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NEWSLETTER

to the
National Reference Laboratories for Antimicrobial Resistance

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Content

Multi-resistance

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The text below is part of 'Editorial: Assessing the antimicrobial susceptibility of bacteria obtained from animals' which is published in J Antimicrob Chemother 2010; 65: 601–604.

(doi:10.1093/jac/dkq037), and in Veterinary Microbiology (doi:10.1016/j.vetmic.2009.12.013).

The term 'multi-resistance' exclusively refers to acquired resistance properties. Bacteria may exhibit intrinsic (primary) resistance to certain antimicrobial agents. Intrinsic resistance may be based on either the lack or the inaccessibility of the antimicrobial target site among the bacteria in question. In other cases, intrinsically resistant bacteria produce inactivating enzymes, such as species-specific b-lactamases, contain multidrug transporters and/or exhibit permeability barriers.[7,8] Such intrinsic resistances must be excluded when describing multi-resistance patterns.

There is no universally accepted definition of 'multi-resistance'. As a consequence, this term is used inconsistently in the literature. The following suggestions are intended to provide guidance for the most accurate presentation of multi-resistance patterns.

(i) If only phenotypic susceptibility testing is performed, resistance to three or more classes of antimicrobial agents can be referred to as multi-resistance. For example, resistance to enrofloxacin, marbofloxacin, difloxacin and orbifloxacin represents resistance to one antimicrobial class, since all agents are fluoroquinolones and resistance is most likely mediated by the same mechanism(s). In the case of fluoroquinolones (and some other antimicrobial classes), resistance to a single representative of this class of antibiotic agent can reasonably be extrapolated to resistance (or reduced susceptibility) to other members of that class. However, single class representatives cannot always be validly defined, e.g.

for b-lactams and aminoglycosides. In these cases, resistance is not a class effect and multiple, diverse resistance mechanisms exist, each of which confers resistance to subgroups of the respective antimicrobial class. Resistance to subgroups should be counted separately, e.g. resistance to streptomycin and spectinomycin is distinct from resistance to gentamicin, kanamycin and/or tobramycin.

(ii) If phenotypic susceptibility testing is supplemented with molecular analysis for the resistance genes present, multi-resistance should be assessed at the molecular level. Bacterial isolates exhibiting the presence of three or more resistance genes or mutations, all of which are associated with a different resistance phenotype (i.e. affecting different antimicrobial classes or subgroups), may be referred to as multi-resistant. Exceptions to this rule would include those cases where a single resistance gene or a gene complex is associated with resistance to structurally and/or functionally different antimicrobial agents, e.g. the gene cfr for resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics,[9] or the erm genes for combined resistance to macrolides, lincosamides and streptogramin B antibiotics.[10]

7 Schwarz S, Cloeckaert A, Roberts MC. Mechanisms and spread of bacterial resistance to antimicrobial agents. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington, DC: ASM Press, 2006; 73–98.

8 Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother 2005; 56: 20–51.

9 Long KS, Poehlsgaard J, Kehrenberg C et al. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob Agents Chemother 2006; 50: 2500–5.

10 Roberts MC, Sutcliffe J, Courvalin P et al. Nomenclature for macrolide and macrolide–lincosamide–streptogramin B resistance determinants. Antimicrob Agents Chemother 1999; 43: 2823–30

Newsletter from HPA

Newsletters on antimicrobial resistance are also being issued from the Health Protection Agency, UK, and are accessible from the agency's website via the link: http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/LaboratoriesAndReferenceFacilities/AntibioticResistanceMonitoringAndReferenceLaboratory/ARMRLNewsletters/

Simple and efficient screening method for the detection of cephalosporin resistant *Escherichia coli*

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Abstract from article available in Bull Vet Inst Pulawy 54, 147-151, 2010 (open access article):

An efficient method for cephalosporin resistance screening in *E. coli* isolated from healthy farm animals has been described. One hundred and twenty nine rectal swabs were streaked on MacConkey agar and selective medium supplemented with cefotaxime. Antimicrobial resistance was tested with broth microdilution and *E. coli* resistant to either/or cefotaxime and ceftazidime were further tested with Etest.

The observed synergy of the compounds allowed confirming the presence of defined cephalosporin resistance phenotypes. The sensitivity of cephalosporin detection by the procedure with MacConkey culture reached merely 16.7% compared to the method with selective supplement medium. Extended spectrum of beta-lactamase producing isolates was found in strains isolated from 15 samples taken from turkeys, broilers, laying hens, and pigs. The ampC-type resistance was noted in *E. coli* from 33 samples originating from the same animal species. None of the resistance phenotypes was observed in cattle isolates. Attention is drawn to possible public health implications of slaughtered farm animals colonised with beta-lactam resistant *E. coli*.

Establishing streptomycin epidemiological cut-off values for *Salmonella* and *E. coli*

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The work on establishing streptomycin epidemiological cut-off values for *Salmonella* and *E. coli* has been ongoing in the EURL-AR network, and is now at the state where the article has been submitted.

The background, discussions and conclusions are in brief that this study was conducted to elucidate the accuracy of the current streptomycin epidemiological cut-off value (ECOFF) for *Escherichia coli* and *Salmonella* spp. Methods: A total of 236 *Salmonella enterica* and 208 *E. coli* isolates exhibiting MICs between 4 mg/L and 32 mg/L were selected from 12 countries.

All isolates were investigated by PCR for the most common streptomycin resistance genes described in *Enterobacteriaceae* (*aadA*, *strA* and *strB*).

Each of the 12 countries provided information on the streptomycin MIC distribution for both species over a one-year period.

Results: Out of 236 *Salmonella* isolates investigated, 32 (13.5%) strains yielded amplicons for *aadA* (n=23), *strA* (n=9), and *strB* (n=11). None of the 60 *Salmonella* isolates exhibiting MIC 4 mg/L harboured any of the resistance genes tested. Of the *Salmonella* isolates exhibiting MICs 8 mg/L, 16 mg/L, and 32 mg/L, 1.6%, 15% and 39%, respectively, tested positive for one or more of the three genes. For most monitoring programs, the streptomycin ECOFF for *Salmonella* spp. is wild type (WT) ≤ 32 mg/L or ≤16 mg/L. A cut of value of WT ≤ 32 mg/L would have resulted in the misclassification of 39% of the strains.

Out of 208 *E. coli* strains investigated, 80 (38.5%) tested positive for *aadA* (n=69), *strA* (n=18) and *strB* (n=31). Of the *E. coli* isolates exhibiting MICs of 4 mg/L, 8 mg/L, 16 mg/L, and 32 mg/L, 3.6%, 17.6%, 53% and 82.3%, respectively, harboured any of the three genes. Based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (ECOFF ≤ 16 mg/L), 53% of the *E. coli* strains presenting MIC 16 mg/L would have been classified as wild-type.

Conclusion: Based on these results, the authors recommend an ECOFF value of WT ≤ 16 mg/L for *Salmonella* and WT ≤ 8 mg/L for *E. coli*. These ECOFF values represent a compromise between MIC distribution of the population and genetic characterization of the resistance genes.

EQAS test strains

The EURL recommends that the NRLs store the EQAS test strains in their strain collection for possible later reference, as the EQAS test strains cover as many as possible of the relevant resistance profiles and mechanisms.

International Collaborative Study on the Occurrence of Plasmid Mediated Quinolone Resistance (PMQR) in *Salmonella* and *E. coli* with a Distinctive Resistance Pattern from Thirteen European countries

Veldman, K; Cavaco, LM; Mevius, D.; Dominguez; Battisti, A; Cerny, T; Franco, A; de Frutos Escobar, C; Guerra, B; Gutierrez, M; Heylen, K; Hopkins, K; Myllyniemi, A; Perrin-Guyomard, A; Schroeter, A; Sunde, M; Wasyl, D; Aarestrup, FM.

Modified from: International collaborative study on the prevalence of plasmid mediated quinolone resistance (PMQR) in *Salmonella* and *E. coli* isolated from humans and animals in Europe. Poster presentation B192 at ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans and the Environment, 8-1 June, 2010, Toronto, Canada p. 118-119.

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[Manuscript in preparation]

Introduction

In the last decade plasmid mediated quinolone resistance (PMQR) is increasingly reported in *Enterobacteriaceae* worldwide, including several European countries. However, studies reporting the occurrence and spread of PMQR-genes in *Enterobacteriaceae* on a European level are lacking.

PMQR-positive isolates display low-level resistance to ciprofloxacin, but remain susceptible to nalidixic acid, which can be used for retrospective studies.

In 2008, the European Reference Laboratory for Antimicrobial Resistance (EURL-AR at the National Food Institute, Denmark) initiated a collaborative study including the European National Reference Laboratories (NRL) within their network. The aim of the study was to collect retrospective information on the prevalence of PMQR in *Salmonella* and *E. coli* isolates in Europe and to identify the genes detected.

Methods

All laboratories within the EURL-AR network were asked to screen their MIC-databases for isolates with a defined PMQR phenotype, retrospectively. The majority of the isolates tested were isolated from 2002 to 2008. The following inclusion criteria were used; for *Salmonella* isolates with ciprofloxacin MICs: 0.125 -1 mg/L and nalidixic acid MICs: 4 – 32 mg/L and for *E. coli*: ciprofloxacin MICs: 0.06 – 1 mg/L and nalidixic acid: 4 – 32 mg/L. Subsequently, all participants were asked to screen their selected isolates with PCR for the following PMQR genes: *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac(6')-1b-cr*. Finally, all amplicons were sequenced to identify the detected resistance genes and respective variants.

Results

Initially, NRL-AR of twenty-one European countries agreed to participate in the study. Subsequently, laboratories of Belgium, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Norway, Poland, Spain, the Netherlands and UK participated in the study.

The retrospective screening resulted in 1215 PMQR suspected *Salmonella* and 333 PMQR suspected *E. coli*, of which 485 and 133, respectively, were genetically characterized.

PMQR-positive *Salmonella* and *E. coli* isolates were identified, respectively in eleven and in four participating countries. PCR testing revealed PMQR genes in 59% (288/485) of the selected *Salmonella* and 15% (20/133) of the selected *E. coli* isolates.

Most PMQR-positive *Salmonella* were detected in isolates from humans (n=102), turkeys (n=118), and poultry (n=39). In *Salmonella*, variants of *qnrB* (n=138) and *qnrS1* (n=125) were most commonly detected. Moreover, *qnrD* (n=22), *qnrA1* (n=3) and *aac(6')-1b-cr* (n=3) were also identified in *Salmonella*. In *E. coli, qnrS1* (n = 19) was identified in isolates from food, poultry, turkeys, cattle and pigs and *qnrB19* in one turkey isolate.

Conclusions

Our study confirms the occurrence of PMQR-positive *Salmonella* isolates of human and animal origin in Europe since 2002. In addition, we report the first *qnrD* in *Salmonella* isolated outside of Asia. Moreover, our findings indicate that in animals in Europe PMQR-positive *E. coli* occur only incidentally.