Antibiotic and quaternary ammonium compound resistance in *Escherichia coli* from calves at the beginning of the fattening period in Switzerland (2017)

A. Hausherr[1,2], J. Becker[2,3], M. Meylan[2], D. Wüthrich[1,2], A. Collaud[1], A. Rossano[1], V. Perreten[1]

[1] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;
[2] Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland;
[3] Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

https://doi.org/10.17236/sat00233

Received: 23.01.2019
Accepted: 21.06.2019

Abstract

In the Swiss veal calf production, antimicrobials and disinfectants are used to control bacterial infectious diseases, leading to a risk of selecting for a resistant bacterial population. While the prevalence of antibiotic resistance in *E. coli* from calves has been monitored at slaughterhouses in Switzerland since 2006, the resistance situation of *E. coli* from young calves entering the fattening period is not known. A total of 100 calves entering the fattening period in 20 geographically distant farms in Switzerland were screened for the presence of *E. coli* using rectal swabs in 2017. Genetic diversity between isolates was determined using repetitive palindromic Polymerase Chain Reaction (*rep*-PCR) revealing a genetically diverse *E. coli* population. Susceptibility to 13 antibiotics and to alkyldimethylbenzylammonium (ADBAC) was determined by the measurement of the minimal inhibitory concentration. Antibiotic and quaternary ammonium compound (QAC) resistance genes were identified using microarray and Polymerase Chain Reaction (PCR). Sixty-four percent of the isolates were resistant to at least one antibiotic, and 52% also exhibited decreased susceptibility to ADBAC. Resistance to more than 3 antibiotics was found in 40% of the isolates. Isolates exhibited resistance to tetracycline (57%) associated with the presence of *tet* genes (*tet*(A), (B), (E), (G)), to sulfonamides (61%) (*sul1*, *sul2*, *sul3*), ampicillin (56%) ($bla_{\text{TEM-1}}$), trimethoprim (32%) (*dfrA*), phenicols (31%) (*catA1, cmlA1, floR*), gentamicin (27%) (*ant(2")-Ia*, *aac(3)-IVa*, *aac(3)-VIa*), and cefotaxime (2%) ($bla_{\text{CTX-M-14}}$ (ESBL)). Mutations in GyrA (S83L) and ParC (S80I) were found in the fluoroquinolone-resistant isolates (6%). All isolates were susceptible to colistin, tigecycline and meropenem. No association between the presence of decreased susceptibility to ADBAC and *qac* genes was observed. In conclusion, antibiotic and QAC resistant

Resistenzlage gegenüber Antibiotika und quaternären Ammoniumverbindungen in *Escherichia coli* von Kälbern zu Beginn der Mastperiode in der Schweiz (2017)

Antibiotika und Desinfektionsmittel werden in der Schweizer Kälbermast eingesetzt, mit dem Ziel Infektionskrankheiten zu bekämpfen. Dieser Einsatz birgt das Risiko der Selektion einer resistenten Bakterienpopulation. Obwohl die Prävalenz der Antibiotikaresistenzen von aus Kälbern an Schweizer Schlachthöfen seit 2006 isolierten *E. coli* überwacht wird, ist die Resistenzsitutation von *E. coli* zu Beginn der Mastperiode bisher nicht bekannt. Im Jahr 2017 wurden *E. coli* aus Kälbern von zwanzig Betrieben aus unterschiedlichen Regionen untersucht. Die dafür nötigen Kottupfer wurden zu Beginn der Mastperiode entnommen. Die genetischen Charakteristika der Isolate wurden mittels repetitiver palindromischer Polymerase Kettenreaktion (*rep*-PCR) bestimmt und weisen auf eine heterogene Population hin. Die Wirksamkeit von 13 Antibiotika sowie von alkyldimethylbenzylammonium (ADBAC) wurde mit Messung der minimalen inhibitorischen Konzentration (MHK) bestimmt. Die Gene, die für die Resistenz gegenüber den Antibiotika und den quaternären Ammoniumverbidungen (QAC) verantwortlich sind, wurden mittels Microarray und Polymerase Kettenreaktion (PCR) identifiziert. Sechsundsechzig der 100 Isolate waren gegen mindestens ein Antibiotikum resistent und 52% wiesen zusätzlich eine verminderte Empfindlichkeit gegen ADBAC auf. Vierzig Prozent der Isolate zeigten Resistenzen gegenüber mehr als 3 Antibiotika, darunter solche gegenüber Tetracyclin (57%) mit Vorhandensein der *tet* Gene (*tet*(A), (B), (E), (G)), Sulfonamide (61%) (*sul1*, *sul2*, *sul3*), Ampicillin (56%) ($bla_{\text{TEM-1}}$),

E. coli are present in the gastrointestinal tract of young calves at the beginning of the fattening period, emphasizing the need for appropriate and reduced use of antibiotics and QAC-containing disinfectants in order to limit further selection of these bacteria during the fattening period.

Keywords: Antibiotic; cattle; disinfectants; Enterobacteriaceae; resistance; QAC

Trimethoprim (32%) (*dfrA*), Phenicol (31%) (*catA1*, *cmlA1*, *floR*), Gentamicin (27%) (*ant(2")-Ia*, *aac(3)-IVa*, *aac(3)-VIa*), und Cefotaxim (2%) ($bla_{\text{CTX-M-14}}$ (ESBL)). In flouroquinolonresistenten Isolaten (6%) wurden Mutationen in GyrA (S83L) and ParC (S80I) gefunden. Alle Isolate waren sensibel gegenüber Colistin, Tigecyclin und Meropenem. Es wurde kein Zusammenhang zwischen verminderter Empfindlichkeit gegenüber ADBAC und *qac* Genen beobachtet.

Im Verdauungsapparat von Kälbern finden sich zu Beginn der Mastperiode *E. coli*, die gegenüber Antibiotika und QAC resistent sind. Dieser Befund verdeutlicht, dass ein angemessener Einsatz von Antibiotika und QAC-haltiger Desinfektionsmittel von Nöten ist, um die weitere Selektion dieser Bakterien während der Mastperiode zu begrenzen.

Schlüsselwörter: Antibiotika; Desinfektionsmittel; Enterobacteriaceae; Resistenz; Rind; QAC

Introduction

In Switzerland, veal and products made thereof belong to one of the favorite traditional meals with 22'262 tons of yearly meat consumption.[15] Almost one hundred percent (99.7%) of the calf meat sold in Switzerland originates from Swiss farms. In the Swiss fattening calf production, calves are merged into groups of up to 40 animals at the age of around 40 days. They are mainly fed with cow milk preparations until they are slaughtered at the age of 150 days in average, up to a maximum of 160 days. At the time of grouping, the calves have an incompletely competent immune system and they also face factors such as stress, new environment and contact with other animals, which favors the development of infectious diseases. During this period, antibiotics are frequently used for metaphylactic or curative treatment of bacterial infections of either individuals or groups of animals to prevent further spread of the disease. In some situations, disinfectants such as quaternary ammonium compounds (QACs) are also used to eliminate pathogenic agents from inanimate materials and direct environment. The use of antibiotics and QACs contributes to the selection of resistances to these antimicrobial agents in both pathogenic and commensal bacteria.[3,11] Both antibiotic and QAC resistance can be localized on the same mobile genetic element such as plasmids, transposons and integrons which can be transferred between bacteria.[22] The use of either QAC or antibiotics in calf husbandry may therefore select for a bacterial population which resists disinfectant and antimicrobial treatment.

Since 2006 prevalence of antibiotic resistance in food-producing animals including calves have been monitored by testing indicator organisms such as *Enterococcus* and *E. coli* isolated from the gastrointestinal tract at slaughter in Switzerland. In the monitoring report of 2016, *E. coli* from calves at slaughter exhibited a high percentage of resistance to antibiotics with up to 40% of the isolates being resistant to sulfonamides (41.6%), tetracyclines (40.5%) and ampicillin (36.8%).[6] However, the resistance situation of *E. coli* isolated from calves entering the fattening period is not known, raising the question whether young calves already carry antibiotic- and QAC-resistant *E. coli*. We therefore determined the antimicrobial phenotype and the molecular nature of the resistance mechanisms of *E. coli* from calves at that early stage of the fattening period independently of possible previous antimicrobial treatment or QAC usage.

Animals, Materials and Methods

A total of 100 randomly selected calves entering the fattening period in 20 geographically distant farms situated within a radius of 12 km to 146 km from Bern in Switzerland were screened for the presence of *E. coli* using rectal swabs between March and May 2017. Antibiotic and QAC usage prior to the fattening period was not recorded. Five samples per farm taken on the same day were further characterized. The samples were taken by veterinarians according to an approved procedure[4] in compliance with the Swiss welfare legislation (authorization for animal experimentation no. BE 71/16). Swabs were immediately placed into vials containing 2 ml of liquid Amies transport medium (Axon Lab AG, Baden, Switzerland) and processed within one day. Samples were spread on selective BROLAC agar plates (Ther-

mo Fisher Scientific, Waltham, USA) and incubated aerobically for 18 to 24 hours at 37 °C. Colonies identified as *E. coli* with Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) (Microflex LT, Bruker Daltonics GmbH, Bremen, Germany) were spread onto trypticase soy agar plates containing 5% sheep blood (TSA- SB; Becton, Dickinson and Company, New Jersey, USA) and incubated overnight at 37 °C. Identification of the purified *E. coli* isolates was confirmed by MALDI-TOF and the isolates were stored in tryptic soy broth with 30% glycerol at -80 °C.

Antimicrobial susceptibility testing and antibiotic resistance gene detection

Minimal inhibitory concentration (MIC) of 13 antibiotics was obtained by broth microdilution in cation-adjusted Mueller-Hinton broth using EUVSEC Sensititre® plates (Thermo Fisher Scientific, Waltham, USA) following guidelines and interpretation criteria of the European Committee of the Antimicrobial Susceptibility testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI).[5,18] The following resistance breakpoints (R) were used: ampicillin (R>8µg/ml), cefotaxime (R>2µg/ml), ceftazidime (R>4µg/ml), chloramphenicol (R>8µg/ml), ciprofloxacin (R>1µg/ml), colistin (R>2µg/ml), gentamicin (R>4µg/ml), meropenem (R>8µg/ml), tigecycline (R>2µg/ml), trimethoprim (R>4µg/ml),[18] nalidixic acid (R≥16µg/ml), sulfamethoxazole (R≥512µg/ml) and tetracycline (R≥16µg/ml).[5] Multidrug-resistance was defined as present in strains exhibiting resistance to three or more antibiotics. Antibiotic resistance genes were detected using the AMR-ve Genotyping Kit (Alere Technologies GmbH, Jena, Germany), except for the trimethoprim resistance genes of the *dfrA* family which were detected using primers identifying groups of *dfrA* genes,[10] as well as primers for the new *dfrA35*[1] (dfrA35-F: 5'-ATGATTTCAATCGTCGTAGCCA and dfrA35-R: 5'-CTCATTCATCTAACACCTTCCTCAC; annealing temperature of 58 °C, elongation time of 30s, 25 cycles) and *dfrA36* genes.[21] Mutations in the fluoroquinolone resistance determining region of GyrA and ParC were detected by amino acid sequences analysis obtained from *in silico* translated PCR products as described previously.[2] The $bla_{\text{CTX-M-14}}$ gene was identified by DNA sequence of a specific PCR product amplified as described previously.[14]

QAC susceptibility testing and QAC resistance gene detection

QAC susceptibility was determined by MIC measurement of alkyldimethylbenzylammonium chloride (ADBAC) using 2-fold serial broth microdilution in cation-adjusted Mueller-Hinton broth with concentrations ranging from 0.5 µg/ml to 512 µg/ml. The presence of genes described to confer resistances to QAC was determined by PCR using specific primers and conditions for *qacE*, *qacEΔ1*, *qacJ*, *qacG* and *sugE(p)* as described.[22] The presence of *qacF/H*, was tested using *Taq*-polymerase and primers *qacF/H-F* (5'-GTTGTAGTTGTGGCTGGCTAC) and *qacF/H-R* (5'-AATGTGCGCTGACCTTGGATA) at an annealing temperature of 58 °C and an elongation time of 30s for 25 cycles.

Genetic diversity

Genetic diversity among isolates was determined using repetitive palindromic PCR (*rep*-PCR).[19] The profiles were analyzed using Bionumerics 7.6 (Applied Maths, Krotnijk, Belgium) (settings: Dice, UPGMA with 0% optimization and 1% band tolerance). Strains were defined as genetically related when they showed ≥90% similarity.

Results

Sampling and genetic diversity

Samples were collected from calves born on the fattening farms (n=53) as well as from calves purchased from other farms (n=47). The average age of the calves at sampling time was 43.36 days. *E. coli* were detected in each of the 5 faecal samples of calves among the 20 farms. The majority of the *E. coli* isolates were genetically diverse as determined by *rep*-PCR which generated 93 different profiles. The *E. coli* found in calves from different farms were genetically different, except for two related *E. coli* found in calves from two different farms. Genetically different *E. coli* were also found in each of the 5 samples in 14 farms, while in 6 farms two to three calves shared genetically identical *E. coli* isolates (Figure 1).

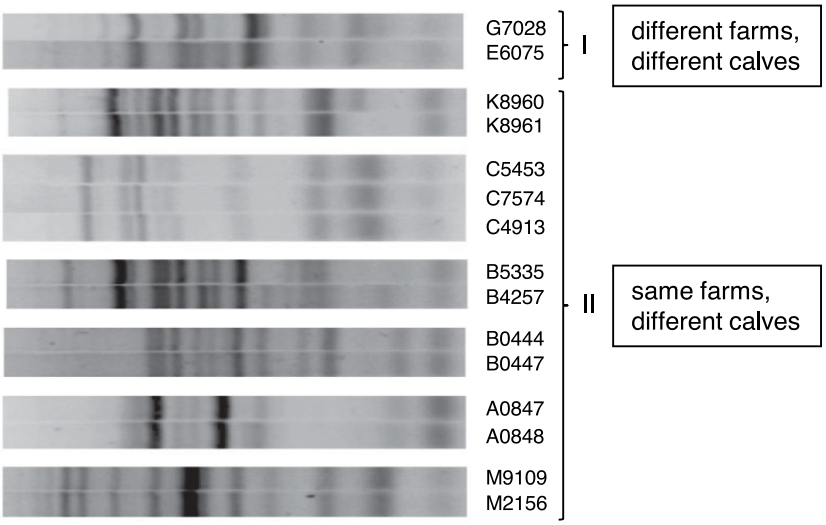

Figure 1: Photograph of *rep*-PCR-fragments analyzed in 2% agarose gel of *E. coli* sharing the same *rep*-PCR profile and genetic relatedness. Letters indicate the different farms and the numbers indicate the *E. coli* isolates. Cluster I shows identical *E. coli* in calves from two different farms, and clusters II show identical *E. coli* from different calves in the same farms.

Antibiotic and QAC resistance

Antimicrobial susceptibility testing revealed that 36% of the isolates were susceptible to all antibiotics tested. The other isolates exhibited resistance to one to eight antibiotics (Table 1). Among them, 40% of the isolates exhibited resistance to more than 3 antibiotics (Table 1).

Resistance to sulfamethoxazole (61%), tetracycline (57%) and ampicillin (56%) were the most frequent, followed by trimethoprim (32%), chloramphenicol (31%), gentamicin (27%), nalidixic acid (11%), ciprofloxacin (6%), cefotaxime (2%). One strain exhibited decreased susceptibility to both ceftazidime and cefotaxime (MIC, 2 µg/ml). All isolates were susceptible to meropenem, colistin and tigecycline (Figure 2). Susceptible and resistant isolates were distributed over all the participating farms.

The acquired resistance genes detected in the resistant isolates consisted of those conferring resistance to tetracycline (*tet*(A), *tet*(B), *tet*(E), *tet*(G)), sulfonamides (*sul1*, *sul2*, *sul3*), trimethoprim (*dfrA1-15-16*, *dfrA12*, *dfrA7-17*, *dfrA8*, *dfrA5-14-25*, *dfrA36*), ampicillin (bla_{TEM-1}, *act1*), phenicols (*catA1*, *cmlA1*, *floR*), gentamicin (*ant(2")-Ia*, *aac(3)-IVa*, *aac(3)-VIa*), and cefotaxime ($bla_{CTX-M-14}$). The mechanism of the strains exhibiting decreased susceptibility to both ceftazidime and cefotaxime remained unexplained. The streptomycin resistance genes *strA*, *strB* and *aadA* were detected in 54 of the isolates either alone or in combination. Amino acid mutations in the fluoroquinolone resistance-determining region of the chromosomal topoisomerase GyrA and ParC were found in the 6 isolates exhibiting resistance to ciprofloxacin. These mutations consisted of a serine to leucine substitution at position 83 in GyrA (S83L) as well as a serine to isoleucine substitution at position 80 in ParC (S80I) (Figure 2). Of note, the trimethoprim resistance phenotype could not be explained by either one of the tested *dfr* gene in three isolates. This suggests the presence of other trimethoprim resistance mechanisms in *E. coli* from calves as it has been recently shown with the discovery of the new *dfrA36* gene within this *E. coli* collection.[21]

MIC of ADBAC for the 100 *E. coli* isolates tested ranged from 1 to 128 µg/ml and were bimodally distributed

Table 1: Distribution of antibiotic and ADBAC resistance of 100 *E. coli* isolated from calves entering the fattening period in Switzerland.

Number of antibiotic resistances	Antibiotic resistance profile	Number of isolates per antibiotic resistance profile	Number of isolates exhibiting resistance to ADBAC	Percentage of isolates per antibiotic resistance profile
0		36	17	
1	TET AMP	1 2	0 1	3%
2	SMX, TET	4	2	4%
3	SMX, TET, AMP SMX, TMP, AMP SMX, TMP, CHL SMX, CHL, AMP	13 1 2 1	7 0 2 0	17%
4	SMX, TET, AMP, GEN SMX, TMP, TET, AMP SMX, TET, CHL, AMP SMX, TMP, CHL, AMP SMX, TET, NAL, AMP	2 2 4 1 1	0 1 3 1 0	10%
5	SMX, TMP, TET, CHL, AMP SMX, TET, FOT, AMP, GEN SMX, TMP, TET, AMP, GEN SMX, TET, CHL, AMP, GEN SMX, TMP, TET, CHL, GEN SMX, CIP, TET, NAL, AMP	3 1 3 2 1 1	2 0 0 2 1 1	11%
6	SMX, TMP, TET, CHL, AMP, GEN	11	9	11%
7	SMX, TMP, TET, NAL, FOT, AMP, GEN SMX, TMP, CIP, TET, NAL, AMP, GEN SMX, TMP, CIP, TET, NAL, CHL, AMP SMX, TMP, TET, NAL, CHL, AMP, GEN	1 1 1 2	0 1 0 1	5%
8	SMX, TMP, CIP, TET, NAL, CHL, AMP, GEN	3	1	3%
Number of resistant isolates		64	52	

ADBAC, alkyldimethylbenzylammonium chloride; AMP, ampicillin; CIP, ciprofloxacin; CHL, chloramphenicol; FOT, cefotaxime; GEN, gentamicin; NAL, nalidixic acid; SMX, sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TMP, trimethoprim.

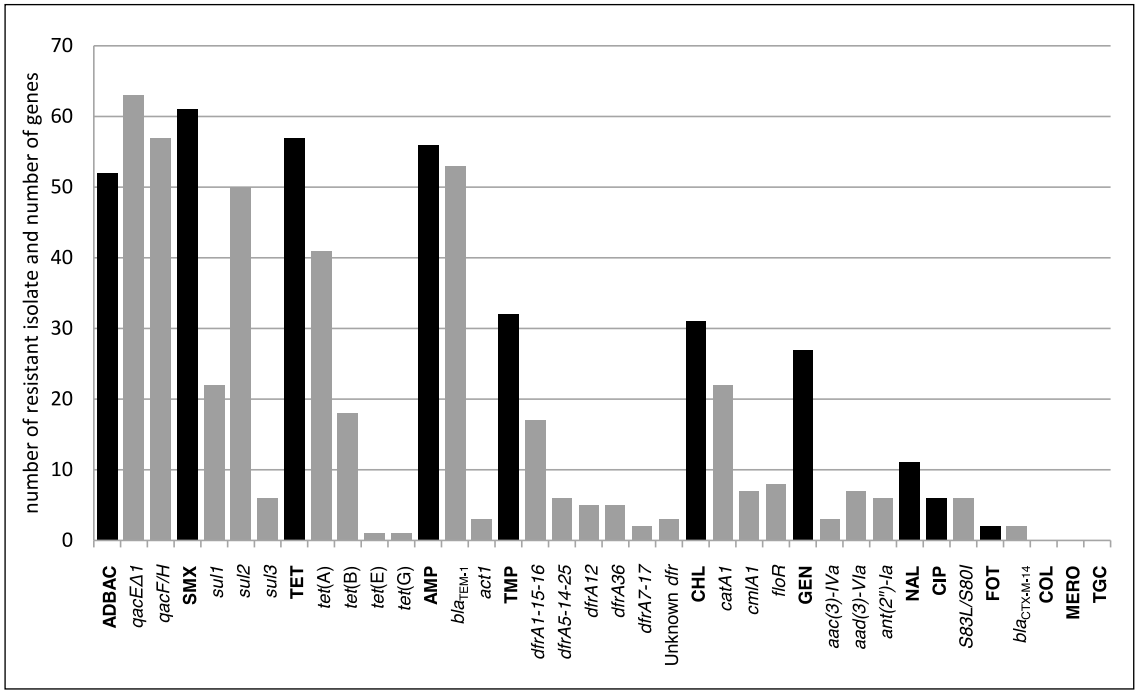

Figure 2: Distribution of antibiotic and QAC resistance phenotype and genotype in *E. coli* (n=100) from calves in Switzerland. Antibiotics: SMX, sulfamethoxazole; TET, tetracycline; AMP, ampicillin; TMP, trimethoprim; CHL, chloramphenicol; GEN, gentamicin; NAL, nalidixic acid; CIP, ciprofloxacin; FOT, cefotaxime; TAZ, ceftazidime; COL, colistin; MERO, meropenem; TGC, tigecycline, ADBAC, alkyldimethylbenzylammonium chloride. Resistance genes: *tet*(A), (B), (E), (G), tetracycline efflux genes; *sul1*, *sul2*, *sul3*, dehydrosulfate synthetase genes for sulfonamide resistance; bla_{TEM-1}, *act1*, β-lactamase genes; *dfrA1-15-16* (*dfrA1, dfrA15* or *dfrA16*), *dfrA12, dfrA7-17* (*dfrA7* or *dfrA17*), *dfrA8, dfrA5-14-25* (*dfrA5, dfrA14* or *dfrA25*), *dfrA36*, dihydropteroate synthase genes for trimethoprim resistance; *catA1*, chloramphenicol O-acetyltransferase gene; *cmlA1*, chloramphenicol efflux gene; *floR*, phenicol exporter gene; *strA, strB, aadA1-2-4* (*aadA1, aadA2* or *aadA4*), streptomycin resistance genes; *aac(3)-IVa, aac(3)-VIa*, aminoglycoside acetyltransferase genes for gentamicin resistance; *ant(2")-Ia*, aminoglycoside nucleotidyltransferase gene for gentamicin resistance; S83L/S80I, serine to leucine substitution at position 83 in GyrA and serine to isoleucine substitution at position 80 in ParC; $bla_{CTX-M-14}$, extended-spectrum-β-lactamase gene conferring resistance to 3rd generation cephalosporins; $qacE_{\Delta}1$, *qacF/H*, quaternary ammonium compound resistance genes.

with 48 isolates having a MIC ≤4µg/ml and 52 isolates having a MIC situated between 8 and 128 µg/ml indicating decreased susceptibility to ADBAC and possible acquisition of a resistance mechanism (Figure 3). Based on this distribution, an epidemiological cut-off was tentatively set at >4 µg/ml to distinguish *E. coli* which exhibit decreased susceptibility to ADBAC from those with lower MICs, classifying 52% of the isolates as non-susceptible to ADBAC. The presence of acquired QAC resistance genes was found in 76 strains with 63 strains containing $qacE_{\Delta}1$, 57 strains containing *qacF/H*, and 43 strains containing both. These QAC genes were found in *E. coli* isolates which exhibited different MIC to ADBAC including both low and high MICs indicating no association between the presence of one of these genes and decreased susceptibility to ADBAC (Figure 3).

Decreased susceptibility to ADBAC was found in strains exhibiting no resistance to antibiotics (17%), as well as in strains exhibiting resistance to one or several classes of antibiotics (33%) (Table 1).

MIC ADBAC (µg/ml)	≤0.5	1	2	4	8	16	32	64	128	256	>512
Number of isolates	0	3	28	17	33	9	8	0	2	0	0
$qacE_{\Delta}1$	0	2	21	12	21	3	1	0	2	0	0
qacF/H	0	1	16	11	26	1	1	0	1	0	0
Others [a)]	0	0	0	0	0	0	0	0	0	0	0

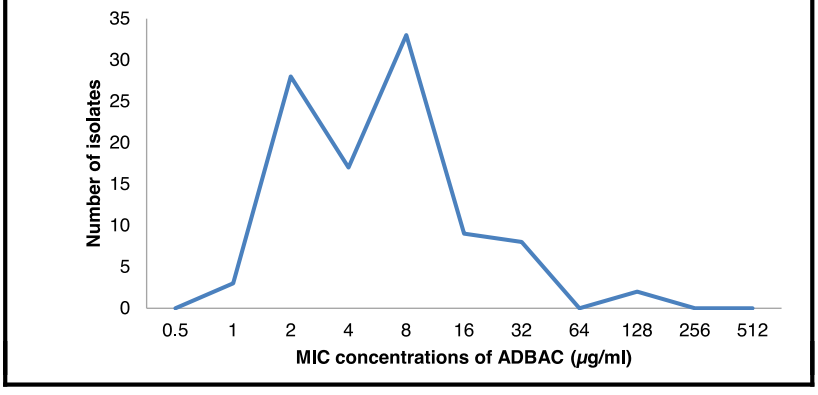

Figure 3: Minimal inhibitory concentration (MIC) of alkyldimethylbenzylammonium chloride (ADBAC) and distribution of *qac* resistance genes in 100 *E. coli* isolates from calves. a) Others consisted of *qacCD, qacG, qacE, qacJ* and *sugE(p)* genes.

Discussion

This study revealed that calves entering the fattening period already carry *E. coli* which exhibit resistance to antibiotics routinely used for the treatment of bacterial infectious diseases in calves such as tetracyclines, β-lactams, sulfonamides and trimethoprim.[13] However, the absence of data concerning antibiotic treatment of calves prior the beginning of the fattening period does not allow for assumptions regarding possible associations between antibiotic usage and resistance in *E. coli* at the animal level. Although the commensal *E. coli* is not the primary target of antimicrobial treatments, it is used as indicator in surveillance programs to determine the prevalence of resistance to antibiotics in the normal flora of animals.[7] The type and proportion of resistance is often corresponding to the classes of antibiotics which are most frequently used. *E. coli* represent also a reservoir of antibiotic resistance genes which can be transferred to pathogenic *Enterobacteriaceae* like e.g. those causing diarrhea in calves, thus jeopardizing treatment. The genes identified among the *E. coli* in the present study are among those which are classically localized on mobile genetic elements,[11] further emphasizing their potential for dissemination. In addition to a high number of *E. coli* resistant to tetracyclines, sulfonamides, trimethoprim and ampicillin, the isolates also showed resistance to critically important antibiotics like fluoroquinolones and cephalosporins. These classes of antibiotics are also used in cattle, e.g. for the treatment of pneumonia and mastitis, and even if they are to supposed to be used less frequently than others, their use also promotes an antimicrobial selection pressure on the commensal bacteria. Besides antimicrobial treatment, the use of milk containing antibiotic residues to feed calves during the withdrawal period is still a common practice in Switzerland,[13] and may also contribute to maintain a resistant population in calves.[20] Additionally, QACs are frequently present as active antimicrobial substance in disinfectants used in animal husbandry and their use can also contribute to the co-selection of QAC and antibiotic resistant bacteria.[17] The presence of several antibiotic and QAC resistance genes in the same bacteria poses the risk of simultaneously selecting for multi-resistance even if only one antibiotic or a disinfectant is being used.[11,16,17] Indeed, the *qacE$_\Delta$1* gene is known to be present on plasmids and integrons together with a wide range of different antibiotic resistance genes including those found in our study like e.g. *sul* (sulfonamides) resistance, *dfrA* (trimethoprim), *bla*$_{\text{TEM-1}}$ (β-lactamase), *tet* (tetracycline efflux), *bla*$_{\text{CTX-M-14}}$ (extended-spectrum-β-lactamase), *cmlA* and *floR* (chloramphenicol and florfenicol resistance).[11,17] More than half of the *E. coli* exhibited decreased susceptibility to the quaternary ammonium compound ADBAC. Despite the wide distribution of *qac* genes, the global mechanism of resistance to QAC remains poorly understood, since a clear association of decreased susceptibility with the presence of resistance genes is not always seen.[9] For instance, the *qacE$_\Delta$1* gene is known to confer only a low level resistance to QAC.[12] This observation was also made during our study where *qac* genes were found in isolates exhibiting low MIC to ADBAC and vice versa, suggesting that other factors are necessarily involved for a higher level of resistance or expression of these genes.[8,17]

Comparing the percentage of resistance to antibiotics found in *E. coli* in calves entering the fattening period from our study with those from the national monitoring of resistance of calves at slaughter in Switzerland revealed similar resistance profiles but the proportion of resistance was up to 20% lower for certain antibiotics at slaughter. In the monitoring reports 2016 and 2018, the proportion of resistance in *E. coli* from calves at slaughter was 40.5% and 41.2% for tetracycline, 36.8% and 38.7% for ampicillin, 41.6% and 46.9% for sulfamethoxazole, 15.8% and 19.1% for trimethoprim, 11.6% and 9.8% for chloramphenicol and 5.8% and 4.6% for gentamicin,[6,7] indicating that the proportion of resistance seems to decrease during the fattening period. Emergence of resistance to critically important antibiotics like the fluoroquinolones and cephalosporins is alarming and should be followed with special attention. Additionally, further studies would be necessary to analyze and follow the evolution of the resistant population at the farm and animal levels.

Conclusion

Our study showed that calves entering the fattening period already harbor antibiotic- and QAC-resistant bacteria among their gut flora. Their selection should be avoided by the unnecessary and inappropriate use of antibiotics and disinfectants, since both may contribute to the selection and maintenance of a resistant *E. coli* population in calf husbandry. It is therefore important to have a sustainable calf production which takes into consideration all factors necessary to prevent the development and spread of diseases to limit the use of antimicrobials at any stage of the calf production.

Acknowledgements

This study was financed by Grant No. 407240_167083 from the Swiss National Science Foundation within the Antimicrobial Resistance National Research Program NRP72 to Mireille Meylan and Vincent Perreten. We are grateful to all participating farmers who kindly made sampling possible.

Résistance d'*Escherichia coli* aux antibiotiques et aux composés d'ammonium quaternaire chez les veaux au début de l'engraissement en Suisse (2017)

Dans la production de veaux en Suisses, des antimicrobiens et des désinfectants sont utilisés pour contrôler les maladies infectieuses bactériennes, ce qui entraîne un risque de sélection d'une population bactérienne résistante. Si la prévalence de la résistance de *E. coli* aux antibiotiques chez les veaux est surveillée dans les abattoirs suisses depuis 2006, la situation de la résistance de *E. coli* chez les jeunes veaux au début de la période d'engraissement n'est pas connue. Un total de 100 veaux entrant dans la période d'engraissement dans 20 exploitations géographiquement éloignées de Suisse ont été testés en 2017 pour détecter la présence de *E. Coli* à l'aide de prélèvements rectaux. La diversité génétique entre les isolats a été déterminée à l'aide de la réaction de polymérase en chaîne répétitive palindrome (*rep*-PCR) révélant une population de *E.coli* génétiquement diversifié. La sensibilité à 13 antibiotiques et au chlorure d'alkyldiméthylbenzylammonium (ADBAC) a été déterminée par la mesure de la concentration inhibitrice minimale. Les gènes de résistance aux antibiotiques et aux composés d'ammonium quaternaire (QAC) ont été identifiés à l'aide d'une puce à ADN et de la réaction de polymérase en chaîne (PCR). Soixante-quatre pour cent des isolats étaient résistants à au moins un antibiotique et 52% présentaient également une diminution de la sensibilité à l'ADBAC. Une résistance à plus de 3 antibiotiques a été trouvée dans 40% des isolats. Les isolats présentaient une résistance à la tétracycline (57%) associée à la présence de gènes *tet* (*tet* (A), (B), (E), (G)), aux sulfonamides (61%) (*sul1, sul2, sul3*), à l'ampicilline (56%) (*bla*$_{\text{TEM-1}}$), au triméthoprime (32%) (*dfrA*), aux phénicols (31%) (*catA1, cmlA1, floR*), à la gentamicine (27%) (*ant(2'')-Ia, aac (3) -IVa, aac (3) -VIa*) et à la céfotaxime (2%) (*bla*$_{\text{CTX-M-14}}$ *(BLSE)*). Les isolats résistants aux fluoroquinolones (6%) présentaient des mutations dans GyrA (*S83L*) et ParC (*S80I*). Tous les isolats étaient sensibles à la colistine, à la tigécycline et au méropénème. Aucune association entre la présence d'une sensibilité diminuée à l'ADBAC et les gènes *qac* n'a été observée.

En conclusion, des *E. coli* résistants aux antibiotiques et aux QAC sont présents dans le tractus gastro-intestinal des jeunes veaux au début de la période d'engraissement, ce qui souligne la nécessité d'un usage approprié et réduit d'antibiotiques et de désinfectants contenant un QAC afin de limiter la sélection ultérieure de ces bactéries au cours de la période d'engraissement.

Mots-clés: antibiotique; bovins; désinfectants; enterobacteriacées; résistance; QAC

Resistenza agli antibiotici e ai composti di ammonio quaternario (QAC) nell'*Escherichia coli* dei vitelli all'inizio del periodo da ingrasso in Svizzera (2017)

Nella produzione svizzera di vitelli da carne si usano antimicrobici e disinfettanti per il controllo delle malattie infettive batteriche, con il rischio di selezionare una popolazione batterica resistente. La prevalenza della resistenza agli antibiotici di *E. coli* è ben monitorata nei macelli svizzeri dal 2006, mentre la situazione dei giovani vitelli resistenti a *E. coli* che entrano nel periodo di ingrasso non è nota. Nel 2017, un totale di 100 vitelli entranti nel periodo di ingrasso provenienti da 20 aziende svizzere distanti tra loro sono stati sottoposti a screening, utilizzando tamponi rettali, per individuare la presenza di *E. coli*. La diversità genetica tra gli isolati à stata determinata usando una reazione a catena della polimerasi palindromica ripetitiva (*rep*-PCR) che ha rivelato una popolazione geneticamente diversa di *E. coli*. La sensibilità a 13 antibiotici e all'alchildimentilbenzilammonio (ADBAC) è stata determinata misurando la concentrazione minima inibitoria. I geni resistenti agli antibiotici e ai composti di ammonio quaternario (QAC) sono stati identificati mediante microarray e PCR. Il 64 percento degli isolati risultavano resistenti ad almeno un antibiotico e il 52% ha mostrato una minore sensibilità all'ADBAC. Nel 40% degli isolati si è rilevata una resistenza a più di 3 antibiotici. Gli isolati hanno mostrato una resistenza alla tetraciclina (57%) associata alla presenza di geni *tet* (*tet*(A), (B), (E), (G)), a sulfonamidi (61%) (*sul1, sul2, sul3*), a ampicillina (56%) (*bla*$_{\text{TEM-1}}$), a trimetoprima (32%) (*dfrA*), a fenicoli (31%) (*catA1, cmlA1, floR*), a gentamicina (27%) (*ant(2")-Ia, aac(3)-IVa, aac(3)-VIa*), e a cefotaxima (2%) (*bla*$_{\text{CTX-M-14}}$ (ESBL)). Le mutazioni nel GyrA (S83L) e ParC (S80I) sono state riscontrate negli isolati resistenti ai fluorochinoloni (6%). Tutti gli isolati erano sensibili a colistina, tigeciclina e meropenem. Nessuna associazione è stata osservata tra la presenza di una ridotta sensibilità all'ADBAC e al *QAC*. In conclusione, l'*E. coli* resistente gli antibiotici e al QAC è presente nel tratto gastrointestinale dei vitelli all'inizio del periodo di ingrasso. Bisogna sottolineare la necessità di un uso appropriato e ridotto di antibiotici e disinfettanti contenenti QAC in modo da limitare l'ulteriore selezione di questi batteri durante il periodo di ingrasso.

Parole chiave: Antibiotico; bovini; disinfettanti; enterobatteriacee; resistenza; QAC

References

[1] Ambrose SJ, Hall RM: Novel trimethoprim resistance gene, *dfrA35*, in IncC plasmids from Australia. *J Antimicrob Chemother* 2019:74(7): 1863-1866.

[2] Brand P, Gobeli S, Perreten V: Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014-2015). *Schweiz Arch Tierheilkd* 2017: 159(7): 373-380.

[3] Buffet-Bataillon S, Branger B, Cormier M, Bonnaure-Mallet M, Jolivet-Gougeon A: Effect of higher minimum inhibitory concentrations of quaternary ammonium compounds in clinical *E. coli* isolates on antibiotic susceptibilities and clinical outcomes. *J Hosp Infect* 2011: 79(2): 141-146.

[4] Cho YI, Yoon KJ: An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *J Vet Sci* 2014: 15(1): 1-17.

[5] Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; twenty-seventh informational CLSI supplement M100-S27. Clinical and Laboratory Standards Institute; Wayne, PA; 2017.

[6] Federal Office of Public Health and Federal Food Safety and Veterinary Office: Swiss Antibiotic Resistance Report 2016. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Bacteria from Humans and Animals in Switzerland. November 2016. FOPH publication number: 2016-OEG-30.

[7] Federal Office of Public Health and Federal Food Safety and Veterinary Office: Swiss Antibiotic Resistance Report 2018. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Bacteria from Humans and Animals in Switzerland. November 2018. FOPH publication number: 2018-OEG-87.

[8] Ioannou CJ, Hanlon GW, Denyer SP: Action of disinfectant quaternary ammonium compounds against *Staphylococcus aureus. Antimicrob Agents Chemother* 2007: 51(1): 296-306.

[9] Jennings MC, Forman ME, Duggan SM, Minbiole KPC, Wuest WM: Efflux pumps might not be the major drivers of QAC resistance in methicillin-resistant *Staphylococcus aureus. Chembiochem* 2017: 18(16): 1573-1577.

[10] Matter D, Rossano A, Limat S, Vorlet-Fawer L, Brodard I, Perreten V: Antimicrobial resistance profile of *Actinobacillus pleuropneumoniae* and *Actinobacillus porcitonsillarum. Vet Microbiol* 2007: 122(1-2): 146-156.

[11] Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DG: Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics* 2015: 16: 964.

[12] Paulsen IT, Littlejohn TG, Rådström P, Sundström L, Sköld O, Swedberg G, et al.: The 3′ conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. *Antimicrob Agents Chemother* 1993: 37(4): 761-768.

[13] Pipoz F, Meylan M: [Calf health and antimicrobial use in Swiss dairy herds: Management, prevalence and treatment of calf diseases]. *Schweiz Arch Tierheilkd* 2016: 158(6): 389-396.

[14] Pitout JD, Hossain A, Hanson ND: Phenotypic and molecular detection of CTX-M-b-lactamases produced by *Escherichia coli* and *Klebsiella* spp. J Clin Microbiol 2004: 42(12): 5715-5721.

[15] Proviande: Der Schlachtvieh- und Fleischmarkt, 2016 (available from: Proviande Genossenschaft, Bern, Switzerland (www.proviande.ch)).

[16] Rodriguez-Blanco A, Lemos ML, Osorio CR: Integrating conjugative elements as vectors of antibiotic, mercury, and quaternary ammonium compound resistance in marine aquaculture environments. *Antimicrob Agents Chemother* 2012: 56(5): 2619-2626.

[17] Tezel U, Pavlostathis SG: Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Curr Opin Biotechnol* 2015: 33: 296-304.

[18] The European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters, version 8.1; http://www.eucast.org; 2018.

[19] Vila J, Marcos MA, Jimenez de Anta MT: A comparative study of different PCR-based DNA fingerprinting techniques for typing of the *Acinetobacter calcoaceticus-A. baumannii* complex. *J Med Microbiol* 1996: 44(6): 482-489.

[20] Würgler-Aebi I: Entwicklung von Resistenzen gegen Makrolid-Antibiotika bei Enterokokken im Kot von Kälbern, gefüttert mit antibiotikahaltiger Milch. Inaugural-Dissertation. Veterinär-Medizinische Fakultät der Universität Bern, Schweiz, 2004.

[21] Wüthrich D, Brilhante M, Hausherr A, Becker J, Meylan M, Perreten V: A novel trimethoprim resistance gene, *dfrA36*, characterized from *Escherichia coli* from calves. *mSphere* 2019: 4(3): pii: e00255-19.

[22] Zou L, Meng J, McDermott PF, Wang F, Yang Q, Cao G, et al.: Presence of disinfectant resistance genes in *Escherichia coli* isolated from retail meats in the USA. *J Antimicrob Chemother* 2014: 69(10): 2644-2649.

Corresponding author

Vincent Perreten
Institute of Veterinary Bacteriology
University of Bern
Länggassstrasse 122
CH-3012 Bern
Switzerland
Phone: ++41 31 631 24 30
Fax: ++41 31 631 26 34
E-Mail: vincent.perreten@vetsuisse.unibe.ch