



# Genotypes and antibiotic resistance of *Campylobacter coli* in fattening pigs

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## ABSTRACT

*Campylobacter coli* is a food-borne zoonotic pathogen causing human gastroenteritis worldwide. The organism is a commensal in the intestine of many food production animals including fattening pigs. The role of the pig as a potential reservoir for *C. coli* affecting human either directly or via poultry has hardly been investigated and genetic characterization of porcine strains is needed to address this question. For this aim multilocus sequence typing (MLST) and *flaB* typing was applied to 256 *C. coli* isolates from faeces of fattening pig collected during 2009 at different slaughterhouses in Switzerland. In addition genotypic resistances towards macrolides and quinolones based on point mutations in the 23S rRNA and *gyrA* genes, respectively, were determined. Of the 67 sequence types (STs) obtained by MLST, 37 were found for the first time. *flaB* typing revealed 46 different types with 14 of them being novel and was useful to further differentiate strains with an identical ST. Quinolone resistance was detected in 33.6% and macrolide resistance was found in 10.6% of isolates. Comparison with 99 *C. coli* pig isolates from 2001 revealed a significant decrease in antibiotic resistance towards both groups of antibiotics and there was high overlap between genotypes of 2001 and 2009. Little overlap of porcine genotypes was found with 97 *C. coli* isolates from poultry collected 2008, however, macrolide resistance was significantly higher in pig isolates. In conclusion, *C. coli* from Swiss pig are heterogeneous containing many novel STs, findings that could reflect the partitioned Swiss pig production with almost no international breed exchange. The antibiotic resistance echoes the use of corresponding drugs in the Swiss livestock production and indicates the efficacy of restrictive application of antibiotics in order to reduce resistances.

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## 1. Introduction

*Campylobacter* is the most common bacterial cause of human gastroenteritis in Europe (European Food Safety Authority, 2011). *Campylobacter jejuni* and *Campylobacter coli* are responsible for approximately 90% and 10% of cases, respectively (Gillespie et al., 2002; Sheppard et al., 2009). Although *C. coli* is less frequently associated with human disease than *C. jejuni* it is still an important food borne pathogen, given the high incidence of the disease. *C. coli* is also more resistant to frequently used antibiotics to

treat humans such as macrolides or quinolones than *C. jejuni* (Engberg et al., 2001; Alfredson and Korolik, 2007; Wirz et al., 2010). Both species asymptotically colonize the intestine of many food production animals but also wildlife and companion animals (Horrocks et al., 2009). *C. coli* is predominantly identified in pigs where its prevalence varies between countries as well as herds (Pezzotti et al., 2003; Payot et al., 2004; Alter et al., 2005; Oporto et al., 2007; Anonymous, 2010; Tadesse et al., 2011). Multilocus sequence typing (MLST) is the method of choice to study molecular epidemiology of *Campylobacter* (Dingle et al., 2001; Miller et al., 2005). Sequencing of the short variable region (SVR) within the flagellin-encoding gene *flaB* is a complementary typing method (Mellmann et al., 2004; Dingle et al., 2008). Resistance to quinolones

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antibiotics is mainly associated with a point mutation in the DNA gyrase gene (*gyrA*) at position C257T while a transition in the 23S rRNA gene at position A2075G is usually responsible for macrolide resistance (Alfredson and Korolik, 2007). In countries like Switzerland with its many mixed farms pig could serve as a possible reservoir for *C. coli* colonizing poultry, which is supposed to be the main source for human infections (Sheppard et al., 2009). The objectives of this study were to obtain an overview of *C. coli* genotypes colonizing fattening pigs in Switzerland, to estimate their macrolide and quinolone resistances and to compare the results to previously investigated isolates from pig and poultry. A multiplex approach including MLST, *flaB* typing and genetic determination of antibiotic resistance towards quinolones and macrolides was applied (Korczak et al., 2009).

## 2. Material and methods

### 2.1. Samples and sample preparation

Isolates of *C. coli* were collected within the antibiotic resistance monitoring program of the Swiss Federal Veterinary Office (FVO) during 2009. A representative selection of pigs, which had been randomly chosen in the 10 largest slaughterhouses proportional to their meat production, was investigated. Thereby 85% of the pork production sites in Switzerland was covered (Anonymous, 2010). One animal per herd was tested with faecal swabs from 350 herds at slaughter being collected and processed at the Centre for Zoonosis, Bacterial Animal Diseases and Antibiotic Resistance in Bern, Switzerland (ZOBA).

Faecal swabs were directly plated on modified charcoal–cefaperazon–desoxycholat agar (mCCDA; Oxoid, Pratteln, Switzerland) and incubated for 48 h at 41.5 °C under microaerophilic conditions. Presumptive *Campylobacter*-colonies on the selective plate were further cultivated on trypticase soy broth agar plates with 5% sheep blood (TSA-S; Oxoid) for 24 h to 48 h at 41.5 °C under microaerophilic conditions. Phenotypic species confirmation was done using routine diagnostic tests, including growth control under microaerophilic conditions at 25 °C and aerobic conditions at 41.5 °C, microscopy, oxidase reaction, catalase reaction, hippurate-hydrolysis, and hydrolysis of indoxyl-acetase (European Commission, 2007). *C. coli* was grown from 256 faecal swabs and the isolates were stored in trypticase soy broth containing 30% glycerol at –80 °C until further analysis. Frozen cultures were cultivated on TSA-S plates for 24–48 h at 37 °C under microaerophilic conditions, transferred into 450 µl of lysis buffer and incubated at 60 °C for 1 h (Korczak et al., 2009). After heat inactivation at 95 °C for 15 min the lysates were stored at –20 °C until further use.

### 2.2. Multiplex PCR and sequencing

Genetic characterization of strains was done according to the protocol of Korczak et al. (2009) with slight modifications. The seven MLST housekeeping genes (*aspA*, *atpA*, *glmM*, *glnA*, *gltA*, *glyA*, *tkt*) as well as *flaB*, 23S rRNA and *gyrA* were amplified by multiplex PCR in three amplification

groups. For amplification of the *flaB* fragment the primer *flaB*-L1 instead of *flaB*-L was used. Each PCR was performed in a 30-µl total volume containing 12 pmol of each primer, 1× FIREPol<sup>®</sup> Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) with additional MgCl<sub>2</sub> up to 5 mM per reaction and 1 µl lysate. The enzymatically purified PCR products were sequenced using the amplification primers.

### 2.3. Antimicrobial susceptibility testing

Isolates with a double peak at position A2075G in the partial 23S rRNA gene sequence were phenotypically tested for macrolide resistance. The minimal inhibitory concentration (MIC) for erythromycin was determined using the NLV44 Sensititre Custom Plate<sup>®</sup> (Trek Diagnostic Systems, OH) according to the manufacturer's instructions. Strains were defined as macrolide resistant if the MIC was >16 µg/ml erythromycin according to EUCAST ([www.eucast.org](http://www.eucast.org)).

### 2.4. Data analysis

Sequence data were entered, edited and analysed in the online-based MLST application for *Campylobacter* provided by SmartGene (Zug, Switzerland) which uses an integrated link to the PubMLST database (<http://pubmlst.org/campylobacter/>) for designation of sequence type (ST) and clonal complex (CC). The *flaB* types were assigned using only the PubMLST database. New alleles and allele combinations were submitted to PubMLST for allele number and ST designation, respectively.

### 2.5. Statistical analysis

The NCSS 2007 software (NCSS, Kaysville, Utah) was used to conduct Fisher's exact test (two-tailed) with the level of significance set at a *P*-value of ≤0.05. For the calculation of confidence intervals (CI) the exact binominal approach was applied. The discriminatory index which describes the ability of a typing method to distinguish between unrelated strains was calculated using Simpson's index of diversity (Hunter and Gaston, 1988). The frequency distribution similarities of STs between different groups of isolates were estimated using the proportional similarity index (PSI) (Feinsinger et al., 1981).

## 3. Results

### 3.1. Typing

The ten target gene fragments of all 256 *C. coli* strains could be successfully amplified and sequenced in the multiplex approach. MLST of these isolates resulted in 67 different STs (Table 1). Thirty ST, containing 193 isolates (75.4%), have been previously defined and 37 were new, encompassing 63 isolates (24.6%). These new STs were submitted to the PubMLST database for number assignment (ST-4935 to ST-4971). Eleven of the new STs resulted from new allele sequences which are *aspA* 282 in ST-4965, *aspA* 283 in ST-4968, *glnA* 382 in ST-4967, *gltA* 320 in ST-4966, *gltA* 321 in ST-4971, *glyA* 417 in ST-4964, *glmM* (*pgm*) 539 in ST-4962 and ST-4970, *glmM* (*pgm*) 540 in ST-4969,

**Table 1**Summary of MLST data, *flaB* types, macrolide and quinolone resistance of *C. coli* isolates from pigs at slaughter collected during 2009.

| CC      | ST   | <i>flaB</i> type | Number of isolates | Macrolide resistant isolates | Quinolone resistant isolates |
|---------|------|------------------|--------------------|------------------------------|------------------------------|
| 828     | 828  | 1476             | 1                  | 0                            | 0                            |
|         | 830  | 1458             | 1                  | 0                            | 0                            |
|         | 854  | 13               | 4                  | 0                            | 2                            |
|         |      | 30               | 2                  | 1                            | 0                            |
|         |      | 34               | 1                  | 0                            | 0                            |
|         |      | 36               | 1                  | 0                            | 0                            |
|         |      | 267              | 1                  | 0                            | 1                            |
|         |      | 319              | 2                  | 0                            | 1                            |
|         |      | 500              | 3                  | 0                            | 2                            |
|         |      | 528              | 2                  | 0                            | 1                            |
|         |      | 915              | 4                  | 0                            | 1                            |
|         |      | 964              | 1                  | 0                            | 0                            |
|         |      | 1258             | 1                  | 0                            | 0                            |
|         |      | 1285             | 1                  | 0                            | 1                            |
|         |      | 1481             | 1                  | 0                            | 0                            |
|         | 890  | 17               | 1                  | 0                            | 1                            |
|         | 1016 | 500              | 7                  | 4                            | 5                            |
|         | 1055 | 1483             | 1                  | 0                            | 0                            |
|         | 1058 | 1488             | 1                  | 0                            | 0                            |
|         | 1061 | 310              | 1                  | 0                            | 1                            |
|         |      | 500              | 4                  | 0                            | 2                            |
|         | 1096 | 13               | 8                  | 0                            | 6                            |
|         |      | 17               | 2                  | 1                            | 2                            |
|         |      | 1486             | 1                  | 0                            | 0                            |
|         | 1100 | 660              | 1                  | 0                            | 0                            |
|         |      | 721              | 2                  | 0                            | 0                            |
|         | 1413 | 17               | 1                  | 0                            | 1                            |
|         |      | 66               | 1                  | 0                            | 1                            |
|         |      | 916              | 1                  | 0                            | 0                            |
|         | 1556 | 66               | 1                  | 0                            | 1                            |
|         |      | 1485             | 1                  | 0                            | 1                            |
|         | 1563 | 528              | 1                  | 0                            | 0                            |
|         | 1579 | 13               | 3                  | 0                            | 1                            |
|         |      | 17               | 1                  | 0                            | 1                            |
|         | 1624 | 267              | 1                  | 0                            | 0                            |
|         | 2139 | 177              | 1                  | 0                            | 0                            |
|         | 2715 | 310              | 3                  | 1                            | 0                            |
|         | 2733 | 13               | 1                  | 0                            | 0                            |
|         |      | 198              | 1                  | 0                            | 0                            |
|         | 3023 | 390              | 1                  | 1                            | 1                            |
|         |      | 500              | 4                  | 3                            | 3                            |
|         | 3336 | 36               | 2                  | 1                            | 0                            |
|         |      | 51               | 1                  | 0                            | 1                            |
|         |      | 198              | 1                  | 0                            | 0                            |
|         |      | 1262             | 1                  | 0                            | 0                            |
|         |      | 1458             | 2                  | 0                            | 2                            |
|         |      | 1475             | 1                  | 0                            | 0                            |
|         |      | 1484             | 1                  | 0                            | 0                            |
|         |      | 882              | 1                  | 0                            | 0                            |
|         | 3337 | 1458             | 1                  | 0                            | 1                            |
|         |      | 17               | 1                  | 0                            | 1                            |
| Unknown | 1049 | 310              | 48                 | 0                            | 3                            |
|         |      | 500              | 1                  | 0                            | 0                            |
|         |      | 1482             | 1                  | 0                            | 0                            |
|         | 1115 | 660              | 1                  | 0                            | 1                            |
|         | 1143 | 310              | 4                  | 1                            | 1                            |
|         |      | 721              | 1                  | 0                            | 1                            |
|         |      | 824              | 1                  | 0                            | 0                            |
|         | 1147 | 500              | 9                  | 1                            | 1                            |
|         | 1421 | 310              | 1                  | 0                            | 1                            |
|         | 1680 | 27               | 1                  | 0                            | 0                            |
|         |      | 500              | 1                  | 0                            | 0                            |
|         | 2737 | 500              | 2                  | 0                            | 0                            |
| Unknown | 2737 | 570              | 1                  | 0                            | 0                            |
|         | 3345 | 310              | 8                  | 3                            | 4                            |
|         |      | 500              | 18                 | 6                            | 5                            |
|         |      | 570              | 1                  | 0                            | 0                            |
|         |      | 660              | 1                  | 0                            | 0                            |
|         |      | 721              | 3                  | 0                            | 1                            |
|         |      | 1479             | 1                  | 0                            | 1                            |

Table 1 (Continued)

| CC | ST   | <i>flaB</i> type | Number of isolates | Macrolide resistant isolates | Quinolone resistant isolates |
|----|------|------------------|--------------------|------------------------------|------------------------------|
|    | 4935 | 17               | 1                  | 0                            | 1                            |
|    | 4936 | 310              | 1                  | 0                            | 1                            |
|    |      | 500              | 2                  | 0                            | 1                            |
|    |      | 660              | 2                  | 0                            | 1                            |
|    |      | 721              | 1                  | 0                            | 1                            |
|    |      | 1474             | 1                  | 0                            | 0                            |
|    | 4937 | 500              | 1                  | 0                            | 0                            |
|    | 4938 | 660              | 1                  | 0                            | 0                            |
|    | 4939 | 310              | 5                  | 0                            | 2                            |
|    |      | 500              | 2                  | 1                            | 2                            |
|    | 4940 | 310              | 2                  | 0                            | 0                            |
|    |      | 500              | 2                  | 0                            | 0                            |
|    |      | 1480             | 1                  | 0                            | 0                            |
|    | 4941 | 310              | 1                  | 0                            | 0                            |
|    |      | 500              | 1                  | 0                            | 1                            |
|    | 4942 | 1477             | 1                  | 0                            | 1                            |
|    | 4943 | 1478             | 1                  | 1                            | 0                            |
|    | 4944 | 500              | 1                  | 0                            | 0                            |
|    | 4945 | 310              | 1                  | 0                            | 1                            |
|    | 4946 | 500              | 2                  | 1                            | 0                            |
|    | 4947 | 500              | 2                  | 0                            | 0                            |
|    | 4948 | 500              | 1                  | 1                            | 1                            |
|    | 4949 | 13               | 1                  | 0                            | 0                            |
|    | 4950 | 1314             | 1                  | 0                            | 0                            |
|    | 4951 | 13               | 1                  | 0                            | 0                            |
|    | 4952 | 721              | 1                  | 0                            | 0                            |
|    | 4953 | 500              | 1                  | 0                            | 0                            |
|    | 4954 | 500              | 1                  | 0                            | 0                            |
|    | 4955 | 290              | 1                  | 0                            | 1                            |
|    | 4956 | 13               | 1                  | 0                            | 0                            |
|    | 4957 | 310              | 1                  | 0                            | 0                            |
|    | 4958 | 500              | 1                  | 0                            | 1                            |
|    | 4959 | 500              | 1                  | 0                            | 1                            |
|    | 4960 | 310              | 2                  | 0                            | 0                            |
|    | 4961 | 310              | 1                  | 0                            | 1                            |
|    |      | 824              | 1                  | 0                            | 1                            |
|    | 4962 | 13               | 5                  | 0                            | 2                            |
|    | 4963 | 500              | 2                  | 0                            | 0                            |
|    | 4964 | 500              | 1                  | 0                            | 0                            |
|    | 4965 | 1459             | 1                  | 0                            | 1                            |
|    | 4966 | 500              | 1                  | 0                            | 1                            |
|    | 4967 | 500              | 1                  | 1                            | 1                            |
|    | 4968 | 500              | 1                  | 0                            | 1                            |
|    | 4969 | 17               | 1                  | 0                            | 1                            |
|    | 4970 | 13               | 1                  | 0                            | 0                            |
|    | 4971 | 1471             | 1                  | 0                            | 1                            |

*tkt* 438 in ST-4961 and finally *tkt* 439 in ST-4963. The other 26 new STs resulted from new combinations of previously described alleles. The three most frequent STs in our study were ST-1049 ( $n = 50/19.5\%$ ), ST-3345 ( $n = 32/12.5\%$ ) and ST-854 ( $n = 24/9.3\%$ ). 38 STs were represented by single isolates. The discriminatory index was 0.93 for MLST. Twenty-two STs, encompassing 89 isolates (34.8%), belonged to CC828. The remaining 45 STs, containing 167 isolates (65.2%) were not part of any known clonal complex.

Analysis of the *flaB* sequences showed 46 different *flaB* types, 14 of them being novel (types 1458 and 1474–1486). Predominant types were 310 (30.9%), 500 (28.5%) and 13 (9.8%), whereas 29 types occurred only once. The discriminatory index of *flaB* typing was 0.81. To further increase the discriminatory ability MLST and *flaB* typing were combined and yielded 116 different genotypes for the 256 isolates (discriminatory index = 0.955). The five most common types accounted for 35.5% of the isolates while 82 types were only found once. Isolates representing ST-854

turned out to be rather heterogeneous regarding the high number of different *flaB* types (13), while ST-1049 was mainly associated with *flaB* type 310 (98% of isolates).

In order to check seasonal distribution of STs they were plotted against months (data not shown). Generally no close seasonal relation of STs could be found with two exceptions: ST-1049 was detected with significantly higher frequency during the six month period from April to September than during the other six months ( $P = 0.012$ ) and ST-3336 was found more frequently during the cold season (January–April and October–December) than from May to September ( $P = 0.01$ ).

### 3.2. Antibiotic resistance

In order to determine antibiotic resistances towards quinolones and macrolides, the partial *gyrA* and the 23S rRNA genes were sequenced and analysed, respectively. The point mutation C257T within the *gyrA* gene, which is

associated with quinolone resistance, was observed in 86 isolates (33.6%; 95% CI: 27.8–39.7%). The transition was found with significantly higher frequency in ST-1096 (72.7% resistant;  $P=0.008$ ), ST-3023 (80% resistant;  $P=0.045$ ) and ST-1016 (71.4% resistant;  $P=0.045$ ). ST-1049, on the other hand, was rarely associated with quinolone resistance (6% resistant;  $P=0$ ).

The point mutation A2075G in the 23S rRNA gene, which is mainly responsible for macrolide resistance, was present in 28 isolates (10.9%; 95% CI: 7.4–15.4%). Six isolates showed a double peak (A and G) at the mentioned site of the gene. They were further tested phenotypically to determine macrolide resistance. Minimal inhibitory concentrations (MIC) for erythromycin of these strains were 0.5 µg/ml, 1 µg/ml, 4 µg/ml (2 strains) and 8 µg/ml (2 strains). Thus these strains could be considered as macrolide sensitive using a breakpoint of erythromycin >16 µg/ml for resistant strains. Macrolide resistance was detected with significantly higher frequency in ST-3023 (80% resistant;  $P=0.0005$ ), ST-1016 (57.1% resistant;  $P=0.003$ ) and ST-3345 (28.1% resistant;  $P=0.003$ ). In contrast ST-1049 was not associated with macrolide resistance (0% resistant;  $P=0.002$ ).

Concerning antibiotic resistance to quinolones there was a higher proportion of resistant strains during the months September–January than during the other months.

### 3.3. Comparison with *C. coli* isolates from other sources

The data generated in this study was compared to that of other 99 Swiss pig isolates that were collected during 2001 within a cross-sectional study assessing antibiotic resistance (Regula et al., 2003) and genotyped in the work of Korczak et al. (2009). The frequency distribution of MLST genotypes between the two groups resulted in a PSI of 0.28. The majority of STs (57.7%) from the sample set 2001 were also represented in the sample set of 2009. At the strain level, the sample set of 2001 showed high overlap with 83.8% of the strains having a genotype equivalent in the sample set of 2009. In isolates collected during 2009 quinolone ( $P=0.005$ ) and macrolide ( $P=0.0001$ ) resistances were detected at a significantly lower frequency than in those of 2001 when 50.5% had been resistant to quinolones and 29.3% to macrolides.

The 256 isolates from pigs were further compared to 97 chicken isolates from 2008. They were collected and genotyped within the framework of the EU baseline study in 2008 investigating the prevalence of *Campylobacter* in broiler flocks (Wirz et al., 2010). The PSI between the two groups was 0.217. Half of the chicken STs (50.0%) were also found with the porcine *C. coli* from 2009 whereas only 24.7% of poultry strains were represented by a genotype equivalent in the porcine collection 2009. In chicken isolates the frequency of macrolide resistance was significantly lower (3.1% resistant;  $P=0.02$ ) than in pig isolates, whereas the difference of quinolone resistance was not significant (26.8% resistant;  $P=0.25$ ). Interestingly, MLST and *flaB* data of the three macrolide resistant chicken isolates from 2008 were identical with at least one pig isolate from 2009.

## 4. Discussion

A systematic study has been carried out on *C. coli* genotypes in Swiss fattening pigs using a multiplex approach including MLST, *flaB*-typing and genetic determination of antibiotic resistance towards quinolones and macrolides. The strains analysed were temporally and geographically representative for the Swiss pig population in 2009. A high diversity was observed among *C. coli* isolates in the Swiss pig population, containing 67 different STs within 256 analysed isolates. Substantial level of diversity in STs of *C. coli* strains from pigs using MLST has been described in several previous studies too (Thakur et al., 2006; Miller et al., 2006; Littrup et al., 2007; Sheppard et al., 2009). Besides diversity also a high number of new STs have been detected in our study indicating that the Swiss *C. coli* population in pigs is to some extent different from that seen in Denmark, United States or United Kingdom where corresponding data is available. A reason for that could be that the Swiss swine population is quite partitioned from other countries and direct exchange of microorganisms is therefore limited. The two most frequent STs in our study were ST-1049 (19.5%) and ST-3345 (12.5%), which have been rarely reported previously. A single isolate of ST-1049 was described for 1996 in a US chicken (Miller et al., 2005). However, isolates with these STs seem to be well adapted to pigs in Switzerland evidenced by their high prevalence in 2009. Other STs found in Swiss pigs were also observed in other countries. As an example, ST-854 also rather frequent in Swiss pigs was the most prominent in UK pigs and also found in the US and Denmark (Thakur et al., 2006; Miller et al., 2006; Littrup et al., 2007; Sheppard et al., 2009). This indicates that internationally spread *C. coli* strains exist and can be isolated at different times from different geographical locations. The only clonal complex found in our study was CC828, which has been previously described to be the predominant clonal complex of two found within *C. coli* isolates so far (Sheppard et al., 2009).

MLST showed a higher discriminatory power than *flaB* typing (0.93 and 0.81, respectively). As seen previously there was no linear relationship between the two independent typing approaches (Korczak et al., 2009; Wirz et al., 2010). Nevertheless, *flaB* typing was helpful to further differentiate strains with the same ST and combined with MLST it increased the discriminatory index. A combination of these two typing methods is useful for short term epidemiology and might also become a reliable possibility to further trace the source of campylobacteriosis in humans (Dingle et al., 2008; Korczak et al., 2009; Wirz et al., 2010).

Increasing resistance of *C. coli* strains towards macrolides and quinolones is a major public health concern, since these two antibiotic classes are used to treat severe and chronic *Campylobacter* infections in humans (Blaser and Engberg, 2008). Phenotypic resistance towards quinolones and macrolides is directly linked to specific point mutations in the *gyrA* and 23S rRNA gene, respectively (Alfredson and Korolik, 2007). The point mutation C257T in *gyrA* referring quinolone resistance was detected in one third of the Swiss porcine *C. coli* in 2009. This corresponds

to the European average, although higher quinolone resistance frequencies have been reported in Spain (~80%) and Italy (~60%) and lower levels in the Netherlands (~4%) and in Denmark (~10%) (European Food Safety Authority, 2010). The macrolide resistance level, based on the mutation A2075G in the 23S rRNA gene, of *C. coli* strains from Swiss pigs was 10.9%, one of the lowest in Europe. Higher proportions of macrolide resistant *C. coli* from pig were detected in Spain and Italy (~60%), Germany and France (~30%), the Netherlands (~20%) and similar proportions were found in Denmark (~10%) (European Food Safety Authority, 2010). Analysing the 23S rRNA gene fragment, six isolates showed a double peak at nucleotide position 2075. These samples were tested for erythromycin resistance by an MIC methodology. Interestingly, each isolate was phenotypically sensitive, using a breakpoint MIC of >16 µg/ml. Ladely et al. (2009) have shown that the MIC of *C. coli* for erythromycin is dependent on the number of copies of the 23S rRNA gene carrying the mutation. Strains with only two of the three copies with the mutation had a twofold decreased MIC compared to those strains having the specific mutation in all three copies. If samples show a double peak at the specific site of the sequence it is therefore advisable to phenotypically check strains for macrolide resistance by determining the MIC as well. Similar to the study of Wirz et al. (2010) in broiler we observed an association between certain STs and resistance towards macrolides and quinolones, respectively.

The sample set of *C. coli* isolates from pigs during 2009 showed substantial overlap of genotypes from a smaller sample set isolated eight years earlier. Far more than 80% of the strains genotyped from 2001 could be retrieved also in 2009. Nevertheless, there was a shift of genotype distribution within the pig population over this time period as reflected by a relatively low PSI. A reason for that could be that the sampling procedures were not the same. In the study of Regula et al. (2003), in totally 88 pig farms faecal samples were collected from the floor of pens containing market-weight finishing pigs. 20 samples of each farm were gathered and cultured pooling five samples on one plate (Regula et al., 2003). Pooling could have the disadvantage that several isolates with different STs have been cultured on the same plate whereas only one ST has been detected by sequencing. Since even individual pigs were shown to contain different genotypes this effect is even more pronounced when pooling faeces from different animals (Thakur and Gebreyes, 2010). In our study faecal samples were collected at the slaughterhouse from individual pigs and directly cultured on agar plates. The long time between the two studies might also have played a role resulting in a shift of genotype distribution. Finally, how much the change of the host background as a consequence of introducing a new breeding programme for Swiss pig producers from 2000 to 2002 also influences the genetics or strain adaptation of *C. coli* remains speculation.

Remarkable was the difference between the two years 2001 and 2009 concerning the percentage of macrolide and quinolone resistant *C. coli* from pig. There was a clear drop of antibiotic resistant *C. coli* during this time period.

Macrolide resistance came down from 50.3% to 33.6% and resistance towards quinolones dropped from 29.3% to 10.9%. This is most probably the result of the ban starting 1999 to use any antimicrobial growth promoters in Switzerland (Boerlin et al., 2001). Moreover, since in 2004 the usage of veterinary medical products including antibiotic agents in production animals has been regulated by law in Switzerland (Der Schweizerische Bundesrat, 2004). Thereby, veterinarians and farmers are legally responsible to document the handling of medical products and this reduces uncontrolled and inappropriate use antibiotics in farm animal production. The development of antimicrobial resistance of *Campylobacter* towards these two antibiotic classes based on their use in veterinary medicine has been documented (Endtz et al., 1991; Juntunen et al., 2011). The current study is a further example on the effectiveness of the controlled use of antibiotics on the occurrence and rate of resistant bacteria.

Comparing the MLST data of isolates to previously investigated chicken samples from 2008 showed that pig and chicken shared some STs of *C. coli* as previously reported (Littrup et al., 2007). There was, however, clearly less genetic overlap between the porcine and poultry groups than between the two pig populations 2001 and 2009. Only about a quarter of the Swiss poultry strains could also be found in the Swiss porcine sample set from 2009. Since the two *C. coli* sample sets were collected in close temporal proximity this difference in genetic populations cannot be attributed to time but reflect genuine differences between *C. coli* populations from Swiss pig and poultry. This also holds true concerning antibiotic resistance towards macrolides which are higher in pig isolates from 2009 than in poultry *C. coli* from 2008, whereas the difference in antibiotic resistance towards quinolones was less pronounced. Enrofloxacin, a fluoroquinolone, is an often and legally used antibiotic for therapeutic treatment of animals, and the one predominantly used in Swiss poultry production. Tylosin, a macrolide, is often applied for therapy in pig but not in poultry production. Thereby the antibiotic resistance reflects the use of these two classes in pig and poultry ([www.swissmedic.ch/archvet-e.asp](http://www.swissmedic.ch/archvet-e.asp)).

In conclusion, *C. coli* from pigs in Switzerland showed a high genetic diversity with more than half of them being novel genotypes. Genotype distribution within the Swiss pig population might be changing over time and *C. coli* genotypes from pig and poultry show limited overlap. Antibiotic resistance of *C. coli* in Swiss pigs towards macrolides and quinolones significantly decreased since their ban as antimicrobial growth promoters and there are also differences in antibiotic resistances between porcine and poultry *C. coli* reflecting the clinical use of corresponding antibiotics. Results indicate that whereas exchange of *C. coli* strains between pig and poultry is possible this might not be occurring on a frequent basis. Further investigations on the role of pig as a direct or indirect source of human infection should also include human *C. coli* isolates. However, these should be collected in a temporally and geographically close context to strains from other sources.



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