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Short communication

PREVALENCE OF SOME GENETIC FACTORS DETERMINING ANTIMICROBIAL RESISTANCE IN COMMENSAL *ESCHERICHIA COLI* ISOLATED FROM BROILERS AND LAYING HENS

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Summary

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The aim of the current study was to determine the prevalence of resistance to some antimicrobial agents in commensal *Escherichia coli* from poultry in Bulgaria. It was performed from June to December 2020 and included 175 strains, 99 of which were isolated from broilers and 76 from laying hens. ESBL phenotypes of isolates were tested for the presence of $bla_{\text{CTX-M-1}}$. The distribution of genes *qnrS*, *qnrA* and *qnrB1* conferring resistance to quinolones was studied. Also, the resistance to tetracycline and the presence of *tetA* gene were investigated. A high percentage of resistance towards tetracycline and ciprofloxacin was observed in commensal *E. coli* isolates from broilers (65.6%, 70.7%, respectively), and a high percentage of resistance to ciprofloxacin (75.0%) in isolates from laying hens. Also, high resistance towards β-lactams ampicillin and amoxicillin/clavulanic acid was observed in isolates from broilers (54.5%, 45.4%), vs 50.0% to ampicillin and 39.5% to amoxicillin/clavulanic acid in isolates from laying hens. Fewer strains resistant to cefotaxime and ceftazidime (8.1 %, 7.1%) were found out in isolates from broilers. None of commensal *E. coli* strains from laying hens were resistant to cefotaxime and ceftazidime. Five of the cephalosporin-resistant *E. coli* from broilers (5.0%) were identified as producers of ESBL, possessing the gene $bla_{\text{CTX-M-1}}$. Sixty-five strains from broilers (65.6%) and 50 strains from laying hens (65.8%) possessed the *qnrS* gene. The *qnrA* and *qnrB1* genes were not detected in ciprofloxacin-resistant *E. coli* isolates. Sixty-five strains from broilers (65.6%) and 33 strains from laying hens (33.0%) possessed the gene *tetA*. The commonest profile of multidrug resistance in *E. coli* isolates from broilers (37.4%) included ampicillin, amoxicillin/clavulanic acid, tetracycline and ciprofloxacin while in isolates from laying hens, resistance to ampicillin, amoxicillin/clavulanic acid and ciprofloxacin predominated (33.0%).

Key words: antimicrobial agents, poultry, resident *Escherichia coli*, resistance

The WHO has defined cephalosporins from third and fourth generations, along with fluoroquinolones, as antimicrobial drugs of critical importance for the therapy of human infectious diseases (FAO/WHO/OIE, 2008). Some resear-

chers affirmed domestic fowl as one of relevant sources for spread of microbial resistance to quinolones in human along the food chain (Kaesbohrer *et al*., 2012, Asai *et al*., 2014). On the other hand, the spread of antimicrobial resistance in resident *E. coli* and enterococci isolated from domestic livestock was outlined as exceptionally appropriate and early indicator for the selective pressure of these drugs in intensive livestock husbandry, respectively for potential transfer of genetic factors along the food chain (EFSA, 2016; 2020).

Unlike the mutation-mediated mechanism, plasmid-determined resistance to fluorinated quinolones in bacteria could be realised by horizontal, conjugation transfer. Usually, plasmids carrying genes of resistance to fluorinated quinolones, possess also genes encoding resistance to other classes of antimicrobial drugs (Robicsek *et al*., 2006). *Qnr* genes may reduce the ability for binding of gyrases and topoisomerase IV to DNA, as well as may influence cleavage enzyme complexes (Xiong *et al*., 2011).

The most spread mechanism of resistance to β-lactam antibiotics in Gram-negative bacteria is associated with expression of β-lactamase enzymes, hydrolysing the β-lactam ring (Ambler, 1980). It is believed that genes encoding the production of extended-spectrum β-lactamases are located mainly in mobile genetic elements (plasmids, integrons, gene cassettes, transposons, insertion sequences), which could be easily mobilised to a variety of bacterial species via horizontal gene transfer (Ewers *et al*., 2012). Plasmids that determine the genetic profile of ESBL types often carry genes encoding resistance to other classes of chemotherapeutics as well – aminoglycosides and fluoroquinolones for instance (Rios *et al*., 2015). In *E. coli* isolates from animals and animal foodstuffs, plasmids carrying genes determining co-resistance to third-generation cephalosporins and fluoroquinolones have been detected (Ghodousi *et al*., 2015; Zurfluh *et al*., 2015). At present, about 17 functional groups of beta-lactamases are associated with four molecular classes (A, B, C, D). Beta-lactamases are grouped according to the variety of substrate profiles, respectively penicillins, cephalosporins, monobactams, carbapenems and their sensitivity to beta-lactamase inhibitors, clavulanic acid, avibactam and EDTA (Bush, 2018). CTX-M-1 is the most prevalent type of the ESBL among *E. coli* isolates from farm animals and from animal foodstuffs (Börjesson *et al*., 2013; Seiffert *et al*., 2013; Zurfluh *et al*., 2014). According to the opinion of different author teams from various regions of the world, domestic fowl are a serious source of *E. coli* producing extended spectrum β-lactamases, ESBL/AmpC (Börjesson *et al*., 2013).

The resistance to tetracyclines is widely spread both among microbial pathogens and commensal bacteria, respectively *tet* genes have been detected among bacteria residing with the urogenital tract, the oral cavity, intestinal tract in men and animals, as well as among bacterial isolates from foods and the environment (Balasubramaniam *et al*., 2014). Efflux proteins encoding resistance to tetracyclines have a similar structure to efflux proteins expressing antimicrobial drug multi-resistance, respectively resistance to quinolones, chloramphenicol and quaternary ammonium compounds (McNicholas *et al*., 1995). *Tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, and *tet*I genes are the most widely prevalent among enterobacteria.

For the 6-month period of the study, from June to December 2020, a total of 220 cloacal swabs – 120 from broiler chickens and 100 from laying hens, were collected in Stuart transport medium. Cloacal swabs were cultivated on selective media of MacConkey agar and incubated for 24-48 hours at 37^0 C. *E. coli isolates* were identified by means of the semi-automated system Crystal (Becton, Dickinson, USA).

For phenotype analysis of resistance of *E. coli* isolates to antimicrobial drugs, the disk diffusion method and the method for determination of MIC were utilized. The chemotherapeutics' concentrations used for the disk diffusion method were as followed: ampicillin (10 μg), ampicillin/clavulanic acid (20/10 μg), ceftazidime (10 μg), cefotaxime (5 μg), gentamicin (10 μg), tetracycline (30 μg) and ciprofloxacin (5 μg), produced by Himedia Biosciences, India. For determination of MIC of chemotherapeutic drugs, Liofilchem® (Italy) MIC Test Strips were used. For analysis of *E. coli* resistance to cefotaxime and ceftazidime, a confirmatory method for MIC determination was also applied with combinations cefotaxime+ clavulanic acid and ceftazidime+clavulanic acid. Interpretation of results obtained by phenotypic methods for determination of resistance to chemotherapeutic drugs was based on the MIC breakpoint published by EUCAST (2021).

For DNA extraction, the commercial kit DNeasy Blood Tissue (Qiagen, Germany) was used, and detection of genes conferring resistance to studied chemotherapeutics, respectively, $bla_{\text{CTX-M-1}}$, *qnrS*, *qnrA* , *qnrB*, and *tetA* was performed by real-time PCR, with TaqMan hydrolysis probes (DNA Assay kits, Qiagen, Germany). The temperature regimen of amplification reaction included an initial activation step, 1× at 95 ^{o}C for 10 min, then followed two steps of 40 cycles each including denaturation and annealing/elongation, 40× denaturation at 95 ^{o}C for 15 sec, annealing/elongation at 60 ^{o}C for 2 min.

The positive DNA control had a C_T value ≤34, whereas the positive control for the amplification reaction – C_T=22±2.

A total of 175 *E. coli* strains – 99 strains from broilers and 76 strains from laying hens, were isolated from the 220 cloacal swabs.

Table 1 presents the results about the prevalence to *E. coli* isolates from broilers and laying hens, resistant to tested chemotherapeutic drugs. A high proportion of resistant *E. coli* strains from broiler chickens were resistant to tetracycline and ciprofloxacin (65.6% and 70.7% respectively). Also, resistance to ciprofloxacin was widely prevalent among isolates from laying hens (75.0%). *E. coli* strains from broilers, resistant to ampicillin and amoxicillin/clavulanic acid were 54.5% and 45.4%, respectively. Among strains from laying hens, ampicillin-resistant ones were 50.0%, whereas 39.5% – to the combination amoxicillin/clavulanic acid. The occurrence of cephalosporin-resistant *E. coli* strains from broiler chickens was low (8.1 %, 7.1%). Among isolates from laying hens, no resistance to third-generation cefotaxime and ceftazidime was found out.

MIC_{90} for ampicillin in *E. coli* isolates from broilers was 32 μg/mL and 8 μg/mL for bacteria from laying hens. For the combination amoxicillin/clavulanic acid, MIC_{90} for broiler strains was 16 μg/mL vs 8 μg/mL for isolates from laying hens. In both broilers' and hens' isolates, MIC_{90} for ciprofloxacin was 0.5 μg/mL. With respect to tetracycline, MIC_{90} values for strains from broilers and laying hens were

Table 1. Resistance distribution among commensal *Escherichia coli* strains isolated from broilers and laying hens (n=175).

Antimicrobial agent	Isolates from broilers (n=99)		Isolates from laying hens (n=76)	
	Number (%)	Confidence limits (CL)	Number (%)	Confidence limits (CL)
Ampicillin	54 (54.5%)	40.7÷60.3	38 (50.0%)	39.4÷61.6
Amoxicillin/clavulanic acid	45 (45.4%)	36.7 ÷55.2	30 (39.5%)	28.9÷50.6
Cefotaxime	8 (8.1%)	3.5 ÷14.1	–	–
Ceftazidime	7 (7.1%)	2.8 ÷12.8	–	–
Gentamicin	15 (15.1 %)	8.6÷22.6	–	–
Tetracycline	65 (65.6%)	54.9÷73.6	25 (33%)	23.0÷43.9
Ciprofloxacin	70 (70.7%)	61.4÷79.2	57 (75%)	64.7÷84.9

Table 2. Resistance phenotypes and genes determining resistance to antimicrobial agents in conmensal *Escherichia coli* strains from poultry

Resistance phenotypes	Genes determining resistance to beta-lactams, tetracycline and quinolones, number (%)				
	$bla_{\text{CTX-M-1}}$	*tetA*	*qnrS*	*qnrA*	*qnrB1*
Broilers (n=99)					
Amp, AMC, T, CIP (n=37)	–	37 (37.4%)	37 (37.4%)	–	–
Amp, AMC, CTX, CAZ, T, CIP (n=8)	5 (5.0%)	8 (8.1%)	8 (8.1%)	–	–
Amp, G, T, CIP (n=9)	–	9 (9.1%)	9 (9.1%)	–	–
Laying hens (n=76)					
CIP (n=25)	–	–	25 (33.0%)	–	–
T (n=12)	–	12 (15.8%)	–	–	–
Amp, AMC, CIP (n=25)	–	–	25 (33.0%)	–	–
Amp, T (n=13)	–	13 (17.1%)	–	–	–
Total (n=175)	5 (2.8%)	79 (45.1%)	104 (59.7%)	–	–

Amp: ampicillin, AMC: amoxicillin/clavulanic acid, T: tetracycline, CIP: ciprofloxacin; CTX: cefotaxime; CAZ : ceftazidime; G: gentamicin.

16 µg/mL and 8 µg/mL, respectively. MIC_{90} value for cefotaxime was 0.25 µg/mL for broiler isolates and 0.125 µg/mL for strains from laying hens, whereas for ceftazidime, isolates from both origins demonstrated a MIC_{90} value of 0.125 µg/mL.

Table 2 presents data related to detected genes associated with resistance to cefotaxime, ceftazidime, ciprofloxacin and tetracycline in commensal *E. coli* isolates. Among all 175 tested strains, 5 (5.0%) carried the $bla_{\text{CTX-M-1}}$ gene. None of strains from laying hens was positive for the genes encoding resistance to cefotaxime and ceftazidime, respectively gene $bla_{\text{CTX-M-1}}$. What is more, none of strains from broiler chickens and hens was positive for the genes *qnrA* and *qnrB1*. The presence of the *qnrS* gene was tested in

104 poultry *E. coli* isolates (59.7%). Seventy-nine of tetracycline-resistant *E. coli* isolates (45.1 %) carried the *tetA* gene.

Thirty-seven (37.4%) of resistant *E. coli* isolates from broilers were characterised with multi-resistance profile including ampicillin, amoxicillin/clavulanic acid, tetracycline and ciprofloxacin, associated with the wide prevalence of the *qnrS* and *tetA* genes. In 25 (33.0%) of resistant isolates from laying hens, multi-resistance profile comprised ampicillin, amoxicillin/clavulanic acid and ciprofloxacin, associated to the higher incidence of the *qnrS* and *tetA* genes.

Fig. 1 presents the amplification plots of $bla_{\text{CTX-M-1}}$ gene determined in *E. coli* strains isolated from broilers.

A number of research reports carried out during the last decade in European countries present and analyse data on the prevalence of plasmid-determined resistance to quinolones among enterobacterial isolates from livestock (Cerquetti *et al*., 2009; Hordijik *et al*., 2011). Thus for instance, the results from an international survey on the occurrence of plasmid-determined resistance to quinolones among *Salmonella* and *Escherichia coli* isolated from animals, food and humans commented the spread of the *qnrS1* gene among 15% of *E. coli* isolates from domestic livestock and food (Veldman *et al*., 2011). In the same report, data from the Netherlands indicated simultaneous presence of the *qnrS1* and $bla_{\text{CTXM-1}}$ genes in *E. coli* isolates from broiler chickens. The authors discussed also the fact that despite the long-term use of fluoroquinolones in livestock husbandry in Europe since the 1980s, the spread of genes conferring plasmid-determined resistance to quinolones among enterobacteria was not broad. They provided arguments in support of the hypothesis that the spread of these genetic determinants among the commensal intestinal microflora could be rather a result from co-selection than related to the use of other chemotherapeutics, as well as to their location in plasmids.

In the Czech Republic, Literak *et al*. (2013) outlined that broiler chickens could be regarded as a source facilitating the spread of resistance to quinolones, as well as the role of community-wide extraintestinal *E. coli* bacteria. Again in the Czech Republic, Röderova *et al*. (2017) reported detection of resistance genes *qnrS1* and *qnrB*19 among 73% of fluoro-

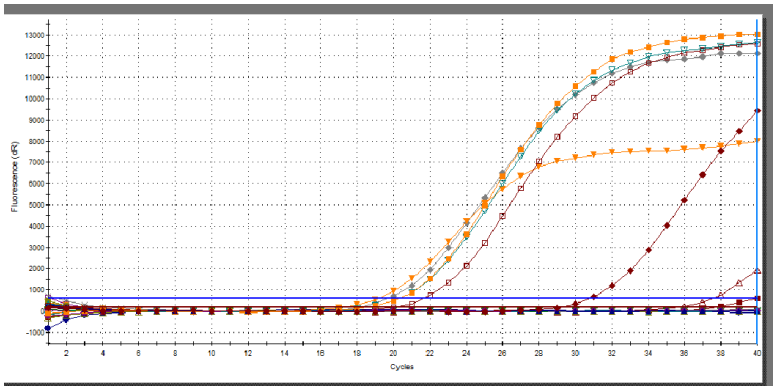

Fig. 1. Amplification plots of gene $bla_{\text{CTX-M-1}}$ determined in *Escherichia coli* strains isolated from broilers.

quinolone-resistant bacterial strains from domestic livestock and wastewater. Specifically for chicken isolates, they found out plasmid-determined resistance to fluoroquinolones in 10.9% of selected *E. coli* strains. The authors determined also a multi-resistance profile including resistance to beta-lactams as well in 95% of *Escherichia coli* isolates.

According to a number of European research teams, a wider prevalence of *E. coli* isolates from broiler chickens that produced extended spectrum beta-lactamases from the CTX-M-1 type, was noted (Randall *et al*., 2011; Dierikx *et al*., 2013; Saliu Eva-Maria *et al*., 2017).

The reports of EFSA and ECDPC from 2016 also affirm that co-resistance to fluorinated quinolones, third-generation cephalosporins and aminopenicillins among commensal *E. coli* isolates from broiler chickens was rather low (0.6%) at the background of resistance to ciprofloxacin in 57.7%, resistance to ampicillin in 64.2% and to cefotaxime and ceftazidime – in 4.2% of strains. The EFSA report from 2020 listed a resistance to ciprofloxacin and cefotaxime in commensal *E. coli* from broilers of 0.8% determined on the base of clinical threshold values and 2.1% on the basis of EUCAST ECOFF values. The multi-resistance profiles of 43.4% of broiler *E. coli* isolates included resistance to tetracycline, sulfamethoxazole, trimethoprim and ampicillin. The observed co-resistance to ciprofloxacin and third-generation cephalosporins was 2.1%, with 31.9% prevalence rate of ESBL-producing commensal broiler chicken *E. coli*. A high prevalence (78.9%) was reported for ciprofloxacin-resistant *E. coli* strains.

The present study has analysed the broader prevalence of resistance to tetracycline and ciprofloxacin, associated with plasmid-determined quinolone resistance and genes *qnrS* and *tetA* (59.7% and 45.1%) among commensal *E. coli* isolates from poultry. Five percent of commensal *E. coli* strains, multi-resistant to beta-lactams, ciprofloxacin and tetracycline also possessed the genes *qnrS* and $bla_{\text{CTXM-1}}$ and *tetA*. In 65 strains (65.6%) from broilers and 50 strains (65.8%) from laying hens with phenotype of ciprofloxacin resistance, the *qnrS* gene was detected. The gene *tetA* was demonstrated in 65 broiler isolates (65.6%) and 25 isolates from laying hens (33%).

In Turkey Aşlantas (2018) also established a high percent of distribution of *tet*A gene (43.4%), retrospectively a low spread of $bla_{\text{CTX-M-1}}$ gene in commensal *E. coli* isolates from broilers. Again in Turkey, Sigirci *et al*. (2019) analysed the prevalence of tetracycline resistance among various members of *Enterobacteriaceae* spp., including *E.coli*, isolated from the faeces of synanthropic birds. The authors found that 65. 2% of isolates were resistant to tetracycline and the *tetA* gene was found in 46.6% of them, respectively. In India Balasubramaniam *et al.* (2014) found a lower (29%) spread of gene *tetA*. In Nigeria, Adelowo *et al*. (2014) observed similar levels (21%) *of tetA* distribution between commensal *E. coli* isolates from birds.

The 9th report of ESVAC (2019) presented data for sales of antimicrobial drugs in livestock husbandry for 2011-2017 in EU member states, Iceland, Norway and Switzerland. Information for Bulgaria shows a total amount of 132.3 mg/population correction units (PUC) with greatest use of tetracycline – 45.6 mg. From comparative point of view, data on groups of chemotherapeutics determined as being of critical importance for medicine, are also interesting. For example, values for third- and fourth-

generation cephalosporins are low – 0.1 mg, and comparable to data from other EU members except for Denmark, Finland, Iceland, The Netherlands and Norway, whose values were lower than 0.01 mg. With regard to fluoroquinolones, national use of 5.7 mg is similar to that of Romania and Spain (4.3 mg, 4.9 mg). Unfortunately, the use of fluoroquinolones in Bulgarian livestock husbandry is far more frequent compared to countries like Sweden, Switzerland and the United Kingdom (0.02 mg, 0.3 mg, 0.2 mg). On the other hand, the EFSA Report (2020) outlined preliminary data referring to the phenotype profile of ESBL-producing *E. coli* isolates – 40.3% of broiler chicken isolates and 32.8% of poultry meat isolates.

In Bulgaria, data regarding the genotype profile of poultry *E. coli* strains producing ESBL are limited. Therefore, the results from the present study could be useful in the context of the official monitoring, which evaluates the commensal *E. coli* isolates from domestic livestock only at the phenotype level.

REFERENCES

Adelowo, O., E. O. Fagade & Y. Agersø, 2014. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, Southwest Nigeria. *Journal of Infection in Developing Countries*, **8**, 1103–1112.

Ambler, R. P., 1980. The structure of β-lactamases. *Philosophical Transactions of the Royal Society Lond B Biological Sciences*, **289**, 321–331.

Asai, T., M. Hiki, M. Ozawa, R. Koike, K. Eguchi, M. Kawanishi, A. Kojima, Y. S. Endoh, S. Hamamoto, M. Sakai & T. Sekiya, 2014. Control of the development and prevalence of antimicrobial resistance in bacteria of food and animal origin in Japan: A new approach for risk management of antimicrobial veterinary medicinal products in Japan. *Food-borne Pathogens and Disease*, **11**, 171–176.

Aslantaş, O., 2018. Antimicrobial resistance among commensal *Escherichia coli* from broilers in Turkey. *Israel Journal of Veterinary Medicine*, **73**, 19–25.

Balasubramaniam, A., M. A. Eswaran, P. Suresh & K. Sukumar, 2014. Detection of tetracycline resistance determinant *tet*A gene and antimicrobial resistance pattern in *Escherichia coli* isolates recovered from healthy layer chickens. *Veterinary World*, **7**, 635–638.

Börjesson, S., M. Egervärn, M. Lindblad & S. Englunda, 2013. Frequent occurrence of extended-spectrum beta-lactamase and transferable AmpC beta-lactamase-producing *Escherichia coli* on domestic chicken meat in Sweden. *Applied and Environmental Microbiology*, **79**, 2463–2466.

Bush, K., 2018. Past and present perspectives on β-lactamases. *Antimicrobial Agents and Chemotherapy*, **62**, e01076-18.

Cerquetti, M., A. Garcia-Fernandez, M. Giufre, D. Fortini, M. Accogli, C. Graziani, I. Luzzi, A. Caprioli & A. Carattoli, 2009. First report of plasmid-mediated quinolone resistance determinant *qnrS1* in *Escherichia coli* strain of animal origin in Italy. *Antimicrobial Agents and Chemotherapy*, **53**, 3112–3114.

Dierikx, C. M., J. A. Van Der Goot, H. E. Smith, A. Kant & D. J. Mevius, 2013. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: A descriptive study. *PLoS ONE*, **8**, e79005.

Ewers, C., A. Bethe, T. Semmler, S. Guenther & L. H. Wieler, 2012. Extended-spectrum β-lactamase and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: A global perspective. *Clinical Microbiology and Infection*, **18**, 646–655.

EFSA, 2016. European Food Safety Authority and European Centre for Disease Preven-

tion and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. *EFSA Journal*, **14**, 4380.

EFSA, 2020. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. *EFSA Journal*, **18**, 6007.

ESVAC, 2019. Ninth Report. Sales of veterinary antimicrobial agents in 31 European countries in 2017 Trends from 2010 to 2017. https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2017_en.pdf (23 March 2022 date last accessed).

EUCAST, 2021. European Committee on Antimicrobial Susceptibility Testing, Clinical breakpoints – breakpoints and guidance, https://www.eucast.org/clinical_breakpoints (23 March 2022 date last accessed).

FAO/WHO/OIE, 2008. Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Report of a meeting held in FAO, Rome, Italy, November 2007, Geneva, Switzerland, https://www.fao.org/3/i0204e/i0204e.pdf (23 March 2022 date last accessed).

Ghodousi, A., C. Bonura, A. M. Di Noto & C. Mammina, 2015. Extended-spectrum β-lactamase, AmpC-producing, and fluoroquinolone-resistant *Escherichia coli* in retail broiler chicken meat, Italy. *Foodborne Pathogens and Disease*, **12**, 619–625.

Hordijik, J. J., A. B. Bosman, A. van Essen-Zandbergen, J. A. Wagenaar & D. Mevius, 2011. *qnrB19* gene bracketed by IS26 on a 40-kilobase IncR plasmid from an *Escherichia coli* isolate from a veal calf. *Antimicrobial Agents and Chemotherapy*, **55**, 453–455.

Kaesbohrer, A., A. Schroeter, B. A. Tenhagen, A. Alt, B. Guerra & B. Appel, 2012. Emerging antimicrobial resistance in commensal *Escherichia coli* with public health relevance. *Zoonoses and Public Health*, **59**, 158–165.

Literak, I., T. Reitschimed, D. Bujankova, M. Dolejska, A. Cizek, J. Bardon, L. Pokludova, P. Alexa, D. Halova & I. Jamborova, 2013. Broilers as a source of quinolone resistant and extraintestinal pathogenic *Escherichia coli* in the Czech Republic. *Microbial Drug Resistance*, **18**, 567–573.

McNicholas, P., M. McGlynn, G. G. Guay & D. M. Rothstein, 1995. Genetic analysis suggests functional interactions between the N- and C-terminal domains of the TetA(C) efflux pump encoded by pBR322. *Journal of Bacteriology*, **177**, 5355–5357.

Randall, L. P., C. Clouting, A. Horton, N. G. Coldham, G. Wu, F. A. Clifton-Hadley, R.H. Davies & C. J. Teale, 2011. Prevalence of *Escherichia coli* carrying extended-spectrum β-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006–2009. *Journal of Antimicrobial Chemotherapy*, **66**, 86–95.

Rios, E., M. C. Lopez, I. Rodriguez-Avial & J. J. Picazo, 2015. Characterization of inhibitor-resistant TEM β-lactamases and mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates. *Microbial Drug Resistance*, **21**, 512–515.

Robicsek, A., G. A. Jacoby & D. C. Hooper, 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infectious Diseses*, **6**, 629–640.

Röderova, M., D. Halova, I. Papousek, M. Dolejska, M. Masarikova, V. Hanulik, V. Pudova, P. Broz, M. Htoutou-Sedlakova, P. Sauer, J. Bardon, A. Cizek, M. Kolar & I. Literak, 2017. Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals, and environment in the Czech Republic. *Frontiers in Microbiology*, **7**, 2147.

Saliu, E.-M., W. Wahjen & J. Zentec, 2017. Types and prevalence of extended-spectrum beta-lactamase producing *En-*

terobacteriaceae in poultry. *Animal Health Research Reviews*, **18**, 46–57.

Seiffert, S. N., M. Hilty & A. Perreten, 2013. Extended-spectrum cephalospsorin- resistant Gram-negative organisms in livestock: an emerging problem for human health? *Drug Resistance Updates*, **16**, 22–45.

Sigirci, B. D., B. Celik, B. B. Kahraman, A. F. Bagcigil & S. Ak, 2019. Tetracycline resistance of *Enterobacteriaceae* isolated from feces of synanthropic birds. *Journal of Exotic Pet Medicine*, **28**, 13–18.

Veldman, K., L. M. Cavaco, D. Mevius, A. Battisti, A. Franco, N. Botteldorn, M. Bruneau, A. Perrin-Guyomard, T. Cerny, C. De Frutos Escobar, B. Guerra, A. Schroeter, M. Gutierrez, K. Hopkins, A. Myllyniemi, M. Sunde, D. Wasyl & F. Aarestrup, 2011. Internal collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *Journal of Antimicrobial Chemotherapy*, **66**, 1278–1286.

Xiong, X., E. H. Bromley, P. Oelschlaeger, D. N. Woolfson & J. Spencer, 2011. Structural insights into quinolone antibiotic resistance mediated by pentapeptide repeat proteins: Conserved surface loops direct the activity of a Qnr protein from a Gram-negative bacterium. *Nucleic Acids Research*, **39**, 3917–3927.

Zurfluh, K., J. Wang, J. Klumpp, M. Nüesch-Inderbinen, S. Fanning & R. Stephan, 2014. Vertical transmission on highly similar *bla* $_{\text{CTX-M-1}}$ harboring plasmids in *Escherichia coli* with different MLST types in the poultry production pyramid. *Frontiers in Microbiology*, **5**, 519.

Zurfluh, K., N. Cernela & R. Stephan, 2015. Quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL) - producing *Escherichia coli* isolated from farm animals in Switzerland. *Schweizer Archives Für Tierheilkunde*, **157**, 59–62.

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