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Whole genome sequence analysis of antimicrobial resistance genes, multilocus sequence types and plasmid sequences in ESBL/AmpC *Escherichia coli* isolated from broiler caecum and meat

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Abstract

Plasmid-encoded extended-spectrum β-lactamase and AmpC gene-carrying *Escherichia coli* (ESBL/AmpC *E. coli*) is an increasing cause of human infections worldwide. Increasing carbapenem and colistin resistance further complicate treatment of these infections. The aim of this study was to assess the occurrence of ESBL/AmpC *E. coli* in different broiler flocks and farms, as well as in broiler meat, in a country with no antimicrobial usage in broiler production. An additional goal was to assess the genetic characteristics of ESBL/AmpC *E. coli* isolates by using whole genome sequencing (WGS). Altogether 520 caecal swabs and 85 vacuum-packed broiler meat samples were investigated at the slaughterhouse level. WGS of the bacterial isolates revealed acquired antimicrobial resistance (AMR) genes, multilocus sequence types (MLST) and plasmid sequences. ESBL/AmpC *E. coli* was identified in 92 (18%) of the caecum and 27 (32%) of the meat samples. ESBL/AmpC *E. coli*-carrying birds derived from six (33%) out of 18 farms. Of the two $bla_{\text{ESBL/AmpC}}$ genes detected by PCR, $bla_{\text{CMY-2}}$ (96%) was predominant over $bla_{\text{CTX-M-1}}$ (4%). Furthermore, WGS revealed an additional AMR gene *sul2*. Carbapenemase, colistin, and other AMR genes were not detected from the isolates of either the caecal or meat samples. Altogether seven MLSTs (ST101, ST117, ST212, ST351, ST373, ST1594 and an unknown ST) and a variety of different plasmid sequences (IncB/O/K/Z, IncI1, IncFII, IncII, IncFIB, IncFIC, IncX1 and an additional set of Col-plasmids) were detected. This is the first study on genomic epidemiology of ESBL/AmpC *E. coli* on broiler farms and flocks with no antimicrobial usage, by using WGS analysis. Results show that ESBL/AmpC *E. coli* occurrence is common both in the ceacum and in the packaged meat. However, compared to other European countries, the occurrence is low and the presence of AMR genes other than $bla_{\text{CMY-2}}$ and $bla_{\text{CTX-M-1}}$ is rare. More studies are needed to understand the ESBL/AmpC *E. coli* occurrence in broiler production to prevent the meat from contamination during slaughter and processing, thereby also preventing zoonotic transmission of ESBL/AmpC *E. coli.* Additionally, more studies are needed to understand the ecology and fitness cost of Enterobacteriaceae plasmids in animal production in order to prevent their acquisition of plasmid-encoded antimicrobial resistance genes such as carbapenem and colistin resistance genes, as this would pose a great hazard to food safety.

Keywords

ESBL, AmpC*, Escherichia coli*, meat, whole genome sequencing, antimicrobial resistance, plasmid, MLST, poultry

1. Introduction

Extended-spectrum β-lactamase and AmpC gene-carrying *Escherichia coli* (ESBL/AmpC *E. coli*) is a major infectious agent and an increasing concern for public health (Pitout and Laupland, 2008). These plasmid-encoded enzymes cause a wide spectrum of β-lactam resistance, including 3rd generation cephalosporins, which limit the possibility of antimicrobial treatment (Pfeifer et al., 2010). Additionally, increasing carbapenem and plasmid-mediated colistin resistance further complicate treatment of these infections (Liu et al., 2016; van Duin and Doi, 2017; Woodford et al., 2014).

The zoonotic risk of ESBL/AmpC *E. coli* has been acknowledged, but the extent of transmission between food-producing animals and humans is an ongoing debate (EFSA, 2011; Lazarus et al., 2015). A possible transmission route of ESBL/AmpC *E. coli* is from food-producing animals to humans is via ingestion of contaminated meat (Lazarus et al., 2015). Among food-producing animals, poultry is considered to be the most important reservoir for ESBL/AmpC *E. coli* (EFSA, 2011). Indeed, ESBL/AmpC *E. coli* is a frequent contaminant in broiler meat worldwide (Borjesson et al., 2013; Casella et al., 2017; Kawamura et al., 2014; Kola et al., 2012; Leverstein-van Hall et al., 2011; Tansawai et al., 2018). It has been proposed that the high occurrence of ESBL/AmpC *E. coli* in broilers is due to the broiler production pyramidal structure, which allows effective spreading of these bacteria (Agerso et al., 2014; Dierikx et al., 2013a; Nilsson et al., 2014). In some European countries, the majority of broilers appear to carry not only ESBL/AmpC *E. coli*, but also carbapenemase-producing *E. coli* and plasmid-mediated *mcr-1* causing colistin resistance (EFSA, 2018; Hasman et al., 2015). Finland is an interesting country to study ESBL/AmpC *E. coli* in broilers because

antimicrobials have not been used for any broiler flocks since 2009. The occurrence of ESBL/AmpC *E. coli* in Finland is among the lowest reported in Europe (EFSA, 2018; Päivärinta, 2016).

The epidemiology of $bla_{ESBL/AmpC}$ genes is complex, since variety of different $bla_{ESBL/AmpC}$ gene families exist, carried by various Enterobacteriacea plasmids, and being able to transfer horizontally between bacterial strains and species. Furthermore, $bla_{ESBL/AmpC}$ genes and their plasmids may also persist in bacterial clones, transmitting within bacterial clones determined by bacterial sequence types (ST) (Berg et al., 2017; Borjesson et al., 2016; de Been et al., 2014; EFSA, 2011; Naseer and Sundsfjord, 2011; Seiffert et al., 2013). For example, in poultry, $bla_{CTX\text{-}M\text{-}1}$ is most often found to be carried by IncI1, IncI, IncFIB plasmids, whereas $bla_{CMY\text{-}2}$ is often carried by IncI, IncK, IncK2, IncA/C plasmids (Borjesson et al., 2016; Carattoli, 2009; Castellanos et al., 2017; Seiffert et al., 2013; Seiffert et al., 2017). In human infections, the most common clone is *E. coli* ST131 carrying $bla_{CTX\text{-}M\text{-}15,}$ often carried by IncFII or multireplicon plasmids that harbour additional FIA and FIB replicons (Naseer and Sundsfjord, 2011).

The aim of this study was to assess the occurrence of ESBL/AmpC *E. coli* in different broiler flocks and farms, as well as in broiler meat, in a country with no antimicrobial usage. An additional goal was to assess the genetic characteristics of ESBL/AmpC *E. coli* isolates by using whole genome sequencing (WGS). Phenotypic antimicrobial susceptibility of the isolates was tested, and ESBL/AmpC genes were screened with PCR. Finally, a set of isolates was subjected to whole genome sequencing in order to identify different antimicrobial resistance genes, STs and plasmid sequences. The possible genetic relatedness of ESBL/AmpC *E. coli* from different broiler farms and flocks and from different sources (i.e. caecum and packaged meat) was also studied.

2. Materials and methods

2.1 Materials

A total of 520 broiler caecal swab samples were collected on nine consecutive days from a Finnish high-capacity slaughterhouse in June 2015. The broilers were eviscerated and the samples were taken from the caecum with a dry swab, and subsequently stored in a tube containing media at 4°C (Amies Charcoal,

Copan Italia S.P.A., Brescia, Italy). Sampled broilers derived from 18 different farms and 52 flocks; from one to five (median 3) flocks per farm were analyzed. Ten broilers were randomly sampled from each flock. A flock consisted of broilers raised in the same hall; they were physically separated but received the same bedding, food and water as the other birds from the same farm. Careful biosecurity measures were followed by the farmers and visitors; hand washing and disinfection, as well as clothes and boot changing when entering and leaving the hall.

Additional samples were taken from 85 vacuum-packed raw broiler meat without marinade intended for consumer use, all from the same high-capacity slaughterhouse. The packages originated from 29 different broiler farms, including the farms and flocks from where the sampled broilers originated. Each package contained meat from only one farm. From one to seven packages (median 2) per farm were analyzed (Table 1). Meat packages were produced on eight different days; two to 21 (median 10) packages were analyzed from each day of production. The meat samples were deep-frozen and shipped on dry ice in isolated packages. At the laboratory, they were melted and analyzed upon arrival.

2.2 Isolation and identification of ESBL/AmpC- producing *Escherichia coli*

Caecal swab samples were enriched in 1 ml of Müller-Hinton broth (Oxoid, Basingstoke, Hampshire, UK) with cefotaxime (1 µg/ml) (USP, Rockville, MD, USA), and incubated at 37°C for 20-24 h. Meat samples (25 g) were enriched in buffered peptone water (225 ml), and incubated at 37°C for 18-22 h. A loopful (10 µl) of enrichment was cultivated on MacConkey agar (Oxoid Basingstoke, Hampshire, UK; Scharlau, Sentmenat, Barcelona, Spain) with cefotaxime (1 µg/ml), and incubated at 37°C for 20-24 h (caecal samples) and at 44°C for 18-22 h (meat samples). One typical lactose positive colony from each sample was further identified as *E. coli* with an Oxidase test (Oxoid, Basingstoke, Hampshire, UK), Gram-staining, and Api20E (Biomerieux, Marly l'Etoile, France).

A disk diffusion test was used to define susceptibility profiles according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards, and epidemiological cutoff values were used to determine the resistance (EUCAST, 2015). Susceptibility to 3rd generation cephalosporins was tested with

cefotaxime 5 µg (Oxoid, Basingstoke, Hampshire, UK), ceftriaxone 30 µg and ceftazidime 10 µg (Neo-Sensitabs; Rosco Diagnostica, A/S, Taastrup, Denmark). The isolates resistant to cefotaxime and/or ceftazidime were further tested with 2nd generation cephalosporin cephamysin (cefoxitin 30 µg) and 4th generation cephalosporin (cefepime 30 µg) (Neo-Sensitabs; Rosco Diagnostica, A/S, Taastrup, Denmark). In addition, isolates resistant to cefotaxime and/or ceftazidime were subjected to a combination disc diffusion test (Rosco Diagnostica, A/S, Taastrup, Denmark). Susceptibility of isolates to meropenem 10 µg was also tested to confirm carbapenem susceptibility.

In addition to 3rd generation cephalosporin resistance, the inhibitory effect of clavulanic acid ≥5 mm, either with cefotaxime or ceftazidime, was used as evidence for the production of ESBL enzyme. Resistance to cefoxitin was used as a criterion for AmpC production. The isolates resistant to 3rd generation cephalosporins with a positive combination disk diffusion test, and also resistant to both cefoxitin and cefepime, were regarded as possible producers of both ESBL and AmpC (ESBL/AmpC producer).

2.3 PCR and sequencing

The isolates that showed phenotypic evidence for producing ESBL and/or AmpC enzymes were further subjected to multiplex PCR according to their phenotypic profile, in order to detect plasmidic bla_{ESBL} and bla_{AmpC} gene families (Dallenne et al., 2010). Multiplex PCR 1 (bla_{TEM}, bla_{SHV}, bla_{OXA}) and multiplex PCR 2 ($bla_{CTX\text{-}M\text{-}Gp1}$, $bla_{CTX\text{-}M\text{-}Gp2}$, $bla_{CTX\text{-}M\text{-}Gp9}$) were used to screen for bla_{ESBL}. Multiplex PCR 3 (bla_{ACC}, bla_{FOX}, bla_{MOX}, bla_{CIT}, bla_{DHA}) was used to screen for bla_{AmpC} (Dallenne et al., 2010). PCR templates were prepared by mixing a loopful of bacteria in 100 µl RNA-free water (Sigma-Aldrich, St. Louis, MO, USA). The mixture was heated to +97°C for ten minutes and centrifuged for three minutes at 13000 rpm. The DyNAzyme II DNA polymerase (Thermo Scientific, Waltham MA, USA) and DyNAzyme Buffer (Thermo Scientific) were used in the PCR. PCR amplicons were visualized on a 2 % TAE-agarose gel with ethidium bromide and Alphaimager®. A representative collection of PCR amplicons (n=19) was selected for further sequencing to detect the gene subtypes.

2.4 Whole genome sequencing and sequence analyses

A random collection of ESBL/AmpC *E. coli* isolates (5 from caecum and 5 from meat) was selected for DNA extraction (Invitrogen, Thermo Fischer Scientific) and whole genome sequencing (WGS). Sequencing was performed by Illumina Miseq with paired end reads and 100X coverage. WGS analysis was performed using the open-access bioinformatic web-tools from www.genomicepidemiology.org for detection of acquired resistance genes (Zankari et al., 2012) and presence of plasmid sequences (Carattoli et al., 2014). Multilocus sequence typing was performed using web-tools from www.genomicepidemiology.org with *Escherichia coli*#1 scheme (Larsen et al., 2012; Wirth et al., 2006).

3. Results

3.1 ESBL/AmpC *Escherichia coli* in broiler caeca

Altogether 92 (18%) of the broiler caecal samples contained ESBL/AmpC producing *E. coli*, carrying $bla_{\text{ESBL/AmpC}}$ gene(s) detected by PCR (Table 1). Sequencing of the PCR products revealed that all isolates were representatives of two different $bla_{\text{ESBL/AmpC}}$ genes; $bla_{\text{CMY-2}}$ (88; 96%) and $bla_{\text{CTX-M-1}}$ (4; 4%).

3.2 ESBL/AmpC *Escherichia coli* in broiler meat

Altogether 27 (32%) of the broiler meat samples contained ESBL/AmpC producing *E. coli*, carrying $bla_{\text{ESBL/AmpC}}$ gene(s) detected by PCR. Sequencing of the PCR products revealed that all isolates were representatives of two different $bla_{\text{ESBL/AmpC}}$ genes; $bla_{\text{CMY-2}}$ (26; 96%) and $bla_{\text{CTX-M-1}}$ (1; 4%).

3.3 ESBL/AmpC *Escherichia coli* in broilers and broiler meat within different flocks and farms

At the farm level, six (33%) of the 18 farms were positive for ESBL/AmpC *E. coli* (Table 1). TÄHÄN TÄRKEIMMÄT TULOKSET TIIVISTETYSTI TAULUKOSTA 1. POISTA TURHA. Of these *E. coli*-positive farms, the number of ESBL/AmpC *E. coli*-positive flocks varied from one to four (25-100%, average 76%) and the number of ESBL/AmpC *E. coli*-carrying broilers varied from two to 32 (7-80 %, average 50%). The ESBL/AmpC *E. coli* occurrence in broilers was 60% or higher in four of the six *E. coli*-positive farms. ESBL/AmpC *E. coli*-carrying broilers were found from 14 (52%) of the 27 flocks. The number of ESBL/AmpC *E. coli*-carrying birds in these positive flocks ranged from two to nine (20-90%, average 66%).

ESBL/AmpC *E. coli* was detected in 27 (32%) of the 85 meat packages; these packages originated from 12 (41%) of the 29 farms (Table 1). From those 12 farms, the occurrence of ESBL/AmpC *E. coli*-positive meat samples varied from 17% to 100% (average 68%). ESBL/AmpC *E. coli* was found on six (75%) of the eight different dates of meat production. On those six days, the proportion of ESBL/AmpC *E. coli*-positive meat samples varied from 13% to 73% (average 40%).

Out of the 18 farms from which both caecum and meat samples were examined, four (22%) harboured ESBL/AmpC *E. coli* in both caecum and meat (Table 1). There were two farms (11%) from which ESBL/AmpC *E. coli* was detected in meat only, and two farms (11%) from which ESBL/AmpC *E. coli* was detected in caecum only. ESBL/AmpC *E. coli* was not detected in meat or caeca from eight (44%) of the farms.

3.4 Whole genome sequencing of ESBL/AmpC *E. coli* isolates

Bioinformatic analyses revealed, that seven phenotypically AmpC-producing isolates possessed bla_{CMY-2} with no other antimicrobial resistance genes (Table 2). Three phenotypically ESBL-producing isolates carried $bla_{CTX-M-1}$ and a sulphonamide resistance gene *sul2*. The results from sequence analyses were in line with the phenotypes and genes detected by PCR. No carbapenem, colistin or other antimicrobial resistance genes were detected. Of the caecal isolates, three different STs were found (ST212, ST1594, and an unknown ST). Of the meat isolates, four different ST types were found, which differed from those from the caecal isolates: ST101, ST117, ST351 and ST373. All of these isolates harboured sequences from two to seven different plasmids (Table 2).

4. Discussion

The current study shows that despite no antimicrobial usage, ESBL/AmpC *E. coli* occurrence is common in broiler caecum (18%) and broiler meat (32%). Moreover, ESBL/AmpC *E. coli* is commonly present in both broiler caecum and meat, indicating possible contamination during meat slaughter and processing. However, the bacterial whole genome sequencing analyses revealed the presence of various plasmid sequences and multiple *E. coli* ST types, which underlines the complex epidemiology of these bacteria.

Fortunately, no carbapenem, colistin or other acquired antimicrobial resistance genes were detected. Specifically, bla_{CMY-2} appears to be the most common ESBL/AmpC gene in Finnish broiler production, and its zoonotic risk needs to be considered. Additionally, our findings suggest that ESBL/AmpC *E. coli* occurrence may vary between different broiler farms without antimicrobial usage; this supports the hypothesis that farm level management can possibly affect the occurrence of ESBL/AmpC *E. coli.* Furthermore, ESBL/AmpC *E. coli* is frequently found in meat, indicating that slaughter and meat processing hygienic aspects need to be considered.

We found an 18% occurrence of ESBL/AmpC *E. coli* in Finnish broilers, in contrast to our previous study that found an occurrence of only 8% (Päivärinta, 2016). A similar increase has also been reported by the Finnish official monitoring program; the occurrence of ESBL/AmpC *E. coli* increased from 7% in 2014 to 14% in 2016 (EFSA, 2018; Evira, 2017). In spite of this increase, the occurrence of ESBL/AmpC *E. coli* in Finnish broilers is still among the lowest in the EU – in which variable levels of ESBL/AmpC *E. coli* prevalence are reported (EFSA, 2018). The increase in ESBL/AmpC *E. coli* occurrence in Finnish broilers is notable in light of the absence of antimicrobial consumption in production broilers. This may reflect the global increase in antimicrobial resistant bacteria, and indicate that ESBL/AmpC *E. coli* potentially has drivers other than antibiotic use.

The relatively low occurrence of ESBL/AmpC *E. coli* in Finnish slaughter broilers may be influenced by multiple factors. Firstly, antimicrobials have not been used for any broiler flocks in Finland since 2009, coccidiostats are the only antimicrobials that are used on these farms. Secondly, the majority of newborn chicks are treated with a competitive exclusion product; the usage of competitive exclusion has been shown to reduce colonization, excretion and transmission of ESBL/AmpC *E. coli* in broiler chicks (Ceccarelli et al., 2017; Nuotio et al., 2013). Thridly, the “all-in-all-out” principle is followed in each hall, and broiler halls are cleaned, dried and disinfected between flocks. Therefore, it is assumed that the strict biosecurity and all-in-all-out system, as well as using competitive exclusion and raising broilers without antimicrobials for years, may all contribute to the low occurrence of ESBL/AmpC *E. coli* in Finnish slaughter broilers.

It is noteworthy that the ESBL/AmpC *E. coli*-carrying birds originated from only six (33%) farms, whereas the other farms were negative. On the majority of the positive farms, all flocks were positive for ESBL/AmpC *E. coli*-carrying birds. In these flocks, from 60 to 80% of birds were positive. Thus, the occurrence of ESBL/AmpC *E. coli* was found to be more frequent on some farms, indicating possible farm- or flock-related factors at the farm level. Similar findings have been reported in other studies (Dame-Korevaar et al., 2017, Dierikx et al., 2013, Huijbers et al., 2016, Mo et al, 2016a). A longitudinal study concerning risk factors for a cephalosporin-resistant *E. coli* broiler flock has been conducted in Norway, where no antimicrobials were used (Mo et al., 2016a). The risk factors for ESBL/AmpC *E. coli* included positive status of previous flocks (most important), number of parent flocks supplying the broiler flock, transportation personnel entering the broiler house, and the lack of disinfection (Mo et al., 2016a).). Further studies are needed to explore the factors affecting the occurrence of ESBL/AmpC *E. coli* at the farm level in order to reduce the number of ESBL/AmpC *E. coli*-positive birds entering the slaughterhouse.

ESBL/AmpC *E. coli* is frequently found from broiler meat; we found an overall occurrence of 32%. In 2016, Finnish reports revealed an occurrence of 22%, which is among the lowest in European countries (EFSA, 2018). Interestingly, the occurrence of ESBL/AmpC *E. coli* in this study is higher in meat than in caecal samples. It should be noted that the enrichment method differed between caecal and meat samples in our study. Nevertheless, this finding agrees with EFSA's report which also revealed a higher occurrence of ESBL/AmpC *E. coli* in broiler meat than caecum (57% vs 47%) (EFSA, 2018). This general trend differs among European countries: in some countries the occurrence of ESBL/AmpC *E. coli* is lower in meat as compared to caecal samples (EFSA, 2018). More studies are needed to investigate the hygienic procedures that may reduce/prevent contamination of meat during slaughter and meat processing. At the slaughterhouse level, significant differences between slaughterhouses have been observed in terms of the effectiveness of hygienic procedures in ESBL/AmpC *E. coli* reduction of fecal contamination. Hence, improvements could be attainable (Pacholewicz et al., 2015; Reich et al., 2016). We found that from two farms, ESBL/AmpC *E. coli* was found from meat packages even when the broiler (caecal) samples from those farms were negative. During broiler slaughter processes, cross-contamination is known to occur, especially during defeathering

(Belluco et al., 2015). In addition, surface samples from both dirty and clean sides of the broiler slaughter line have been shown to be positive for ESBL/AmpC *E. coli*, which may cause contamination of the incoming carcasses (Gregova et al., 2012). Cross-contamination can also occur easily during cutting and packaging of meat. As the current study employed sample enrichment, the quantity of ESBL/AmpC *E. coli* may not be concluded. In other studies, the number of ESBL/AmpC *E. coli* have been shown to reduce significantly during the slaughter process, and are in line with generic *E. coli* (Pacholewicz et al., 2015). Thus, generic *E. coli* concentrations on carcasses may be used to implement control strategies for ESBL/AmpC *E. coli.* The effect of slaughter operations on indicator bacteria – including *E. coli* – has been thoroughly studied, and it has been shown that scalding in particular effectively lowers the number of *E. coli* on carcasses (Belluco et al., 2015). EFSA has highlighted the flock-based risk categorization in food chain information, and the classification of slaughterhouses based on their capability to reduce carcass–fecal contamination (EFSA, 2012). It should be noted that pets eating raw broiler meat may be an important route to humans and should therefore be taken into careful consideration (Baede et al., 2017).

Whole genome sequencing (WGS) of the isolates revealed two caecal isolates with similar MLST types (ST1594), which came from the same farm but two different flocks. Both of these isolates carried $bla_{\text{CTX-M-1}}$ and *sul*2 resistance genes and plasmids Col 156, ColpVC, IncFIB, IncFII and IncI1. Another farm also had *E. coli* ST1594 but carried a different resistance gene, $bla_{\text{CMY-2}}$, and a different plasmid profile ColpVC and IncB/O/K/Z. These findings indicate that both clonal transfer of $bla_{\text{CTX-M-1}}$-carrying *E. coli* ST1594 as well as horizontal transfer of $bla_{\text{CMY-2}}$ plasmids between different *E. coli* ST-types may occur. ST1594 has previously been found to carry $bla_{\text{CMY-2}}$ from broilers in Denmark and from broiler meat in Sweden (Agerso et al., 2014; Borjesson et al., 2013). In a recent Swedish study, it was shown that without antimicrobial selection pressure, certain *E. coli* lineages (ST38) carrying $bla_{\text{CMY-2}}$ were able to survive (Myrenas et al., 2018). A recent study from the Netherlands showed that on a test farm without antimicrobial selective pressure, *E. coli* carrying the plasmid IncA/C vanished from birds of the parental generation (Dame-Korevaar et al., 2017). In a Norwegian study, plasmid stability systems in broiler meat isolates were detected with the IncK plasmid containing $bla_{\text{CMY-2}}$, hypothesized to cause the persistence and clonal spread of *E. coli* ST38 (Mo et

al., 2016b). Some lineages and plasmids likely have other fitness properties since ESBL/AmpC-carrying plasmids survive without antimicrobial selective pressure. This may enable some lineages and plasmids to persist at farms without antimicrobial usage.

5. Conclusion

Our results show that despite no antimicrobial usage, ESBL/AmpC E. *coli* occurrence is common (18%) in broilers in Finland. Moreover, ESBL/AmpC E. *coli* is commonly found from broiler meat (32%). However, compared to other European countries, the occurrence is low (EFSA 2018) and the presence of AMR genes other than bla_{CMY-2} and $bla_{CTX-M-1}$ is rare; no carbapenem, colistin or other acquired antimicrobial resistance genes are found. The presence of various plasmid sequences and multiple *E. coli* ST types underlines the complex epidemiology of these bacteria. More studies are needed to understand the transmission and persistence of these acquired AMR genes and plasmids in different *E. coli* ST types. Both reducing the ESBL/AmpC *E. coli* occurrence on the farm level and preventing meat contamination during slaughter and meat processing are important factors for further studies in order to diminish ESBL/AmpC *E. coli* spreading in the food chain with possible zoonotic risk. In addition to farm level, more studies are needed throughout the broiler production chain to investigate the transmission routes of ESBL/AmpC *E. coli*.

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Table 1. Presence of ESBL/AmpC-producing *Escherichia coli* in broiler caeca and meat.

Farm	No. of broilers studied per farm	No. of broilers positive for ESBL/AmpC *E. coli* on a farm (%)	No. of flocks studied per farm	No. of flocks positive for ESBL/AmpC *E. coli* on a farm (%)	No. of meat packages studied per farm	No. of meat samples positive for ESBL/AmpC *E. coli* per farm (%)
1	20	0 (0)	2	0 (0)	6	0 (0)
2	10	0 (0)	1	0 (0)	1	0(0)
3	50	0 (0)	5	0 (0)	-[a]	-
4	30	18 (60)	3	3 (100)	2	2(100)
5	10	0 (0)	1	0 (0)	1	0 (0)
6	20	0 (0)	2	0 (0)	3	0 (0)
7	20	14 (70)	2	2 (100)	4	4 (100)
8	30	0 (0)	3	0 (0)	1	0 (0)
9	20	0 (0)	2	0 (0)	1	0 (0)
10	30	0 (0)	3	0 (0)	2	2 (100)
11	40	0 (0)	4	0 (0)	-	-
12	30	0 (0)	3	0 (0)	1	0 (0)
13	30	2 (7)	3	1 (33)	3	0 (0)
14	30	18 (60)	3	3 (100)	1	0 (0)
15[b]	40	32 (80)	4	4 (100)	2	1 (50)
16	20	0 (0)	2	0 (0)	7	3 (43)
17	40	8 (20)	4	1 (25)	2	1 (50)
18	50	0 (0)	5	0 (0)	4	0 (0)
19	-	-	-	-	6	4 (67)
20	-	-	-	-	4	3 (75)
21[c]	-	-	-	-	6	3 (50)
22	-	-	-	-	3	2 (67)
23	-	-	-	-	6	1 (17)
24	-	-	-	-	1	0 (0)
25	-	-	-	-	3	0 (0)
26	-	-	-	-	1	1 (100)
27	-	-	-	-	3	0 (0)
28	-	-	-	-	6	0 (0)
29	-	-	-	-	2	0 (0)
30	-	-	-	-	1	0 (0)
31	-	-	-	-	2	0 (0)
All (%)	520	92 (18)	52	14 (27)	85	27 (32)

[a] Not determined.

[b] In addition to bla_{Cmy-2}-carrying *E. coli*, the farm was also positive for $bla_{CTX-M-1}$-carrying *E. coli*. Those $bla_{CTX-M-1}$-carrying *E. coli* isolates (n=4) were found from flocks 1 and 3.

[c] In addition to bla_{Cmy-2}-carrying *E. coli*, one meat package from this farm was positive for $bla_{CTX-M-1}$ *E. coli*.

Table.

Isolate[a]	Origin	Resistotype	β-lactamase gene[b]	Other AMR genes(s)[c]	Plasmids[d]											MLST[e]
					Col8282	Col156	ColpVC	Col(MG8282)	IncB/O/K/Z	IncFIB	IncI1	IncII(pHN7A8)	IncFII	IncFIC(FII)	IncX1	
5	Meat	AmpC	*bla*$_{CMY-2}$	None	+	+	+	-	+	-	-	-	-	-	-	ST373
33	Meat	AmpC	*bla*$_{CMY-2}$	None	+	+	+	-	+	-	-	-	-	-	-	ST373
34	Meat	AmpC	*bla*$_{CMY-2}$	None	-	+	-	+	+	+	+	+	+	-	-	ST117
47	Meat	AmpC	*bla*$_{CMY-2}$	None	-	-	-	-	-	+	+	-	-	+	-	ST101
53	Meat	ESBL	*bla*$_{CTX-M-1}$	*sul2*	-	-	-	-	-	+	+	-	+	-	+	ST351
A12	Caecum	ESBL	*bla*$_{CTX-M-1}$	*sul2*	+	-	+	-	-	+	+	-	+	-	-	ST1594
C15	Caecum	AmpC	*bla*$_{CMY-2}$	None	-	-	+	-	+	-	-	-	-	-	-	ST1594
M11	Caecum	AmpC	*bla*$_{CMY-2}$	None	-	-	-	-	-	+	+	-	+	-	-	ST unknown
Q11	Caecum	ESBL	*bla*$_{CTX-M-1}$	*sul2*	+	-	+	-	-	+	+	-	+	-	-	ST1594
W20	Caecum	AmpC	*bla*$_{CMY-2}$	None	-	-	-	-	+	+	-	-	+	-	-	ST212

[a] Isolates were sequenced using Illumina Hiseq, paired end reads with 100 X coverage.

[b] Phenotype was defined by using EUCAST cutoff values (EUCAST, 2015). Genotype was defined by using PCR with small modifications and sequencing (Dallenne et al., 2010).

[c] Presence of *bla*$_{ESBL/AmpC}$ genes was determined from whole genome sequences; raw sequence reads were analyzed using ResFinder with 95% treshold for identification (Zankari et al., 2012). All results revealed 100% homology matching to the database.

[c] AMR; antimicrobial resistance. Presence of antimicrobial resistance genes was determined from whole genome sequences; raw sequence reads were analyzed using ResFinder with 95% threshold for identification (Zankari et al., 2012). All results revealed 100% homology matching to the database.

[d] %ID; percent of nucleotide identity matching to the database containing replicon sequences. Presence of Enterobacteriaceae plasmids was determined from whole genome sequences; raw sequence reads were analyzed using PlasmidFinder (Carattoli et al., 2014). Replicon sequence nucleotide identity from 95% to 100% were included.

[e] Multi Locus Sequence Type of *Escherichia coli* was determined from whole genome sequences; raw sequence reads were analyzed using Multi Locus Sequence Typer 2.0 with *Escherichia coli#1* as a reference (Larsen et al., 2012).

Highlights

- ESBL/AmpC *E. coli* common in broiler meat even in production without antimicrobials
- WGS reveals complex epidemiology of ESBL/AmpC plasmids and *E. coli* ST types
- To reduce ESBL/AmpC *E. coli* on farms and to preventmeat contamination is important