Quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolated from farm animals in Switzerland

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The extensive and widespread use of antimicrobials increases the selection of antimicrobial resistant microorganisms. Cephalosporins of the 3rd- and 4th-generation and (fluor)quinolones are broad-spectrum antimicrobial compounds and of critical importance. In Switzerland, 231 kg and 335 kg of the total amount of 56'277 kg antimicrobials sold for farm animals in 2012 were cephalosporins and fluoroquinolones, respectively (ARCH-Vet 2012, 2013). In the last few years, the prevalence of *Enterobacteriaceae* isolates of human and animal origin showing resistance to higher generation cephalosporin and fluoroquinolones has increased (Lautenbach et al., 2001; Sorlozano et al., 2007; Shaheen et al., 2013). In contrast to the β-lactam resistance mechanism in higher generation cephalosporin resistant *Enterobacteriaceae* that are mainly plasmid-encoded enzymes inactivating the β-lactam-ring, the main mechanisms of quinolone resistance is the accumulation of mutations in the chromosomally encoded DNA gyrase and DNA topoisomerase IV genes. Besides, plasmid-mediated quinolone resistance (PMQR) determinants, including e.g. target protection proteins of the *qnr* gene family, the aminoglycoside acetyltransferase gene variant *aac-(6')-Ib-cr* (for *c*iprofloxacin *r*esistance) and the efflux pump gene *qepA*, contribute to a reduced susceptibility phenotype (Strahilevitz et al., 2009).

Since it has been previously stated (Strahilevitz et al., 2009) that there is a link between the occurrence of PMQR genes and those encoding ESBLs, the purpose of this study was to identify and characterize the quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL)- producing *Escherichia coli* isolated from healthy farm animals in Switzerland.

Isolates, antimicrobial susceptibility testing and characterization of the quinolone resistance mechanisms

A collection of 90 extended-spectrum β-lactamase producing *Escherichia coli* isolates from healthy farm animals (9 pig fecal samples, 17 cattle fecal samples, 5 sheep fecal samples and 59 chicken fecal samples from crates of different flocks) in Switzerland (Geser et al., 2012) was screened for reduced susceptibility to quinolones by the disc diffusion assay according the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2011).

For isolates with increased level of resistance to quinolones, minimal inhibitory concentrations (MICs) of nalidixic acid (NA) and ciprofloxacin (CIP) were determined using Etest strips (bioMérieux, Marcy l'Etoile, France) and the criteria according to the CLSI (CLSI, 2011). Moreover, amplification of the quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*, *parC* and *parE* genes and PCR based detection of the plasmid-mediated quinolone resistance (PMQR) markers (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr* and *qepA*) was performed as described elsewhere (Shaheen et al., 2013). Custom-sequencing was performed by Microsynth (Balgach, Switzerland) and the nucleotide and translated protein sequences were analyzed with CLC Main Workbench 6.6.1.

Co-transferability of PMQR markers and bla_{ESBL} was tested by conjugation experiments which were performed with plasmid-free recipient strain *E. coli* HK225 strain (*Strep*^{r}, Rif^r) (Kayser and Morenzoni, 1982). Briefly, single colonies of the donor and recipient were inoculated in LB broth (Difco Laboratories) and grown overnight at 37 °C. Subsequently, equal volumes of the donor and recipient cultures were mixed and incubated overnight at 37 °C without shaking. Serial dilutions were

then plated on LB agar (Difco Laboratories) selection plates supplemented with a combination of 600 µg/ml streptomycin (Sigma-Aldrich, Switzerland), 100 µg/ml rifampicin (Sigma-Aldrich, Switzerland) and 10 µg/ml cefotaxime (Sigma-Aldrich, Switzerland). PCR was performed to identify the resistance genes acquired by the transconjugants.

Results and Discussion

A total of 25 of 90 extended-spectrum β-lactamase-producing *Escherichia coli* strains isolated from fecal samples of farm animals during a previous study (Geser et al., 2012) showed increased levels of nalidixic acid (NA) resistance according to CLSI criteria (CLSI, 2011). Based on the Etest MIC values, twelve isolates were classified as resistant to NA, four were resistant to NA and showed intermediate resistance to CIP and nine isolates were resistant to both tested (fluoro)quinolones (Tab. 1). The findings of this study correlate well with the data of ESBL/AmpC-producing *Enterobacteriaceae* with reduced susceptibility to (fluoro)quinolones published in the ARCH-Vet 2012 report (ARCH-Vet 2012, 2013) with the exception of ciprofloxacin resistant isolates from chicken samples which have not been found in this study.

The target gene mutations of the 25 isolates were not uniformly distributed among the animal species, but corresponded to those detected in quinolone resistant *E. coli* in a study performed in Ireland on farm animal isolates (Karczmarczyk et al., 2011). In our strain collection, isolates from chicken origin (chicken crates) all showed only a single mutation in *gyrA* leading to the amino acid (aa) substitution Ser83→Leu. In contrast, all isolates from cattle harboured two mutations in *gyrA* (aa substitutions: Ser83→Leu, Asp87→Asn) and all of them but one showed an additional mutation in *parC* (aa substitution: Ser80→Ile). Four cattle isolates showed an additional mutation in *parE* (aa substitution: Ser458→Ala). Vogt and colleagues (Vogt et al., 2014) isolated recently ciprofloxacin resistant ESBL-producing

Table 1: Detection of target gene mutations, prevalence of PMQR and MIC values of ciprofloxacin and nalidixic acid for isolates described in this study

		Target gene mutations					MIC values [µg/ml][a]			
Isolate	Origin	GyrA		ParC	ParE	PMQR	NAL	CIP	ESBL type	Additional resistance pattern[b]
Rd 52	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile	Ser458→Ala	*aac-6′-Ib-cr*	>256	>32	CTX-M-15	AM, CF, CTX, GM, TE, SXT
Rd 53	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile	Ser458→Ala	*aac-6′-Ib-cr*	>256	>32	CTX-M-15	AM, CF, CTX
Rd 142	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile	Ser458→Ala	*aac-6′-Ib-cr*	>256	>32	CTX-M-117	AM, CF, CTX, GM, TE, S, C, SXT
Rd 124	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile	Ser458→Ala		>256	32	CTX-M-15	AM, CF, CTX, S
Rd 115	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	24	CTX-M-14	AM, CF, CTX, C, SXT
Rd 116	cattle	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	16	CTX-M-14	AM, CF, CTX, GM, TE, S, C, SXT
Rd 136	cattle	Ser83→Leu	Asp87→Asn				>256	3	CTX-M-1	AM, CF, CTX, TE
Sf 108	sheep	Ser83→Leu	Asp87→Asn	Ser80→Ile	Ser458→Ala	*aac-6′-Ib-cr*	>256	>32	CTX-M-15	AM, CF, CTX, GM, TE, S, SXT
Sf 100	sheep	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	12	CTX-M-14	AM, CF, CTX, GM, TE, S, C
Sf 102	sheep	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	12	CTX-M-14	AM, CF, CTX, GM, TE, S, C
Sf 2	sheep	Ser83→Leu					>256	0.38	CTX-M-1	AM, CF, CTX, TE, S, C
Sw 17	pig	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	3	CTX-M-1	AM, CF, CTX, TE, S
Sw18	pig	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	3	CTX-M-1	AM, CF, CTX, S, C
Sw 16	pig	Ser83→Leu	Asp87→Asn	Ser80→Ile			>256	3	CTX-M-1	AM, CF, CTX, GM, TE, S, SXT
Sw 65	pig	Ser83→Leu					>256	0.19	CTX-M-1	AM, CF, CTX, TE
Sw 14	pig	Ser83→Leu					>256	0.125	CTX-M-1	AM, CF, CTX, TE
Sw 13	pig	Ser83→Leu					>256	0.094	CTX-M-1	AM, CF, CTX, TE, S, SXT
H 77	chicken	Ser83→Leu					>256	0.25	SHV-12	AM, CF, CTX, TE, SXT
H 88	chicken	Ser83→Leu					>256	0.25	CTX-M-1	AM, CF, CTX, SXT
H 17	chicken	Ser83→Leu					>256	0.125	CTX-M-1	AM, CF, CTX, TE
H 35	chicken	Ser83→Leu					256	0.25	CTX-M-1	AM, CF, CTX, TE, SXT
H 34	chicken	Ser83→Leu					256	0.125	SHV-12	AM, CF, TE, SXT
H 23	chicken	Ser83→Leu					256	0.19	TEM-52	AM, CF, CTX
H 2	chicken	Ser83→Leu					256	0.094	SHV-12	AM, CF, CTX, TE, C
H 84	chicken	Ser83→Leu					96	0.19	CTX-M-1	AM, CF, CTX, TE, SXT
HK225[c]	–	–					4	0.023	–	S, Rif

[a] NAL, nalidixic acid; CIP, ciprofloxacin.
[b] AM, ampicillin; C, chloramphenicol; CF, cephalothin; CTX, cefotaxime; GM, gentamicin; TE, tetracycline; S, streptomycin; SXT, trimethoprim-sulfamethoxazole.
[c] *E. coli* recipient strain used for conjugation experiments.

E. coli from Swiss chicken meat harbouring the same aa substitution in GyrA (Ser83→Leu, Asp87→Asn) but a different substitution in ParC (Ser80→Arg). The isolates from sheep and swine showed mainly two substitutions in GyrA (Ser83→Leu and Asp87→Asn) and one in ParC (Ser80→Ile). No mutations were found in *gyrB* in any isolates. A single mutation in the QRDR of *gyrA* is sufficient to generate a high-level quinolone, and a double mutation even a high-level fluoroquinolone resistance phenotype (Vila et al., 1994).

Plasmid-mediated quinolone resistance (PMQR) determinants were so far rarely found in isolates from food producing animals (Kirchner et al., 2011; Liu et al., 2011). The *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qepA* genes were not detected in this study. However, we describe one *E. coli* isolate from a sheep (Sf 108) and three isolates from cattle (Rd52, Rd53 and Rd142.11), which harbor the ciprofloxacin-resistance variant of the aminoglycoside acetyltransferase, AAC(6')-Ib-cr. The contribution of PMQR determinants to the fluoroquinolone resistance phenotype is low, thus the transconjugation experiments were performed by the selection for bla_{ESBL}, since it has been shown that *acc(6')-Ib-cr* is often located on the same plasmid as the ESBL genes (Strahilevitz et al., 2009). Transconjugation was possible for two (Rd52 and Sf108) of the four isolates by selection for the co-transferred $bla_{\text{CTX-M-15}}$ gene. Although the contribution of AAC(6')-Ib-cr to the resistance level to certain fluoroquinolones (e.g. ciprofloxacin and norfloxacin) is low, it is proposed that it may promote the accumulation of mutations in DNA gyrase and DNA topoisomerase IV genes (Poirel et al., 2012a). So far, the modified acetyltransferase was frequently detected in *E. coli* isolates harboring $bla_{\text{CTX-M-15}}$ from companion animal (Gibson et al., 2010; Shaheen et al., 2013), and in human *E. coli* isolates (Poirel et al., 2012b; Strahilevitz et al., 2009).

In summary, the presence of *E. coli* co-expressing (fluoro)quinolone resistance along with extended-spectrum β-lactamases in healthy livestock is worrisome, as they might spread into the farm environment and along the food chain. Although these strains belong the common gut microflora and are usually unproblematic for their carrier, their occurrence is an important veterinary public health issue. Isolates characterized during this study exhibit resistance mechanisms to the two most frequent used antimicrobial classes in human medicine. Besides harbouring PMQR determinants and bla_{ESBL} genes, these bacterial isolates exhibit resistances to other antimicrobial classes such as for example sulfonamides and tetracyclines. These multi-drug resistant bacteria are an important reservoir for antimicrobial resistance genes, from where the resistance determinants might be mobilized by plasmids, transposon or other mobile genetic elements and integrated into pathogenic (zoonotic) species.

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References

ARCH-Vet 2012: Bericht über den Vertrieb von Antibiotika in der Veterinärmedizin und das Antibiotikaresistenzmonitoring bei Nutztieren in der Schweiz. Swissmedic/BVET, 2013.

Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne PA. 2011

Geser N., Stephan R., Hächler H.: Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet. Res. 2012, 8: 21.

Gibson J. S., Cobbold R. N., Heisig P., Sidjabat H. E., Kyaw-Tanner M. T., Trott D. J.: Identification of Qnr and AAC(6′)-1b-cr plasmid-mediated fluoroquinolone resistance determinants in multidrug-resistant *Enterobacter* spp. isolated from extraintestinal infections in companion animals. Vet. Microbiol. 2010, 143, 329–36.

Karczmarczyk M., Martins M., Quinn T., Leonard N., Fanning S.: Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. Appl. Environ. Microbiol. 2011, 77, 7113–7120.

Kayser F. H., Morenzoni G., Homberger F.: Activity of cefoperazone against ampicillin-resistant bacteria in agar and broth dilution tests. Antimicrob. Agents Chemother. 1982, 22, 15–22.

Kirchner M., Wearing H., Teale C.: Plasmid-mediated quinolone resistance gene detected in *Escherichia coli* from cattle. Vet. Microbiol. 2011, 148, 434–5.

Lautenbach E., Strom B. L., Bilker W. B., Patel J. B., Edelstein P. H., Fishman N. O.: Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae.* Clin. Infect. Dis. 2001, 33, 1288–94.

Liu B.-T., Wang X.-M., Liao X.-P., Sun J., Zhu H.-Q., Chen X.-Y., Liu Y.-H.: Plasmid-mediated quinolone resistance determinants oqxAB and aac(6′)-Ib-cr and extended-spectrum β-lactamase gene blaCTX-M-24 co-located on the same plasmid in one *Escherichia coli* strain from China. J. Antimicrob. Chemother. 2011, 66, 1638–9.

Poirel L., Cattoir V., Nordmann P.: Plasmid-mediated quinolone resistance; interactions between human, animal, and environmental ecologies. Front. Microbiol. 2012a, 3, 1–7.

Poirel L., Cattoir V., Nordmann P.: Plasmid-Mediated Quinolone Resistance; Interactions between Human, Animal, and Environmental Ecologies. Front. Microbiol. 2012b, 3, 24.

Shaheen B. W., Nayak R., Foley S. L., Boothe D. M.: Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates recovered from companion animals in the USA. J. Antimicrob. Chemother. 2013, 68, 1019–1024.

Sorlozano A., Gutierrez J., Jimenez A., de Dios Luna J., Martínez J. L.: Contribution of a new mutation in *parE* to quinolone resistance in extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates. J. Clin. Microbiol. 2007, 45, 2740–2.

Strahilevitz J., Jacoby G. A., Hooper D. C., Robicsek A.: Plasmid-mediated quinolone resistance: a multifaceted threat. Clin. Microbiol. Rev. 2009, 22, 664–689.

Vila J., Ruiz J., Marco F., Barcelo A., Goñi P., Giralt E., Jimenez de Anta T.: Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. Antimicrob. Agents. Chemother. 1994 38: 2477–2479.

Vogt D., Overesch G., Endimiani A., Collaud A., Thomann A., Perreten V.: Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat. Microb. Drug Resist. 2014 Oct;20(5): 485–94.

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