnrA1* (1 %). As for nalidixic acid, the mutations were all *gyrA* mutations, except for a few *parC* (p.A56T) mutations. The *gyrA* mutations were dominated by *gyrA* (p.S83L) (92 %), in combination with *gyrA* (p.D87N) in 75 % of the cases.

COLISTIN. 97 isolates harbored a total of 97 colistin resistance genes: *mcr-1.1* (94.8%), *mcr-1.12* (1.0 %), *mcr-3.2* (1.0 %), *mcr-4.2* (1.0 %), *mcr-4.6* (1.0 %) and *mcr-9* (1.0 %).

GENTAMICIN. 67 isolates harbored a total of 72 gentamicin resistance genes: *aac3*-genes (95.8 %), *ant2*"-gener (2.8 %), *rmtB* (1.4 %). The majority of the *aac3*-genes were *aac3-IV* (41 %) and *aac3-IId* (35%).

NALIDIXIC ACID. 91 isolates were detected with mutations conferring resistance to nalidixic acid. All mutations were *gyrA* mutations, except for 2 isolates with a *parC* (p.A56T) mutation. The *gyrA* mutations were dominated by *gyrA* (p.S83L) (86 % of the isolates), in combination with *gyrA* (p.D87N) in 50 % of the cases.

SULFAMETHOXAZOLE. 216 isolates harbored a total of 304 sulfonamide genes: *sul2* (51.0 %), *sul3* (26.6 %) and *sul1* (22.4 %).

TEMOCILLIN. No resistance genes or mutations were detected.

TETRACYCLINE. 222 isolates harbored a total of 281 tetracycline resistance genes: *tetA* (61.2 %), *tetB* (19.9 %), *tetM* (17.1 %), *tetY* (1.1 %) and *tetD* (0.7 %).

TIGECYCLINE. No resistance genes or mutations were detected.

TRIMETHOPRIM. 174 isolates harbored a total of 192 trimethoprim genes: *dfrA12* (32.3 %), *dfrA1* (27.1 %), *dfrA14* (13.0 %), *dfrA17* (13.0 %), *dfrA5* (8.9 %), *dfrA8* (1.6 %), *dfrA36* (1.6 %), *dfrA7* (1.0 %) and *dfrA19* (0.5 %).