DTU Food
National Food Institute

NEWSLETTER

to the
National Reference Laboratories
for Antimicrobial Resistance

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Content

Tentative breakpoint for quinopristin/dalfopristin susceptibility testing of *Enterococcus faecium*

Agersø Y (EURL-AR) and Kahlmeter G (EUCAST)

Susceptibility testing of *E. faecium* against quinopristin/dalfopristin in the range 0.5-64 mg/L is included in the EU legislation 'Commission Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria' (2013/652/EU). Issues regarding the availability of the drugs for susceptibility testing have been solved and quinupristin/dalfopristin are now included in the testing panels. But testing of quinopristin/dalfopristin is not straightforward. Especially, the interpretation criteria are not well defined and recently EUCAST decided to remove the epidemiological cut-off values until more data are available. Quinopristin and dalfopristin are both members of the streptogramin class of antimicrobial agents. They are protein synthesis inhibitors used in combination in the proportion 30% - 70%, respectively. The instability of the compounds also challenges the susceptibility testing. This combination of two compounds and the instability probably influences the MIC distributions. Additionally, more knowledge is needed about the resistance mechanisms because isolates that are regarded as resistant based on phenotypic tests often do not contain known resistance genes.

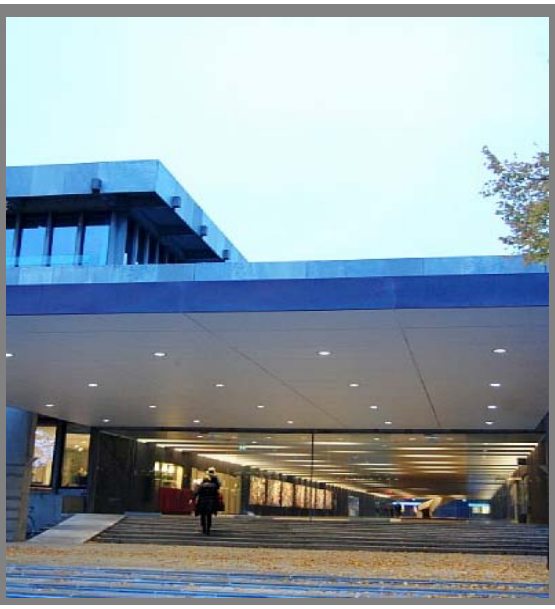
With the data currently available, EURL-AR recommends a tentative breakpoint for interpretation of quinopristin/dalfopristin resistance in *E. faecium* of >4 mg/L, whereas isolates with MIC=4 mg/L should be reported as intermediate resistant. The MIC distributions and distributions based on disc diffusion are shown on the EUCAST webpage (www.eucast.org) and is it expected that more data will be made available in the near future.

Further reading:

Hwang IY, Ku HO, Lim SK, Lee KJ, Park CK, Jung GS, Jung SC, Park YH, Nam HM (2010). Distribution of streptogramin resistance genes and genetic relatedness among quinupristin/dalfopristin-resistant *Enterococcus faecium* recovered from pigs and chickens in Korea. Res Vet Sci. 89:1-4.

Anette M. Hammerum, Yvonne Agersø, Lourdes Garcia-Migura, Anne Mette Seyfarth, Lone J. Porsbo, Hanne-Dorthe Emborg, Lars Bogø Jensen (2009). Evaluation of the quinupristin/dalfopristin breakpoints for *Enterococcus faecium*. International Journal of Antimicrobial Agents 34: 281–291.

Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA Journal 2012;10(6):2742 [64 pp.]. doi:10.2903/j.efsa.20



News flash from the EURL-AR: EURL-AR workshop, April 7-8, 2014

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.

Comparison of antimicrobial susceptibility tests with *in silico* detection of resistance genes

Zankari E (EURL-AR)

We have used the web-tool ResFinder to identify acquired antimicrobial resistance genes in whole-genome sequencing data from 200 isolates, covering four bacterial species, originating from Danish pigs (1). The four bacterial species included in the study was, *Salmonella* Typhimurium, *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium*. ResFinder results were then compared with results from phenotypic antimicrobial susceptibility tests using EUCAST epidemiological cut-off values.

The *E. coli* and *Salmonella Typhimurium* isolates were, respectively, tested for susceptibility to 16 and 17 different antimicrobial agents belonging to 7 different antimicrobial classes. *E. faecalis* were tested for susceptibility to 14 antimicrobial agents belonging to 9 different classes and *E. faecium* for susceptibility to 15 antimicrobial agents belonging to 10 different classes.

A total of 3051 different phenotypic tests were performed for the entire dataset; 482 tests led to the categorizing of isolates as resistant and 2569 as susceptible. Only in seven cases (six *E. coli* isolates and one *E. faecalis* isolate) disagreement between tested and predicted susceptibility were observed corresponding to a concordance of 99.74%. All of the seven disagreements were false positive, meaning that ResFinder identified a resistance gene but the isolate was tested phenotypically susceptible to the corresponding antimicrobial agent. All six disagreements in *E. coli* were related to spectinomycin resistance, ResFinder identified an *aadA1* gene with 100% identity, whereas the isolates were tested phenotypically susceptible to spectinomycin (five with MIC 64 mg/L and one with MIC 32 mg/L).

The last case of disagreement was an *E. faecalis* isolate which contained *aac(6′)-aph(2′′)* (ID 100%), but was tested phenotypically susceptible to gentamicin (MIC 32 mg/L).

A high concordance, 99.74%, between phenotypic and predicted antimicrobial susceptibility was observed. Thus, antimicrobial resistance testing based on whole-genome sequencing can be used as an alternative to conventional phenotypic methods.

ResFinder-2.0: 1st of November 2013 we released a new modified version of ResFinder. In ResFinder-1.4 (the old version) a gene in the genome has to cover at least 2/5 of the length of the resistance gene in the database to be outputted, in the new version it is possible for the user to change this value. In addition, if different alleles of the same gene are present at different loci of the genome, all loci will now be outputted.

The web server ResFinder-2.0 is freely available at: http://cge.cbs.dtu.dk/services/ResFinder-2.0/

Further reading:

(1) Zankari E., Hasman H., Kaas R. S., Seyfarth A. M., Agersø Y., Lund O., Larsen V. M., Aarestrup F. M. 2012. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. J. Antimicrob Chemother. 68: 771–777. (http://jac.oxfordjournals.org/content/68/4/771.full.pdf+html)

New legislation on harmonized monitoring on AMR in the EU

Peran i Sala R (EU Commission)

The strengthening of surveillance systems on antimicrobial resistance (AMR) and the use of antimicrobials in the production of animals and food are essential parts of the European Commission's five year action plan against the rising threats of antimicrobial resistance.

Harmonized data is the key element to a better understanding of the epidemiology of AMR. Comparable and harmonized data is also needed for risk assessment and risk management decisions, and for the evaluation of the effectiveness of the measures taken.

In order to review and harmonize the surveillance systems on AMR in the European Union, the European Commission requested the European Food Safety Authority (EFSA) to publish a scientific report revising the existing technical specifications on the monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter*, indicator commensal *Escherichia coli* and *Enterococcus* spp. transmitted through the food production system.

This report as well as other scientific opinions of EFSA such as the scientific opinion on extended spectrum β-lactamases (ESBL) have all been considered in the recently adopted *Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (Decision 2013/652/EU)* that will apply from the 1st of January 2014.

The new legislation lays down detailed rules for all Member States (MS) implementing the harmonized monitoring of AMR. Those rules include elements such as prioritizing the combinations of bacterial species, food-producing animal species and food products that are the most relevant from a public health perspective but also interpretation of results and reporting of AMR data. In order to minimize the burden of each MS, the monitoring is based on biological samples or isolates collected by the competent authority when checking the compliance with Regulation 2073/2005 on microbiological criteria for foodstuffs and in the framework of other already established national control programmes. Additionally, the legislation includes specific requirements for the harmonized monitoring and reporting of ESBL, ampC β-Lactamases (ampC) and carbapenemase-producing bacteria in certain food-producing animal populations and in certain food types.

The technical annex of the legislation describes the detailed rules for the sampling (origin of the isolates, sampling frequency, sampling size, randomised sampling design), analysis of the isolates (antimicrobials, epidemiological cut-off values and concentration ranges to be used for antimicrobial susceptibility testing of the isolates), for the specific monitoring of ESBL- or ampC- or carbapenemase-producing *Salmonella* and *E. coli*, and the reporting of the results.

A training course for National Reference Laboratories was held at the EURL-AR facilities in Kgs. Lyngby, Denmark, from Monday 25th to Friday 29th November 2013 to introduce the new legislation on AMR monitoring. The training provided basic knowledge and tools for executing the tasks laid down in the new legislation to ensure that all MS would be able to comply with the legislation.

Reference:

Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (Decision 2013/652/EU); http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:303:0026:0039:EN:PDF.

News flash from the EURL-AR: One representative per Member State

In relation to the activities arranged by the EURL-AR, the EU Commission has requested an adjustment in the expenditure of the financial means in relation to proficiency tests, workshops and training courses.

Following these adjustments, upcoming invitations to these activities will be extended to one participant to represent the Member State (MS). Where possible, the invitation will also be extended to additional representatives, in these cases the conditions for this will be stated in the invitation or the prenotification.

The changes in relation to this issue will be introduced from 2014.

Follow up on tentative colistin epidemiological cut-off value for *Salmonella* spp. and evaluation on serovar level for *S.* Enteritidis and *S.* Dublin

Agersø Y (EURL-AR) and Kahlmeter G (EUCAST)

Susceptibility testing of *Salmonella* spp. against colistin in the range 1-16 mg/L is included in the EU legislation 'Commission Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria' (2013/652/EU). But neither testing nor interpretation of the test result is straightforward. The issue was partly described in the EURL-AR newsletter published in December 2012 and further discussed at the annual EURL-AR workshop in April 2013. MIC determination of colistin MICs in plastic trays is under investigation. Colistin binds to plastic which influences MICs. A joint CLSI and EUCAST subcommittee is expected to produce recommendations for both committees and for CDC and ECDC over the next year. These may change MIC determination recommendations, pharmacokinetics and pharmacodynamics. Based on the new information, breakpoints (clinical and ECOFFs) will be reviewed by EUCAST and CLSI.

With the current recommendations, EURL-AR recommends that *Salmonella* spp. with MIC >2 mg/L for colistin is evaluated on serovar level with a tentative epidemiological cut-off value for *S.* Enteritidis and *S.* Dublin of WT ≤8 mg/L. The MIC results from three different sources in the range 2-16 mg/L are shown on the EUCAST webpage (www.eucast.org) and it is expected that more data on serovar level will be made available in the near future.

Interesting reports published

The ESVAC 2011 report with data from 25 countries was published on the EMA web page and is available for public access: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2013/10/WC500152311.pdf

Revised ESVAC reflection paper on collecting data on consumption of antimicrobial agents per animal species, on technical units of measurement and indicators for reporting consumption of antimicrobial agents in animals is published and is available for your information (Home/Regulatory/Veterinary medicines/Antimicrobial resistance/ESVAC/Revised reflection paper on collecting data)

The report *'Antibiotic resistance threats in the United States, 2013'* gives a first-ever snapshot of the burden and threats posed by the antibiotic-resistant germs having the most impact on human health – link: http://www.cdc.gov/drugresistance/threat-report-2013/index.html

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The US Food and Drug Administration (FDA) is implementing a voluntary plan with industry to phase out the use of certain antibiotics for enhanced food production – link: http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM378197.pdf

Merry Christmas!

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

This past year we have enjoyed good collaboration with you and look forward to continuing this in the year to come.

Best wishes for a wonderful holiday and a very happy New Year!

The EURL-AR team