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NEWSLETTER

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Content

In silico detection of resistance genes in bacteria

Hasman, H (EURL-AR)

We have recently developed a web-based tool called *ResFinder* for identifying resistance genes in bacteria using sequencing data (1). This data can both originate from regular PCR-amplified (assembled) Sanger-sequencing in Fasta format or from various next-generation sequencing (NGS) platforms. The *ResFinder* database contains 1862 different resistance genes belonging to 1411 gene groups and covering most of the known resistance genes previously published to be present in bacteria. The genes confer reduced susceptibility towards one or more of the following antimicrobial classes: Aminoglycosides, beta-lactams, flouroquinolones, fosfomycins, fusidic acid, glycopeptides, macrolide-lincosamide-streptograminB, phenicols, rifampicin, sulphonamides, tetracyclins and trimethoprim. The *ResFinder* web-tool has initially been verified using the same tested on a set of 1862 Genbank files, which was used to create the database and then further tested on 23 fully sequenced (draft) bacterial genomes originating from 5 different bacterial species (*E. coli, K. pneumoniae, S. enterica, S. aureus* and *V. cholera*), where phenotypic data were available. This evaluation showed very good agreement between detected genes and susceptibility results.

These results show that genotypic detection of resistance genes is a promising alternative to phenotypic testing. Especially in relation to monitoring of resistance, this would allow further separation of resistant bacteria into genetic subgroups, which has not been possible (or has been problematic) when using phenotypic methods. A study examining the power of *ResFinder* in monitoring of antimicrobial resistance is currently being conducted at the EURL-AR. At the moment, *ResFinder* is not able to detect acquired chromosomal mutations in target genes or intrinsic resistance determinants. However, these are issues we are currently working on to improve. The *ResFinder* web server is freely available at: http://cge.cbs.dtu.dk/services/ResFinder/.

Further reading:

(1) Zankari E., H. Hasman, S. Cosentino, M. Vestergaard, S. Rasmussen, O. Lund, F. M. Aarestrup, M. V. Larsen. 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67:2640-2644. (http://jac.oxfordjournals.org/content/67/11/2640.full.pdf+html)

More focus on screening for carbapenemase producing bacteria from food producing animals

Agersø Y (EURL-AR)

The occurrence of *Enterobacteriacae* resistant to carbapenems is a growing threat in human medicine as carbapenems are last line antimicrobial agents for treatment of infections caused by multidrug resistant Gram-negative bacteria in humans (EFSA, 2012). The prevalence of carbapenemase producing bacteria in food producing animals is not known, but lately carbapenemase producing *E. coli* has been detected in livestock pigs and carbapenemase producing *Salmonella enterica* subsp. *enterica* has been detected in both livestock pigs and poultry (Fischer et al., 2012a, 2012b). Carbapenemase-producing *Acinetobacter* spp. were also very recently reported from cattle in France (Poirel et al., 2012). The presence and possible spread of carbapenemase producing bacteria in production animals is thus considered extremely important for the assessment of potential zoonotic risks and has therefore been included in the latest report of harmonized monitoring of antimicrobial resistance by EFSA, where it is recommended that phenotypic testing for carbapenem resistance in *Salmonella* and *E. coli* is performed consistently (EFSA, 2012).

Photo: Colourbox

The detection of carbapenem resistance is not straightforward, since carbapenemases belong to several different classes of beta-lactamases and no single test is likely to give high sensitivity as well as high specificity for all types of enzymes (EFSA, 2012; Nordmann and Poirel, 2012). Three carbapenems: Meropenem, imipenem and ertapenem can be used for screening (EFSA, 2012). Ertapenem is believed to detect all types but produces false positives, whereas some bla_{OXA} carbapenemases has a low hydrolytic activity against imipenem and meropenem and will be overlooked in a phenotypic screening. Moreover, the suggested clinical breakpoint and EUCAST ECOFF are also under debate (Nordmann and Poirel, 2012). Due to these difficulties with the phenotypical detection of carbapenem resistance, supplementary tests such as screening for resistance genes may be necessary. Carbapenemases in *Enterobacteriaceae* are mostly of the bla_{KPC}, bla_{VIM}, bla_{IMP} bla_{NDM} and bla_{OXA-48} types and are for several types not always expressed phenotypically (Fischer et al., 2012a, 2012b; Nordmann and Poirel, 2012). Phenotypical and genotypical detection of carbapenemase producing bacteria in food producing animals and meat will be discussed at the annual EURL-AR workshop in April 2013.

Further reading:

Fischer J, Rodríguez I, Schmoger S, Friese A, Roesler U, Helmuth R, Guerra B. Escherichia coli producing VIM-1carbapenemase isolated on a pig farm. J Antimicrob Chemother. 2012 ;67:1793-5.

Fischer J, Rodríguez I, Schmoger S, Friese A, Roesler U, Helmuth R, Guerra B. Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. J Antimicrob Chemother. Ahead of print.

EFSA.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.

Poirel L, Berçot B, Millemann Y, Bonnin RA, Pannaux G, Nordmann P. Carbapenemase-producing Acinetobacter spp. in Cattle, France. Emerg Infect Dis. 2012;18:523-5.

Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. JAC. 2012. Ahead of print.

Quinopristin / dalfopristin susceptibility

Agersø Y (EURL-AR)

Susceptibility testing of enterococci and staphylococci for quinupristin/dalfopristin has been done for several years and is also recommended by EFSA in two recent publications on harmonised monitoring and reporting of antimicrobial resistance in EU (EFSAa, EFSAb). 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. They are used to treat human enterococci and staphylococci infections and confer cross-resistance to previously used growth promoters. Lately, the availability of these drugs for susceptibility testing has become a problem for several NRL-ARs. Therefore, both the relevance and possibilities for conducting susceptibility testing in enterococci and staphylococci for quinupristin/dalfopristin will be discussed at the EURL-AR workshop in April 2013.

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

Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant Staphylococcus aureus in food-producing animals and food. EFSA Journal 2012;10(10):2897 [56 pp.]. doi:10.2903/j.efsa.2012.2897

Expert workshop on an EU protocol for harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* from humans

Hendriksen RS (EURL-AR) and Westrell T (ECDC)

On 29-30 May 2012, an expert workshop on an EU protocol for harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* from humans was held at ECDC in Stockholm, Sweden. The workshop was attended by experts on antimicrobial susceptibility testing for *Salmonella* and *Campylobacter* in human and veterinary microbiology services including representatives from EFSA, EUCAST, the Food- and Waterborne Diseases and Zoonoses network (FWD net), EARS-Net, the EU-RL for *Campylobacter* and the EURL-AR, as well as external scientific experts.

The aim of the meeting was to review the range of antimicrobial agents used for monitoring and to define the standardisation and harmonisation needs. This included discussions on in vitro antimicrobial susceptibility testing, interpretation, and reporting on human clinical isolates of *Salmonella* and *Campylobacter* with the aim to enable production of comparable data between countries regarding human infections and with animal/food isolates for monitoring of resistance at EU level.

One goal of the meeting was to discuss the need for technical guidance about the range of antimicrobial agents and key resistance determinants to be monitored in comparison with the recently published guidelines from EFSA (EFSA, 2012) issued subsequent to the meeting. The antimicrobials currently under EU-level surveillance for human *Salmonella* and *Campylobacter* isolates were reviewed and a list of priority antimicrobials to include in testing was set up, with some antimicrobials removed and others added to the current list.

Ranges and antimicrobials for ESBL-detection in human *Salmonella* isolates were discussed. Overall, there was agreement with the stepwise strategy based on EFSA's proposal, however, with modifications. It was agreed to wait with the final suggestions until the recommendations of the EUCAST Subcommittee on resistance mechanisms are published at the end of 2012.

The conclusions from the meeting will be put forward to the FWD network for decision in early 2013.

Further reading:

European Food Safety Authority; 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.2012.2742. Available online: www.efsa.europa.eu/efsajournal

Photo: Vibeke Hempler

EURL-AR workshop, April 4-5, 2013

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

The second day of the workshop will be a joint meeting between the EURL-AR network and the ECDC-FWD-network (public health sector) from where relevant experts will be invited to participate.

We are looking very much forward to this opportunity for networking and collaboration!

Shortly, an official invitation with further details will follow.

Please book the days in your calendar.

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

Agersø Y (EURL-AR)

The interpretive criteria are crucial when determining bacteria as either resistant or sensitive to an antimicrobial drug. The European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2011) presents a clinical breakpoint and an epidemiological cut-off value of >2 mg/L for colistin, whereas *Salmonella* isolates with a Minimal Inhibitory Concentration (MIC) ≤2 mg/L should be reported as sensitive or wildtype. The epidemiological cut-off value is defined based on MIC population distributions of *Salmonella* spp. [www.EUCAST.org].

In a recent study minimal inhibitory concentration (MIC) population distributions for colistin for *Salmonella* on subtype level was determined. Furthermore, it was also investigated if differences in MIC for colistin could be explained by mutations in *pmrA* or *pmrB*, encoding proteins involved in processes that influence the binding of colistin to the cell membrane (Agersø *et al*. 2012).

MIC distributions for colistin at serotype level showed that *S.* Dublin followed by *S.* Enteritidis were less susceptible than 'other' *Salmonella* serotypes originating from humans and *S.* Typhimurium of animal/meat origin. MIC was ≤1 mg/L for 98.9% of 'other' *Salmonella* serotypes originating from humans, 99.4% of *S.* Typhimurium, 61.3% of *S.* Enteritidis and 12.1% of *S.* Dublin isolates (Figure 1). Interestingly, *S.* Dublin and *S.* Enteritidis belong to the same O-group (O:1,9,12) suggesting that surface lipopolysaccharides (LPS) of the cell (O-antigen) play a role in colistin susceptibility. The epidemiological cut-off value >2 mg/L for colistin suggested by EUCAST is placed inside the distribution for both *S.* Dublin and *S.* Enteritidis. All tested *S.* Dublin isolates, regardless of colistin MIC value, had identical *pmrA* and *pmrB* sequences.

It was therefore concluded that missense mutations are not necessarily involved in increased MICs for colistin. Increased MICs for colistin seemed to be linked to specific serotypes (*S.* Dublin and *S.* Enteritidis). It is therefore recommended that *Salmonella* with MIC >2 mg/L for colistin are evaluated on serovar level. In 2012, laboratories in the EU reported their colistin MIC results for *S.* Enteritidis and *S.* Dublin to the EURL-AR and MIC results from three different sources in the range 2-16 was obtained and epidemiological cut-off value for *S.* Enteritidis and *S.* Dublin is under development at the EUCAST homepage.

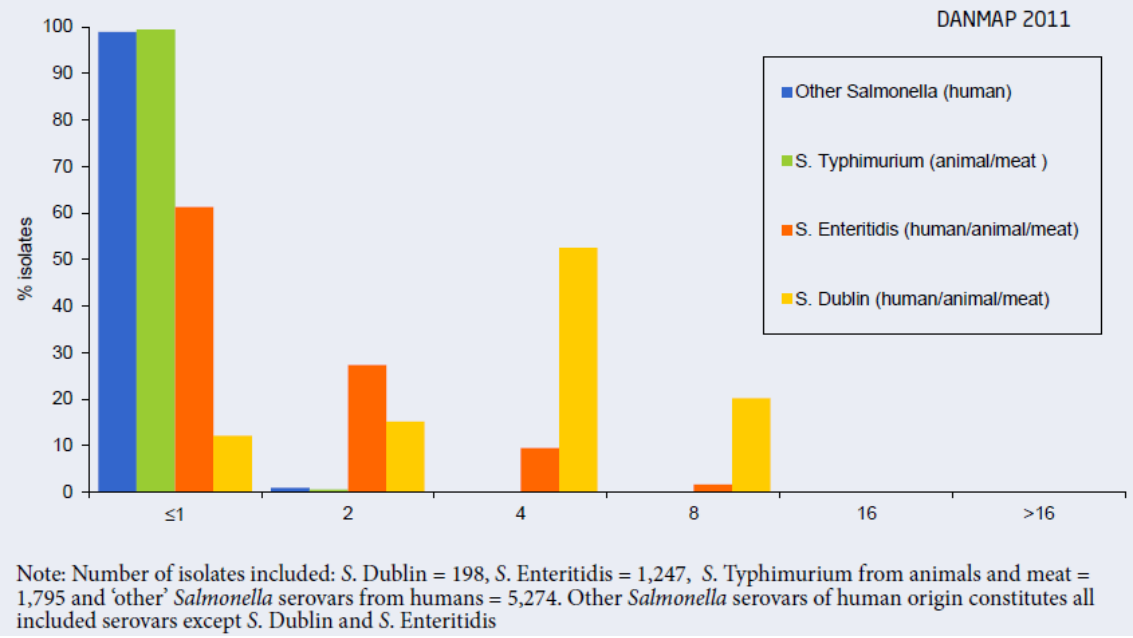
Further reading:

Agersø Y, Torpdahl M, Zachariasen C, Seyfarth A, Hammerum AM, Nielsen EM. Tentative colistin epidemiological cut-off value for *Salmonella* spp. Foodborne Pathog Dis. 2012; 9 :367-9.

DANMAP 2011. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032

Sun S, Negrea A, Rhen M, Andersson DI. Genetic analysis of colistin resistance in *Salmonella* enterica serovar Typhimurium. Antimicrob Agents Chemother. 2009; 53:2298-305.

Figure 1: MIC distribution (%) of *Salmonella* serovars from humans and animals/meat, Denmark (reprint from DANMAP 2011)



Note: Number of isolates included: *S.* Dublin = 198, *S.* Enteritidis = 1,247, *S.* Typhimurium from animals and meat = 1,795 and 'other' *Salmonella* serovars from humans = 5,274. Other *Salmonella* serovars of human origin constitutes all included serovars except *S.* Dublin and *S.* Enteritidis