Characterization of third-generation cephalosporin-resistant *Escherichia coli* from slaughter calves and fattening pigs: A pilot study for monitoring antimicrobial resistance by whole genome sequencing in Switzerland

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Charakterisierung von Dritt-Generation Cephalosporin-resistenten *Escherichia coli* bei Schlachtkälbern und Mastschweinen: Eine Pilotstudie zur Überwachung von antimikrobiellen Resistenzen durch Ganzgenomsequenzierung in der Schweiz

Die Ganzgenomsequenzierung (Whole Genome Sequencing, WGS) wurde 2022 als zusätzliche Methode zur phänotypischen Antibiotika-Empfindlichkeitsprüfung mittels Bouillon-Mikrodilution in das Schweizer Programm zur Überwachung von Antibiotikaresistenzen eingeführt, um Dritt-Generation Cephalosporin-resistente (3GC-R) *Escherichia coli* zu charakterisieren. Blinddarmproben von Schweizer Schlachtkälbern und Mastschweinen sowie Rind- und Schweinefleisch aus dem Schweizer Einzelhandel von 2021 wurden nach europäisch harmonisierten Protokollen auf das Vorhandensein von 3GC-R *E. coli* untersucht. Im Jahr 2021 wurden 3GC-R *E. coli* in 23,8 % der Schlachtkälber, 5,9 % der Mastschweine und in 0 % der Fleischproben nachgewiesen. Ein Vergleich der Ergebnisse der phänotypischen Resistenzbestimmung mit den Ergebnissen der WGS ergab eine sehr hohe Übereinstimmung hinsichtlich der phänotypischen Resistenzen und den detektierten zugrundeliegenden molekularen Mechanismen (99 %). Die Resistenz gegen Dritt-Generation Cephalosporine (3GCs) war hauptsächlich mit dem Vorhandensein von $bla_{\text{CTX-M-15}}$ in *E. coli*-Isolaten von Kälbern und $bla_{\text{CTX-M-1}}$ in *E. coli*-Isolaten von Schweinen sowie mit Mutationen im *ampC*-Promotor (g.-42 C>T) in *E. coli*-Isolaten von beiden Tierarten verbunden. Die WGS-Daten wurden ferner für eine phylogenetische Analyse auf der Grundlage von Multi-Locus-Sequenztypen (MLST) und Kerngenom-MLST (cgMLST) verwendet. Es zeigte sich, dass die aus Schweizer Schlacht-

Summary

Whole genome sequencing (WGS) was introduced into Swiss antimicrobial resistance monitoring in 2022 as an additional method to phenotypic antimicrobial susceptibility testing by broth microdilution to characterize presumptive third-generation cephalosporin-resistant (3GC-R) *Escherichia coli*. Caecal samples from Swiss slaughter calves and fattening pigs, as well as beef and pork meat from Swiss retail taken in 2021, were analyzed for the presence of 3GC-R *E. coli* according to European harmonized protocols. In 2021, 3GC-R *E. coli* was detected in 23,8 % of slaughter calves, 5,9 % of fattening pigs, and 0 % of meat. Comparative analysis of the antimicrobial resistance results obtained by phenotypic measurement and those obtained by the detection of corresponding underlying molecular mechanisms by WGS showed very high agreement (99 %). Resistance to third-generation cephalosporins (3GCs) was mainly associated with the presence of $bla_{\text{CTX-M-15}}$ in *E. coli* isolates from calves and $bla_{\text{CTX-M-1}}$ in *E. coli* isolates from pigs and mutations in the *ampC*-promoter (g.-42 C>T) in *E. coli* isolates from both animal species. Moreover, WGS data were used for phylogenetic analysis based on multi locus sequence types (MLST) and core genome MLST(cgMLST) revealing that 3GC-R *E. coli* isolated from Swiss slaughter calves and fattening pigs were genetically diverse. In this study, it was shown that using WGS alone to monitor antimicrobial resistance could detect trends in known molecular antimicrobial resistance mechanisms while also providing other valuable information about the isolates, such as genetic relatedness. However, only by combining phenotypic susceptibility testing and WGS early detection of previously unknown resistance mechanisms will be possible.

Keywords: AmpC, cattle, CTX-M, ESBL, multidrug-resistant, swine

kälbern und Mastschweinen isolierten 3GC-R *E. coli* genetisch unterschiedlich waren. In dieser Studie wurde gezeigt, dass WGS zur Überwachung der Antibiotikaresistenz es ermöglicht, Trends bei bekannten molekularen Antibiotikaresistenzmechanismen zu erkennen und gleichzeitig andere wertvolle Informationen über die Isolate, wie z. B. die genetische Verwandtschaft, zu erhalten. Allerdings ist nur durch die Kombination von phänotypischen Empfindlichkeitstests und WGS eine Früherkennung bisher unbekannter Resistenzmechanismen möglich.

Schlüsselwörter: AmpC, CTX-M, ESBL, Multiresistenz, Rind, Schwein

Introduction

Escherichia coli is a Gram-negative bacterium of the *Enterobacterales* order and a commensal of the intestinal tract of humans and animals, that can cause opportunistic infections. Under pressure of β-lactam antimicrobial usage, selection of third-generation cephalosporin-resistant (3GC-R) *E. coli* and acquisition of the respective resistance mechanisms may occur in both humans and animals.[3] Resistance of *E. coli* to third-generation cephalosporins (3GCs) is mediated by acquired extended-spectrum β-lactamases conferring genes (ESBL) or mutations in the promoter region of the chromosomal *ampC* gene (AmpC β-lactamases).[3] While chromosomal *ampC* mutations are not transferable, a broad spectrum of genes conferring resistance to 3GCs has been found on mobile genetic elements that can be exchanged between bacteria.[8]

In human medicine, 3GC-R *Enterobacterales* pose a serious problem when involved in infections because of the restricted therapeutic options necessitating the use of last resort antimicrobials.[39] The extent of dissemination of 3GC-R bacteria in clinical cases was highlighted in a study by Cassini et al., which examined data from the European Antimicrobial Resistance Surveillance Network (EARS-Net). Cassini et al. found that in 2015, of 670,000 patients in Europe infected with invasive antimicrobial resistant bacteria, 297,000 were affected with 3GC-R *E. coli* which was fatal in 9,100 cases.[5] Moreover, in 2020, approximately 137,000 invasive *E. coli* from humans in 29 European countries were analyzed. The mean percentage of 3GC-R *E. coli* was 14,9 % with rates for each country ranging broadly from 5,8 % to 41,4 %.[37]A prevalence of 10 % was determined for Switzerland.[37]

Food-producing animals colonized with 3GC-R *E. coli* can form a reservoir for resistant bacteria and resistance genes, including those for critically important antimicrobials. These can potentially be transmitted to humans via numerous routes of transmission, including handling and/or consumption of contaminated meat, direct contact between livestock and humans, or animal excreta.[14] In this context, coordinated and comprehensive national, European and global action plans have been developed.[10,38]

In 2014, the European Commission implemented European-wide harmonized monitoring of antimicrobial resistance in zoonotic and commensal bacteria in livestock, beef, pork and poultry, based on technical specifications provided by the European Food Safety Authority (EFSA).[12] This monitoring program includes the analysis of the prevalence and phenotypic antimicrobial resistance pattern of 3GC-R *E. coli* in caecal samples at slaughterhouse and fresh meat at retail (2013/652/EU). Since 2015, the Swiss antimicrobial resistance monitoring program has followed this European-wide harmonized program. Since the beginning of this program, the prevalence of 3GC-R *E. coli* in Swiss slaughter calves remained high at 37,6 % in 2015 and 33,2 % in 2019, whereas the prevalence of 3GC-R *E. coli* in fattening pigs decreased from 26 % in 2015 to 13 % in 2019. The prevalence of 3GC-R *E.coli* in beef and pork was consistently very low (< 0,7 %) (Table 1).

Since 2021, the European Commission has recommended whole genome sequencing (WGS) as an alternative method to phenotypic testing by broth microdilution on a voluntary basis (EU2020/1729). The increasing use of WGS technology has recently provided new insights into the emergence and spread of antimicrobial resistant organisms and the variety of underlying molecular mechanisms. Moreover, WGS permits the determination of genetic relatedness between isolates using, e.g. multi locus sequence typing (MLST) and core genome MLST (cgMLST), which provide useful information for epidemiological investigations. Therefore, the role of food-producing animals as reservoirs and vectors for antimicrobial resistant bacteria to humans can be better understood.[21]

In 2022, WGS was introduced as an additional method to phenotypic testing in the Swiss antimicrobial resistance monitoring program at the Center for Zoonoses, Bacterial Animal Diseases and Antibiotic Resistance (ZOBA), Insti-

tute for Veterinary Bacteriology (IVB), University of Bern. To evaluate the potential of WGS for antimicrobial resistance monitoring, 3GC-R *E. coli* isolated in 2021 from Swiss slaughter calves and fattening pigs were sequenced using next generation sequencing short read technology. The resulting data were compared to phenotypic antimicrobial susceptibility results and used for molecular characterization of resistance genes and genetic relatedness between the 3GC-R *E. coli* isolates.

Materials and Methods

Sample Collection

Stratified random samples were collected within the framework of the Swiss national antimicrobial resistance monitoring program 2021, resulting in 294 calf and 298 porcine caecal samples. Sampling at slaughterhouses was spread evenly throughout the year at the seven largest cattle (CA-CF and CP) and six largest pig slaughterhouses (PA-PE and CP). Every slaughterhouse collected a number of samples proportional to the number of animals of the species slaughtered per year. The samples of these slaughterhouses together are representative of at least 60 % of the total number of slaughter calves and fattening pigs slaughtered annually in Switzerland. With the exception of five calf samples, which came from two slaughter batches, each of the respective individual samples were taken from different slaughter batches.

In 2021, 307 beef samples (266 samples of Swiss origin and 41 samples of foreign origin) as well as 310 Swiss pork samples (min. 50 g) were taken from fresh, chilled, packed and untreated meat at the retail level by cantonal laboratories. Samples were collected in all 26 Swiss cantons. The sampling scheme considered each canton's population density and market shares of the retailers. Moreover, the proportion of imported and domestically produced meat within each meat category was included in the sampling plan.

Caecal samples and fresh meat were sent immediately after sampling under cooled conditions to ZOBA, IVB, University of Bern, for analysis.

Isolation of 3GC-R *E. coli*

1 g ± 0,1 g of caecal content was added to 9 ml, and 25 ± 0,5 g of fresh meat was added to 225 ml buffered peptone water, respectively (Axonlab AG, Baden, Switzerland). The mixtures were incubated aerobically at 37 ± 1 °C for 18–22 h. A loopful (10 µl) of the suspension was spread onto MacConkey agar containing 1 µg/ml cefotaxime (Tritium Microbiologie B.V., Eindhoven, The Netherlands) and incubated aerobically at 44 ± 0,5 °C for 18–22 h as recommended by the European Reference Laboratory for antimicrobial resistance (EURL-AR), Denmark.[15] One lactose-positive colony per plate was recultered onto MacConkey agar with 1 µg/ml cefotaxime and an additional colony was spread on Tryptone™ Soy Agar with 5 % Sheep Blood (TSA-SB) (Becton and Dickinson Company, Franklin Lakes, NJ, US) and incubated aerobically at 37 ± 1 °C for 18–22 h. Species identification was performed by Matrix-Assisted Laser-Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics GmbH, Bremen, Germany) using the direct transfer protocol according to the manufacturer's instructions. *E. coli* isolates were cryopreserved at -80 °C in tryptone soy bouillon containing 30 % glycerol.

Table 1: Prevalence of ESBL / AmpC *Escherichia (E.) coli* from caecal samples of slaughter calves and fattening pigs between 2015 and 2019 in the framework of the Swiss antimicrobial resistance monitoring program

Year	Slaughter calves			Fattening pigs		
	Number of caecal samples (n)	Total number of ESBL-/AmpC *E. coli* (n)	ESBL-/AmpC *E. coli* Prevalence (%)(95 % CI)	Number of caecal samples (n)	Total number of ESBL-/AmpC *E. coli* (n)	ESBL-/AmpC *E. coli* Prevalence (%)(95 % CI)
2015	298	112	37,6 (32,1–43,4)	300	77	25,7 (20,8–31,0)
2017	304	101	33,2 (28,0–38,8)	296	52	17,6 (13,4–22,4)
2019	298	98	32,9 (27,6–38,5	306	40	13,1 (9,5–17,4)
Year	**Beef**			**Pork**		
2015	298	1	0,3 (0,01–1,86)	301	3	1,0 (0,21–2,88)
2017	299	2	0,6 (0,08–2,4)	302	1	0,3 (0,01–1,83)
2019	309	1	0,3 (0,01–1,79)	310	2	0,7 0,08–2,31)

AmpC, AmpC-producing β-lactamases; ESBL, extended-spectrum β-lactamases; CI, confidence interval.

Minimum inhibitory concentration

For antimicrobial susceptibility testing, the isolates were grown on TSA-SB aerobically at 35 ± 1 °C for 18 ± 2 h. Minimum inhibitory concentrations (MICs) were determined by broth microdilution in cation adjusted Mueller-Hinton Broth (CAMHB) using Sensititre™ EUVSEC3 and EUVSEC2 plates following the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org). The plates were incubated aerobically at 35 ± 1 °C for 18 ± 2 h. *Pseudomonas aeruginosa* CCUG 17619 (=ATCC27853) and *Acinetobacter baumanii* 2012-70-100-69, recommended by the EURL-AR were used for quality control and showed MICs within the acceptable range.

MICs were interpreted as wild-type and non-wild-type using the following EUCAST epidemiological cut-off (ECOFF) values for *E. coli*: amikacin (> 8 mg/L), ampicillin (> 8 mg/L), azithromycin (> 16 mg/L), cefepime (> 0,25 mg/L), cefotaxime (> 0,25 mg/L), cefotaxime/clavulanic acid (> 0,25 mg/L), cefoxitin (> 8 mg/L), ceftazidime (> 0,5 mg/L), ceftazidime/clavulanic acid (> 0,5 mg/L), chloramphenicol (> 16 mg/L), ciprofloxacin (> 0,06 mg/L), colistin (> 2 mg/L), gentamicin (> 2 mg/L), imipenem (> 0,5 mg/L), meropenem (> 0,06 mg/L), nalidixic acid (> 8 mg/L), temocillin (> 16 mg/L), tetracycline (> 8 mg/L), tigecycline (> 0,5 mg/L) and trimethoprim (> 2 mg/L). Because of the lack of ECOFFs, the following interpretive criteria recommended by EFSA[13] were used for ertapenem (> 0,06 mg/L) and sulfamethoxazole (> 64 mg/L).

Phenotypic classification of the 3GC-R *E. coli* isolates as either presumptive ESBL phenotype or AmpC phenotype was performed based on the EFSA categorization scheme.[11] According to this scheme, the ESBL phenotypes show MIC values against cefotaxime or ceftazidime > 1 mg/L, against meropenem ≤ 0,12 mg/L, and against cefoxitin ≤ 8 mg/L. In addition, ESBL showed a threefold decrease in MIC values against cefotaxime or ceftazidime in combination with clavulanic acid. AmpC phenotypes show MIC values against cefotaxime or ceftazidime > 1 mg/L, against meropenem ≤ 0,12 mg/L and against cefoxitin >8 mg/L. With the AmpC phenotype, the addition of clavulanic acid does not result in a decrease in the MIC against cefotaxime or ceftazidime.

Whole genome sequencing (WGS)

DNA extraction was performed from a lawn of bacterial colonies grown on TSA-SB aerobically at 35 ± 1 °C for 18 ± 2 h according to the Nextera™ DNA Flex Microbial Colony Extraction Demonstrated Protocol (1000000035294 v01, Illumina Inc., San Diego, CA, US). DNA quality control was performed according to the Illumina DNA Prep Reference Guide (Illumina) and quantification of DNA yield was performed using Invitrogen™ Qubit™ 3,0 Fluorometer (Thermo Fisher Scientific). DNA library preparation was performed by using Illumina® DNA Prep, (M) Tagmentation Kit and IDT® for Illumina® DNA/RNA UD Indexes following the manufacturer's instructions (Illumina). All libraries were sequenced on an Illumina NovaSeq 6000 SP flow cell platform (300 cycles) at the Next Generation Sequencing Platform, Institute of Genetics, Vetsuisse Faculty, University of Bern. The Illumina raw reads were deposited into the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA926902 with BioSample accession numbers SAMN32885924-SAMN32886010.

Analysis of WGS data

For quality filtering, trimming and removing adapters from the raw reads Trimmomatic v0.39[1] was used. Assemblies were created using Unicycler v0.4.9b.[36] Resistance mechanisms were identified by ResFinder EFSA (access date 24 May 2022) using default settings (threshold of 90 % identity (ID) and 60 % length (minimum percentage length of the resistance gene)) of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) and the AMRFinder tool incorporated into Ridom SeqSphere+ (version 7.7, Ridom GmbH, Münster, Germany). The output of ResFinder analysis concerning presence (including all three categories) and absence was included in the analysis. Sequence typing and cgMLST were performed based on a 2520 allele scheme using Seqsphere+. A core genome based phylogeny was constructed with the Neighbor Joining method[30] calculated pairwise ignoring missing values using SeqSphere+. Phenotypic testing and sequencing were repeated for all isolates for which the phenotypic and genotypic results were incongruent.

Confidence intervals were calculated by Ausvet Epitools Epidemiological Calculators using the Clopper-Pearson method (http://epitools.ausvet.com.au).

Results

Slaughter Calves

From 294 calf caecal samples, 70 3GC-R *E. coli* were isolated, which corresponds to a prevalence of 23,8 % (95 % confidence interval (CI) 19,1–29,1 %). The 3GC-R *E. coli* were isolated from calves slaughtered in six out of the seven Swiss slaughterhouses (CA to CE and CP) (Table 2). The slaughter calves came from 58 different farms in 17 different cantons (Figure 1). For four samples, information on the location of the farm was not available.

In line with the defined 3GCs resistance mechanisms, all 70 3GC-R *E. coli* isolates exhibited MICs above the ECOFF for ampicillin, cefotaxime and/or ceftazidime.

Forty-six of the 70 3GC-R *E. coli* isolates (65,7 % [95 % CI 53,4–76,7 %]) were phenotypically classified as presumpti-

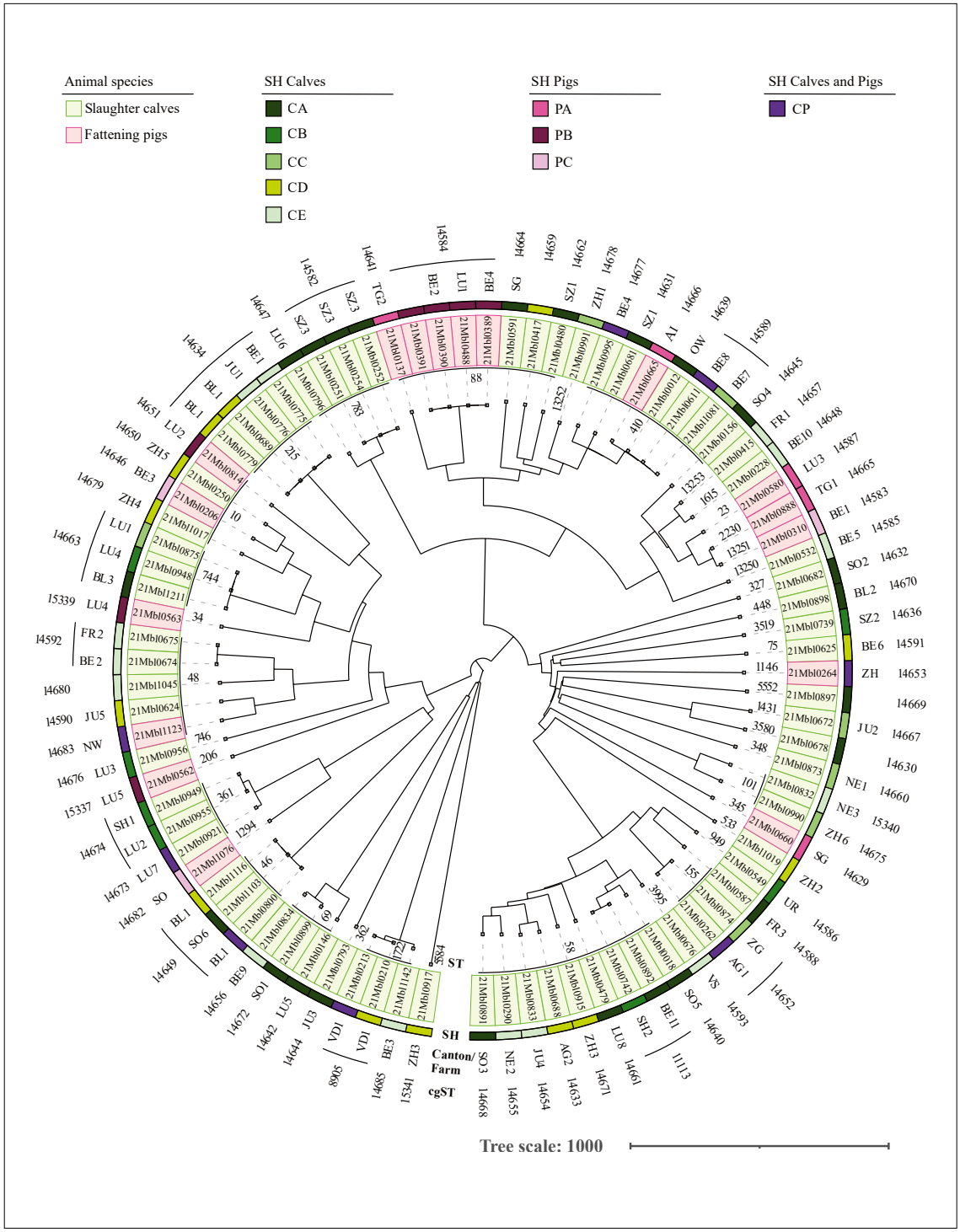

Figure 1: Phylogenetic neighbor-joining tree of 87 sequenced third-generation cephalosporin-resistant *Escherichia coli* isolates from caecal samples of Swiss slaughter calves and fattening pigs based on cgMLST. The phylogenetic tree was created by Ridom SeqSphere+ (version 7,7, Ridom GmbH, Münster, Germany) and visualized by iTol (https://itol.embl.de/). *E. coli* isolates from caecal samples of slaughter calves have a green background; those from caecal samples of fattening pigs have a pink background. ST: sequence type, isolates belonging to the same ST are marked with a dash. SH: Slaughterhouse. SH with C are slaughterhouses where calves were slaughtered, numbered from A-E. SH with P are slaughterhouses where pigs were slaughtered, numbered from A-D. SH with CP is a slaughterhouse where calves and pigs are slaughtered. Canton: AG, Aargau; AI, Appenzell Innerrhoden; BE, Bern; BL, Basel-Landschaft, FR, Fribourg; JU, Jura; LU, Luzern; NE, Neuchâtel; NW, Nidwalden; OW, Obwalden; SG, Sankt Gallen; SH, Schaffhausen; SO, Solothurn; SZ Schwyz, TG, Thurgau; UR, Uri; VD, Vaud; VS, Valais, ZG, Zug; ZH, Zürich. Numbers (1–11) behind the cantons represent the different farms from that canton. cgST: core genome sequence typing, isolates belonging to same cgST are marked with a dash. Empty spaces: no information for this isolate was available.

Table 2: Genetic characteristics and antimicrobial resistance mechanisms of third-generation cephalosporin-resistant *Escherichia coli* isolates from caecal samples of Swiss slaughter calves.

Antimicrobials			FOX	FOT and/or TAZ										FEP							AMI	GEN		CHL							TMP							AZI	CIP								NAL		SMX			TET								TGC
ECOFF (mg/L)			> 8	> 0.25 / > 0.5										> 0.25							> 8	> 2		> 16							> 2							> 16	> 0.06								> 8		> 64			> 8								> 0.5
Total number of detected resistance mechanisms (n)			21	2	1	1	6	2	1	7	28	1	21	2	1	1	6	2	1	7	28	1	1	1	4	6	12	4	11	1	1	3	16	1	6	1	5	4	7	1	5	1	1	7	3	4	2	9	1	1	1	2	1	1	1	1	19	9	1	1	1	2	1	5	17	24	8	2	25	7	7	3	11	3	1	3
Isolates	Origin	ST	*ampC*-promoter (g.-42 C>T)	*ampC*-promoter (g.-32 T>A)	*bla* DHA-1	*bla* ACI-1	*bla* CTX-M-1	*bla* CTX-M-3	*bla* CTX-M-9	*bla* CTX-M-14	*bla* CTX-M-15	*bla* CTX-M-27	*ampC*-promoter (g.-42 C>T)	*ampC*-promoter (g.-32 T>A)	*bla* DHA-1	*bla* ACI-1	*bla* CTX-M-1	*bla* CTX-M-3	*bla* CTX-M-9	*bla* CTX-M-14	*bla* CTX-M-15	*bla* CTX-M-15, *bla* OXA-1	*bla* CTX-M-27	*aac(6')-Ib-cr*	*ant(2")-Ia*	*aac(3)-IIa*	*aac(3)-IId*	*aac(3)-IV*	*catA1*	*catA1 / cmlA1*	*catB3 / floR / cml*	*catA1 / floR*	*floR*	*cmlA1*	*cmlA1 / floR*	*catA1 / cmlA1 / floR*	*dfrA1*	*dfrA5*	*dfrA12*	*dfrA12, dfrA14*	*dfrA14*	*dfrA14, dfrA1*	*dfrA16*	*dfrA17*	*dfrA36*	*mph* (A)	*mef*(C)/*mph* (G)	GyrA S83L D87N / ParC S80I	GyrA S83L D87N/ParC S80I*aac(6')-Ib-cr3*	GyrA S83L, D87N / ParC S80I / qnrS1	GyrA D87Y	GyrA S83L / ParE L416F	ParC S80I / *qnrS1*	*qnrA1*	*qnrB4*	*qnrS1*	GyrA S83L D87N / ParC S80I	GyrA S83L D87N/ParC S80I*aac(6')-Ib-cr3*	GyrA S83L, D87N / ParC S80I / qnrS1	GyrA D87Y	GyrA S83L / ParE L416F	*qnrS1*	*sul1*	*sul1,sul2*	*sul2*	*sul2, sul3*	*sul3*	*tet* (A)	*tet* (A), *tet* (B)	*tet* (A), *tet* (M)	*tet* (A), *tet* (Y)	*tet* (B)	*tet* (B), *tet* (X6)	*tet* (A), *tet* (B), *tet* (M)	*tet* (X6)
21Mbl0250	CD	10																																																																
21Mbl1017	CD	10																						b																																							
21Mbl0800	CP	46																																																											
21Mbl1103	CA	46																																																										
21Mbl1116	CD	46																																																										
21Mbl0624	CD	48																																																										
21Mbl0674	CE	48								a																																																		
21Mbl0675	CE	48																																																										
21Mbl1045	CE	48																																																										
21Mbl0018	CA	58																																																										
21Mbl0290	CE	58																																																										
21Mbl0479	CA	58																																																										
21Mbl0688	CD	58																																																										
21Mbl0742	CB	58																																																										
21Mbl0833	CE	58																																																										
21Mbl0891	CA	58																																																										
21Mbl0892	CA	58																																																										
21Mbl0915	CD	58																																																										
21Mbl0146	CA	69																																																										
21Mbl0834	CE	69																																																										
21Mbl0899	CA	69																																																									
21Mbl0625	CD	75																																																										
21Mbl0417	CD	88																																																										
21Mbl0480	CA	88																																																										
21Mbl0591	CA	88																																																										
21Mbl0832	CE	101																																																										
21Mbl0990	CC	101																																																										
21Mbl0262	CP	155																																																										
21Mbl0587	CA	155																																																										
21Mbl0874	CC	155																																																										
21Mbl0689	CD	215																																																										
21Mbl0775	CE	215																																																										
21Mbl0776	CE	215																																																										
21Mbl0779	CD	215																																																										
21Mbl0682	CA	327								a														c																																			
21Mbl0873	CC	348																																																										
21Mbl0921	CP	361																																																									
21Mbl0949	CB	361																								e																																	
21Mbl0955	CB	361																																																									
21Mbl0793	CA	362																																																									
21Mbl0012	CA	410																																																									
21Mbl0156	CA	410																																																									
21Mbl0611	CP	410																																																									
21Mbl0681	CA	410																																																									
21Mbl0995	CP	410																																																									
21Mbl1081	CC	410																																																									
21Mbl0898	CA	448																																																									
21Mbl1019	CD	533																																																									
21Mbl0875	CC	744																																																									
21Mbl0948	CB	744																																																									
21Mbl1211	CA	744																																																									
21Mbl0956	CB	746																																																									
21Mbl0251	CA	783																																																									a
21Mbl0252	CA	783																																																									a
21Mbl0254	CA	783																																																									i
21Mbl0796	CA	783																																																									
21Mbl0549	CB	949				a																																																					
21Mbl0672	CC	1431																																																									
21Mbl0228	CE	1615																																																									
21Mbl0210	CD	1722																																																									
21Mbl0213	CP	1722																																																									
21Mbl1142	CE	1722																																																									
21Mbl0739	CB	3519																																																									
21Mbl0678	CA	3580																																																									
21Mbl0676	CE	3995																																																									
21Mbl0897	CA	5552																																																									
21Mbl0917	CD	5584																																																									
21Mbl0532	CE	13250																																																									
21Mbl0991	CC	13252																																																									
21Mbl0415	CE	13253																																																									

ECOFF, Epidemiological cut-off according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST); Antimicrobials: AMI, amikacin; AZI, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; FOT, cefotaxime; FOX, cefoxitin; GEN, gentamicin; NAL, nalidixic acid; SMX, sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim. Origin: C, cattle slaughterhouse A-E; CP, cattle and pig slaughterhouse. ST, sequence type. Green: agreement between the detected minimum inhibitory concentration and the genetically analyzed resistance mechanism found by whole genome sequencing. Red: discrepancy between the detected minimum inhibitory concentration and the genetically analyzed resistance mechanism found by whole genome sequencing. Minimum inhibitory concentration: a = 0.5 mg/L; b = 1 mg/L; c = 2 mg/L; d = ≤ 1 mg/L, e = ≤ 0.05 mg/L, f = 1 mg/L, g = 8 mg/L; h = 32 mg/L; i = ≤ 0.25 mg/L.

ve ESBL- producing *E. coli* and exhibited MICs above the ECOFF for cefepime. Twenty-four of the 70 3GC-R *E. coli* isolates (34,3 % [95 % CI 23,4–46,6 %]) were phenotypically classified as presumptive AmpC- producing *E. coli* and exhibited MICs below the ECOFF for cefepime (Table 2). The most frequent ESBL genes detected were $bla_{\text{CTX-M-15}}$ (n = 28), $bla_{\text{CTX-M-14}}$ (n =7), and $bla_{\text{CTX-M-1}}$ (n = 6). The mutation in the *ampC* promoter gene g.-42 C>T was found to be the most frequent underlying resistance mechanism in 21 out of 24 *E. coli* isolates phenotypically classified as presumptive AmpC producers (Table 2).

The 70 3GC-R *E.coli* isolates also exhibited MICs above the ECOFFs for non-β-lactam antimicrobials such as tetracycline (n = 57; 81,4 % [95 % CI 70,3–89,7 %]), sulfamethoxazole (n = 55; 78,6 % [95 % CI 67,1–87,5 %]), chloramphenicol (n = 40; 57,1 % [95 % CI 44,8–68,9 %]), ciprofloxacin (n = 36; 51,4 % [95 % CI 39,2–63,6 %]), trimethoprim (n = 33; 47,1 % [95 % CI 35,1–59,5 %]), gentamicin (n = 23; 32,9 % [95 % CI 22,1–45,1 %]), nalidixic acid (n = 14; 20 % [95 % CI 11,4–31,3 %]), azithromycin (n = 5; 7,1 % [95 % CI 2,4–15,9 %]) and tigecycline (n =1, 1,43 % [95 % CI 0,04–7,7 %]).

All 70 3GC-R *E. coli* isolates showed wild-type MICs to amikacin, colistin, ertapenem, imipenem, meropenem and temocillin.

Among the non-β-lactam antimicrobials, the following resistance conferring genes were most commonly found: *tet*(A) (tetracycline, n = 43), *sul2* (sulfamethoxazole, n = 49), *floR* (chloramphenicol, n = 27), *qnrS1* (ciprofloxacin, n = 21), *dfrA12* (trimethoprim, n = 8), *aac(3)-IId* (gentamicin, n = 12), GyrA S83 L, D87N/ParC S80I (ciprofloxacin and nalidixic acid, n = 11), *mph*(A) (azithromycin, n = 4) and *tet*(X6) (tigecycline, n =3) (Table 2).

In nine isolates, an antimicrobial resistance gene was detected, but they exhibited MICs below the ECOFF for the corresponding antimicrobial (Table 2, highlighted in red). Additionally, a *mcr-9*gene was detected in one isolate (21Mbl0682) that exhibited a wild-type phenotype to colistin.

The 70 3GC-R *E. coli* belonged to 32 different STs and 54 different cgSTs (Table 2, Figure 1). Although several STs occurred several times, no predominant STs were observed. Three 3GC-R *E. coli* isolates were identified as new STs (ST13250, ST13252 and ST13253).

The ST was, however, neither correlated to the resistance mechanisms nor the sampling location (Table 2).

CgMLST analysis confirmed that the 3GC-R *E. coli* isolates were genetically diverse (Figure 1). Three isolates (21Mbl0251, 21Mb0252, 21Mb0254) and two isolates (21Mbl0689, 21Mbl0779) belonging to cgST 14582 and 14634, respectively, were isolated from samples derived from the same slaughterhouses. In the phylogenetic tree based on cgMLST, there was no clear separation between isolates from slaughter calves and fattening pigs. However, 3GC-R *E. coli* isolates from slaughter calves and fattening pigs were found to belong to the same ST in four cases (ST10, ST48, ST88 and ST410).

Fattening Pigs

From 289 porcine caecal samples, 17 3GC-R *E. coli* were isolated, which corresponds to a prevalence of 5,9 % (95 % CI 3,5–9,3 %). The 3GC-R *E. coli* were isolated from pigs slaughtered in four out of the six sampled Swiss slaughterhouses (PA to PC and CP) (Table 3). The fattening pigs came from 16 different farms in eight different cantons (Figure 1). For one sample, information on the location of the farm was not available.

In line with the defined 3GCs resistance mechanisms, all 17 3GC-R *E. coli* isolates exhibited MICs above the ECOFF for ampicillin, cefotaxime and ceftazidime.

Twelve of the 17 3GC-R *E. coli* isolates (70,6 % [95 % CI 44,0–89,7 %]) were phenotypically classified as presumptive ESBL-producing *E. coli*. Ten presumptive ESBL-producing *E. coli* exhibited MICs above the ECOFF for cefepime and two exhibited MICs below the ECOFF for cefepime (21Mbl0562 and 21Mbl0563) (Table 3). Five of the 17 3GC-R *E. coli* isolates (29,4 % [95 % CI 10,3–56,0 %]) were phenotypically classified as presumptive AmpC-producing *E. coli* and exhibited MICs below ECOFF for cefepime (Table 3).

The ESBL genes detected were $bla_{\text{CTX-M-1}}$ (n = 7), $bla_{\text{CTX-M-15}}$ (n =2), $bla_{\text{SHV-12}}$ (n = 2) and $bla_{\text{CTX-M-32}}$ (n = 1). The resistance conferring mutation detected in all five *E. coli* isolates phenotypically classified as presumptive AmpC producers was the mutation in the *ampC*-promoter gene g.-42 C>T (Table 3).

The 17 3GC-R *E.coli* isolates also exhibited MICs above the ECOFFs for non-β-lactam antimicrobials such as sulfamethoxazole (n = 12; 70,6 % [95 % CI 44,0–89,7 %]), trimethoprim (n = 10; 58,8 % [95 % CI 32,9–81,6 %]), tetracycline (n = 9; 52,9 % [95 % CI 27,8–77,0 %]), ciprofloxacin (n =4; 23,5 % [95 % CI 9,6–47,2 %]), gentamicin (n = 2; 11,8 % [95 % CI 1,5–36,4 %])and chloramphenicol (n = 1; 5,9 % [95 % CI 0,1–28,7 %]).

All 17 3GC-R *E. coli* isolates showed wild-type MICs to amikacin, azithromycin, colistin, ertapenem, imipenem, meropenem, nalidixic acid, temocillin and tigecycline.

Among the non-β-lactam antimicrobials, the following resistance conferring genes were most commonly found: *sul2*

Characterization of third-generation cephalosporin-resistant *Escherichia coli* from slaughter calves and fattening pigs: A pilot study for monitoring antimicrobial resistance by whole genome sequencing in Switzerland

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Table 3: Genetic characteristics and antimicrobial resistance mechanisms of third-generation cephalosporin-resistant *Escherichia coli* isolates from caecal samples of Swiss fattening pigs.

Antimicrobials			FOX	FOT and/or TAZ					FEP				GEN		CHL		TMP					CIP	SMX			TET		
ECOFF (mg/L)			> 8	> 0.25 / > 0.5					> 0.25				> 2		> 16		> 2					> 0.06	> 64			> 8		
Total number of detected resistance mechanisms (n)			5	7	2	1	2	5	7	2	1	2	1	1	1	1	3	1	2	3	1	4	1	9	2	7	1	1
Isolates	Origin	ST	*ampC* -promoter (g.-42 C > T)	*bla* CTX-M-1	*bla* CTX-M-15	*bla* CTX-M-32	*bla* SHV-12	*ampC* -promoter (g.-42 C > T)	*bla* CTX-M-1	*bla* CTX-M-15	*bla* CTX-M-32	*bla* SHV-12	*aaac(3)-IId*	*aaac(3)-IV*	*catA1*	*catB2*	*dfrA1*	*dfrA12*	*dfrA14*	*dfrA17*	*dfrA21 / dfrA13*	*fosA7*	*sul1*	*sul2*	*sul1 / sul2*	*tet* (A)	*tet* (A) / *tet* (Y)	*tet* (B)
21Mbl0206	PC	10																										
21Mbl0814	PB	10																										
21Mbl0580	PA	23																										
21Mbl0563	PB	34										a																
21Mbl1123	CP	48																										
21Mbl0137	PA	88																										
21Mbl0389	PB	88																										
21Mbl0390	PB	88																										
21Mbl0391	PB	88																										
21Mbl0488	PB	88																										
21Mbl0562	PB	206										a																
21Mbl0660	PA	345														b												
21Mbl0665	PA	410																										
21Mbl0264	CP	1146																										
21Mbl1076	PC	1294																										
21Mbl0888	PA	2230																										
21Mbl0310	PC	13251																										

ECOFF, Epidemiological cut-off according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST); Antimicrobials: CHL, chloramphenicol; CIP, ciprofloxacin; FOT, cefotaxime; FOX, cefoxitin; GEN, gentamicin; SMX, sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TMP, trimethoprim. Origin: P, pig slaughterhouse A-E; CP, cattle and pig slaughterhouse. ST, sequence type. Green: agreement between the detected minimum inhibitory concentration and the genetically analyzed resistance mechanism found by whole genome sequencing. Red: discrepancy between the detected minimum inhibitory concentration and the genetically analyzed resistance mechanism found by whole genome sequencing. Minimum inhibitory concentration: a = 0.25 mg/L; b = ≤ 8 mg/L.

(sulfamethoxazole, n = 11), *dfrA1* and *dfrA17* (trimethoprim, n = 3), respectively, *tet*(A) (tetracycline, n = 8), *qnrS1* (ciprofloxacin, n = 4), *aac(3)-IId* and *aac(3)-IV* (gentamicin, n = 1), respectively, and *catA1* (chloramphenicol, n = 1) (Table 3).

Three isolates showed MICs below the ECOFF for the corresponding antimicrobial, but an associated resistance gene was detected (Table 3, highlighted in red).

The 17 3GC-R *E. coli* belonged to 12 different STs and 14 cgSTs (Table 3, Figure 1). No predominant ST was detected. However, ST88 was found in five isolates (21Mbl0137, 21Mbl0389, 21Mbl0390, 21Mbl0391 and 21Mbl0488) derived from samples originating from different slaughterhouses. One 3GC-R *E. coli* isolate (21Mbl0310) was identified as a new ST (ST13251).

However, for the slaughter calf isolates, the STs were not correlated with resistance mechanisms or sampling locations (Table 3).

Phylogenetic analysis confirmed that the 3GC-R *E. coli* isolates were genetically diverse, except for four isolates (21Mbl0389, 21Mbl0390, 21Mbl0391 and 21Mbl0488) that had the same cgST (14584). These were isolated from animals from different farms but slaughtered at the same slaughterhouse (PB) (Figure 1).

Fresh meat

Third generation cephalosporin resistant *E. coli* was not detected in the 307 (95 % CI 0,00–1,19 %) beef samples or in the 310 (95 % CI 0,00–1,18 %) pork samples.

Discussion

In the framework of Swiss antimicrobial resistance monitoring, WGS was used to identify antimicrobial resistance genes and determine the genetic relatedness of 3GC-R *E. coli* from caecal samples of Swiss slaughter calves and fattening pigs. The isolates were obtained from selective culture, which permitted targeted isolation of 3GC-R *E. coli* and determination of their prevalence in calves and pigs at slaughterhouses and in meat at retail. Resistance to 3GCs was confirmed by MIC determination prior to subsequent WGS. In contrast to previous years, the prevalence of 3GC-R *E. coli* in caecal samples of Swiss slaughter calves decreased from 32,9 % in 2019 to 23,8 % in 2021. A comparable trend is seen in Swiss fattening pigs, where the prevalence of 3GC-R *E. coli* in caecal samples decreased from 13,1 % in 2019 to 5,9 % in 2021. In 2019, the mean prevalence of 3GC-R *E. coli* from caecal samples of slaughter calves and fattening pigs in nine and 28 European countries, respectively, was 46,2 % and 42,7 %, with marked differences between countries.[11] Other European countries also showed a statistically significant decreasing trend in recent years comparable to Switzerland.[11] This trend goes along with an overall strong decrease in antimicrobial consumption in veterinary medicine in European countries since 2016.[9] A possible explanation for the pronounced decline in the prevalence of 3GC-R *E. coli* in Swiss slaughter calves and fattening pigs could be the ban on dispensing third- and fourth-generation cephalosporins as well as fluoroquinolones for stockpiling in 2016 and the specification, that these may only be used for therapy after consultation of an antibiogram confirming therapeutic efficacy.[32] Significantly fewer 3GC-R-positive *E. coli* isolates were found in the pigs (n = 17) than in the calves (n = 70). A reason for the generally higher prevalence of 3GC-R *E. coli* in slaughter calves could be feeding with milk derived from dairy cows treated with antimicrobials.[2,27] Studies have shown that the prevalence of antimicrobial resistant *E. coli* is higher in young calves, but decreases with animal age.[19,35] Feeding waste milk was identified as the most important risk factor for a high prevalence of 3GC-R *E. coli* in calves.[2] Interestingly, Nüesch-Inderbinden et al. showed that the husbandry system had an effect on the prevalence of 3GC-R *E. coli* in calves raised on organic versus conventional dairy farms, as ESBL-producing *E. coli* were only found on conventional farms.[26] For pigs, both husbandry and age at the time of sampling appear to play a role in the prevalence of 3GC-R *E. coli*. Antimicrobial consumption was shown to be higher in fattening pigs belonging to specialized fattening herds than in fattening pigs belonging to combined breeding-fattening herds.[22] The prevalence of 3GC-R *E. coli* was shown to be the highest in suckling pigs and decreases with increasing pig age.[22, 25] Within the Swiss antimicrobial resistance monitoring program, the prevalence of 3GC-R *E. coli* in beef and pork has always been very low (<0,7 %). In 2019, the mean prevalence of 3GC-R *E. coli* in 28 European countries was low with 5,2 % in beef and 6,8 % in pork, and marked differences were observed between the European countries for both beef and pork.[11] Contamination of meat could occur at slaughter posing a potential hazard to humans,[4] but based on the results of this study and previous Swiss monitoring data,[16] it can be assumed that the risk of transmission of 3GC-R *E. coli* via beef or pork to humans is negligible. However, bacteria other than 3GC-R *E. coli* may be present in meat[24] and bacteriological status should be checked within quality control management.

Implementation of WGS analysis confirmed the presence of acquired cephalosporin resistance genes known to be associated with mobile genetic elements. However, it also revealed the presence of mutations in the chromosomal *ampC* promoter of one third of the isolates from calves and pigs. Given the chromosomal nature of this resistance trait, chromosomal AmpC *E. coli* producers do not pose the risk of disseminating 3GC resistance to other bacteria including pathogenic *E. coli* from pigs and cattle. On the other hand, the remaining 3GC-R *E. coli* in slaughter calves and fatten-

ing pigs from Switzerland contained genes of the CTX-M family. The most frequently detected ESBL gene in *E. coli* from slaughter calves in this study was *bla*$_{\text{CTX-M-15}}$. In another recent Swiss study, *bla*$_{\text{CTX-M-15}}$ was also the most frequently detected ESBL-gene in healthy Swiss calves from organic and conventional farms.[26] In Switzerland[29] and as confirmed by different studies in other countries, the *bla*$_{\text{CTX-M-15}}$ gene is widely distributed in human isolates.[7,23,28] In contrast, in calf samples collected between 2007 and 2017, *bla*$_{\text{CTX-M-14}}$ was found mainly in the United Kingdom[7] and *bla*$_{\text{CTX-M-1}}$ in the Netherlands,[6] indicating that acquisition of genes is local and likely depends on the gene pool present in each sampling setting. In Swiss fattening pigs, the ESBL gene *bla*$_{\text{CTX-M-1}}$ was detected in twelve of the 17 3GC-R *E. coli* isolates. Moor et al. investigated 3GC-R *E. coli* in Swiss fattening pigs isolated in 2018 to 2019, with *bla*$_{\text{CTX-M-14}}$ being the most frequently detected ESBL-gene.[25] Moreover, Moor et al. found the *bla*$_{\text{CTX-M-1}}$ gene in only five isolates from fattening pigs out of 121 3GC-R *E. coli* isolates. Interestingly, in pig samples collected between 2007 and 2018, *bla*$_{\text{CTX-M-1}}$ was found mainly in the Netherlands[6], *bla*$_{\text{CTX-M-15}}$ in Portugal[17] and *bla*$_{\text{CTX-M-32}}$ in Cuba.[20] Here again, the diversity of the CTX genes in different countries and within the same country further indicates the broadness of the *bla*$_{\text{CTX-M}}$ reservoir and the ability of *E. coli* to acquire these genes. Further WGS-based monitoring of 3GC-R *E. coli* from different food-producing animals including poultry will determine the trend of resistance mechanisms to 3GC in *E. coli* over time in the different animal settings. In addition to 3GC resistance mechanisms, 3GC-R *E. coli* also contained mechanisms conferring resistance to other antimicrobial classes, which may also be transferred to other bacteria. For instance, fluoroquinolones are on the list of highest priority critically important antibiotics (HPCIA).[37] In this study, 51,4 % of the 3GC-R *E. coli* from Swiss slaughter calves and 23,5 % of the 3GC-R *E. coli* from fattening pigs showed non-wild-type MIC to ciprofloxacin, with *qnrS1* being the most common resistance gene detected. This high prevalence in both animal species may occur due to the use of enrofloxacin, which is recommended and used as a second-line antimicrobial for some diseases.[34] With the prohibition of stockpiled fluoroquinolones, an attempt is being made in Switzerland to counteract these high resistance rates.[31] In 2016, polymyxins were added to the list of HPCIA.[39] Since decades, colistin has been used in pig production for gastrointestinal disorders because it is not absorbed from the intestinal mucosa. However, all 3GC-R *E. coli* isolates from Swiss slaughter calves and fattening pigs in this study and in the past showed wild-type MIC to colistin. Only one 3GC-R *E. coli* isolate from a calf contained the *mcr-9* gene, but this gene does not confer colistin resistance in field strains of *E. coli* and *Salmonella* spp.[33] According to the World Health Organization (WHO)[37], the recent emergence of carbapenem-resistant *E. coli* is of serious concern in human medicine. In this study, as well as in the past, all 3GC-R *E. coli* showed wild-type MICs to meropenem, imipenem and ertapenem, and in this study, no genes conferring carbapenem resistance were detected. Based on the results of this study and the results of the European monitoring program, which includes specific detection of carbapenem-resistant *E. coli* and *Klebsiella* spp., it can be assumed that the risk of transmission of carbapenemase-producing *E. coli* to humans via direct contact with slaughter calves and fattening pigs or via meat is currently negligible, but still needs to be carefully monitored.

WGS analysis also permitted to determine whether acquisition of 3GC resistance mechanisms is limited to specific clonal lineages or expanded to a larger population of genetically diverse *E. coli*. MLST and cgMLST revealed that the 3GC-R *E. coli* isolates from caecal samples of Swiss slaughter calves and fattening pigs were generally genetically diverse, indicating independent acquisition of genes. Several isolates belonging to the same ST could be further distinguished by cgMLST. This analysis revealed that some clones were identified in different samples from the same slaughterhouse and/or the same farm, but also in different samples from different slaughterhouses and different farms. One cause of this spread could be the animal trade. For example, calves and piglets originating from different birthing farms and encountering each other at a fattening farm may exchange such 3GC-R *E. coli* during transport from the different birthing farms to the fattening farm or during their stay at the fattening farm. If the samples are from the same slaughterhouse, it is more likely that contamination occurred during sampling at the slaughterhouses. To determine a possible cause with certainty, the origin of such 3GC-R *E. coli* would have to be traced back to understand the route of spread among slaughter calves and fattening pigs. The use of cgMLST provided an additional discriminatory method, which allowed better distinction between isolates of the same ST. However, in general, the use of MLST already showed that the isolates were genetically diverse with a few exceptions. For European harmonized WGS monitoring, it should be defined which bioinformatics tools should be used so that the generated data, such as resistance mechanisms, ST or cgST are comparable between countries.

Overall, this comparative study showed high agreement (99 %) between the results of phenotypic testing by broth microdilution and confirmation by genotypic analysis with WGS, which makes WGS a good instrument for the monitoring of known antimicrobial resistance mechanisms in 3GC-R *E. coli*. However, besides the isolate with the *mcr-9* gene, 13 3GC-R *E. coli* isolates, which contained resistance mechanisms, showed MIC values below the ECOFFs of the corresponding antimicrobial. Alterations in the promoter sequences upstream of the detected genes could be a reason for these discrepancies.[18] However, this was not investigated in more detail. The high level of agreement also demonstrated that using WGS alone to monitor antimicro-

bial resistance could reveal trends in known molecular antimicrobial resistance mechanisms while providing other valuable information about the isolates, such genetic relatedness. However, the exclusive use of WGS analyses hinders the discovery of new antimicrobial resistance mechanisms, highlighting the advantage of combining phenotypic testing by broth microdilution with genotypic testing by WGS.

Phylogenetic analysis demonstrated that the 3GC-R *E. coli* found in this study were genetically diverse and that there are no predominant clones circulating in the Swiss slaughter calf and fattening pig production systems. The inclusion of WGS into the antimicrobial resistance monitoring program represents an important step for the genetic characterization of antimicrobial resistant bacteria isolated from animals and provides a baseline for future One health molecular epidemiology studies.

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Caractérisation d'*Escherichia coli* résistants aux céphalosporines de troisième génération provenant de veaux de boucherie et de porcs d'engraissement: Une étude pilote pour la surveillance de la résistance aux antibiotiques par séquençage du génome entier en Suisse

Le séquençage du génome entier (Whole Genome Sequencing, WGS) a été introduit dans la surveillance suisse de la résistance aux antibiotiques en 2022 en tant que méthode supplémentaire aux tests phénotypiques de sensibilité aux antibiotiques pour caractériser les *Escherichia coli* résistants aux céphalosporines de troisième génération (3GC-R). Des échantillons de cæcum pris en 2021 à l'abattoir de veaux et de porcs suisses, ainsi que de viande de bœuf et de porc provenant de détaillants suisses ont été analysés pour détecter la présence d'*E. coli* 3GC-R conformément aux protocoles européens harmonisés. En 2021, les *E. coli* 3GC-R ont été détectés dans 23,8 % des veaux d'abattage, 5,9 % des porcs d'engraissement et 0 % dans la viande. Les résultats de résistance aux antibiotiques obtenus par mesure phénotypique et ceux obtenus par la détection des mécanismes moléculaires sous-jacents concordaient à 99 %. La résistance aux céphalosporines de troisième génération était principalement associée à la pré-sence de blaCTX-M-15 dans les isolats d'*E. coli* provenant de veaux et de blaCTX-M-1 dans les isolats d'*E. coli* provenant de porcs et à des mutations dans le promoteur *ampC* (g.-42 C>T) dans les isolats d'*E. coli* provenant des deux espèces animales. Les données WGS ont également été utilisées pour une analyse phylogénétique basée sur les types de séquences multilocus (MLST) et MLST du génome de base (cgMLST) révélant que les *E. coli* 3GC-R isolés des veaux et des porcs suisses étaient gé-

Caratterizzazione dell'*Escherichia coli* resistenti alle cefalosporine di terza generazione provenienti da vitelli da macello e suini da ingrasso: uno studio pilota per il monitoraggio della resistenza antimicrobica mediante sequenziamento dell'intero genoma in Svizzera

Il sequenziamento dell'intero genoma (WGS) è stato introdotto nel monitoraggio della resistenza antimicrobica in Svizzera nel 2022 come metodo aggiuntivo ai test di suscettibilità antimicrobica fenotipica mediante micro-diluizione in brodo per caratterizzare l'*Escherichia coli* presuntivamente resistente alle cefalosporine di terza generazione (3GC-R). Campioni del ceco provenienti da vitelli da macello e da suini da ingrasso svizzeri, nonché di carne di manzo e di maiale provenienti dalla vendita al dettaglio in Svizzera prelevati nel 2021, sono stati analizzati per la presenza di *E. coli* 3GC-R secondo i protocolli armonizzati europei. Nel 2021, l'*E. coli* 3GC-R è stata rilevata nel 23,8 % dei vitelli da macello, nel 5,9% dei suini da ingrasso e nello 0 % della carne. L'analisi comparativa dei risultati della resistenza antimicrobica ottenuti con la misurazione fenotipica e quelli ottenuti con l'individuazione dei corrispondenti meccanismi molecolari sottostanti mediante WGS ha mostrato una molto elevata concordanza (99 %). La resistenza alle cefalosporine di terza generazione (3GC) è stata associata principalmente alla presenza di $bla_{\text{CTX-M-15}}$ negli isolati di *E. coli* provenienti dai vitelli e di $bla_{\text{CTX-M-1}}$ negli isolati di *E. coli* provenienti dai suini e di mutazioni nel promotore *ampC* (g.-42 C>T) negli isolati di *E. coli* di entrambe le specie animali. Inoltre, i dati WGS sono stati utilizzati per l'analisi filogenetica basata su tipi di sequenza multi locus (MLST) e core genome MLST (cgMLST), rivelando che

nétiquement divers. Dans cette étude, il a été démontré que l'utilisation du WGS seul pour surveiller la résistance aux antibiotiques pouvait détecter des tendances dans les mécanismes moléculaires connus de la résistance aux antibiotiques tout en fournissant d'autres informations précieuses sur les isolats, comme la parenté génétique. Cependant, ce n'est qu'en combinant les tests de sensibilité phénotypique avec le WGS que la détection pré-coce de mécanismes de résistance inconnus sera possible.

Mots clés: AmpC, BLSE, bovins, CTX-M, multirésistants, porcins

gli isolati di *E. coli* 3GC-R provenienti dai vitelli da macello e dai suini da ingrasso svizzeri erano geneticamente diversi. In questo studio è stato dimostrato che l'utilizzo della sola WGS per monitorare la resistenza antimicrobica può rilevare delle tendenze nei meccanismi molecolari noti di resistenza antimicrobica, fornendo al contempo altre informazioni preziose sugli isolati, come la parentela genetica. Tuttavia, solo combinando test di suscettibilità fenotipica e WGS sarà possibile individuare precocemente meccanismi di resistenza precedentemente sconosciuti.

Parole chiave: AmpC, CTX-M, ESBL, multiresistenza, bovini, suini

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