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# Contamination of chicken meat with extended-spectrum beta-lactamase producing- *Klebsiella pneumoniae* and *Escherichia coli* during scalding and defeathering of broiler carcasses

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ABSTRACT

Extended-spectrum beta-lactamase- (ESBL-) producing *Klebsiella (K.) pneumoniae* and *Escherichia (E.) coli* are of critical importance in human and veterinary medicine. Animal food products, especially broiler chickens, are discussed as a possible source for the exposure of humans with antibiotic resistant bacteria. Although the occurrence and vertical transmission of ESBL-/AmpC-producing Enterobacteriaceae in the broiler production has been reported before, detailed investigations concerning the dissemination along the slaughter processing line are missing.

In this study, we investigated cross-contamination with ESBL-producing Enterobacteriaceae during the processing of two different broiler flocks in one slaughterhouse. The ESBL-status during the fattening period of the flocks was determined and environmental samples from the slaughterhouse were taken before processing of the respective flocks. These isolates were compared to those found in samples from the carcasses after processing using whole genome sequencing. Phylogenetic analyses of seven ESBL-producing *K. pneumoniae* and 14 E. *coli* revealed close relationships between isolates from scalding water and the defeathering machine, respectively, which were collected before the processing of the broiler flocks, to those isolates found in samples from skin and filet of the respective flock carcasses. In conclusion, using high resolution molecular data we found evidence for the cross-contamination of carcasses with ESBL-producing Enterobacteriaceae during scalding and defeathering in the slaughterhouse.

## 1. Introduction

Bacterial resistances against beta-lactam antibiotics, especially 3rd generation cephalosporins, are of major concern in Public Health due to limitations in the treatment of infections (Pitout and Laupland, 2008). Extended-spectrum beta-lactamase- (ESBL-) producing Enterobacteriaceae are frequently detected in humans (Coque et al., 2008), animals (farm, wild and companion animals) (Ewers et al., 2012; Guenther et al., 2017; Rubin and Pitout, 2014) as well as in environmental settings (surface, hospital and wastewater) (Franz et al., 2015; Jorgensen et al., 2017). Among these, particularly high numbers of ESBL-producing Enterobacteriaceae are reported for broiler chickens (Bortolaia et al., 2010; Randall et al., 2011; Zogg et al., 2016). It was found that ESBL-producers can spread along the broiler production chain (Dierikx et al., 2013; Projahn et al., 2017) and that broiler (breeder) chickens get colonized even without antibiotic treatment (Daehre et al., 2018; Huijbers et al., 2016; Mo et al., 2014; Projahn et al., 2018). There are also a variety of reports on the contamination of chicken meat with ESBL-producing Enterobacteriaceae (Borjesson et al., 2013; Cohen Stuart et al., 2012; Egea et al., 2012; Kim et al., 2005; Kola et al., 2012); however, there are only limited studies which investigated the occurrence of ESBL-producers during the processing of broiler chickens in the slaughterhouse (Pacholewicz et al., 2015; Reich et al., 2016; von Tippelskirch et al., 2018). A high variability of ESBL-producing Enterobacteriaceae in the slaughterhouses and changing compositions of these resistant bacteria along the production chain has

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**Table 1**
*K. pneumoniae* and *E. coli* isolates derived from samples before and during processing of broiler flocks F and G, respectively, selected for next generation sequencing from the original study.

| Isolate | Sampling | Sample | Species, Phylogroup | MLST | ESBL gene | Flock |
|---|---|---|---|---|---|---|
| 10143 | Defeathering machine 2 | Swab sample | *K. pneumoniae* | ST-2762 | $bla_{SHV-2}$ | F |
| 10144 | Defeathering machine 2 | Swab sample | | | | |
| 10145 | Skin sample 1 | 25 g breast skin | | | | |
| 10146 | Skin sample 1 | 25 g breast skin | | | | |
| 10147 | Skin sample 2 | 25 g breast skin | | | | |
| 10148 | Skin sample 2 | 25 g breast skin | | | | |
| 10150 | Defeathering machine 1[a] | Swab sample | | | | |
| 10151 | Defeathering machine 2 | Swab sample | *E. coli*, A/C | ST-361 | $bla_{CTX-M-15}$ | G |
| 10152 | Defeathering machine 2 | Swab sample | | | | |
| 10153 | Skin sample 1 | 25 g breast skin | | | | |
| 10154 | Skin sample 1 | 25 g breast skin | | | | |
| 10155 | Skin sample 2 | 25 g breast skin | | | | |
| 10156 | Skin sample 2 | 25 g breast skin | | | | |
| 10157 | Fillet sample 1 | 25 g fillet sample | | | | |
| 10158 | Fillet sample 1 | 25 g fillet sample | | | | |
| 10159 | Fillet sample 2 | 25 g fillet sample | | | | |
| 10160 | Fillet sample 2 | 25 g fillet sample | | | | |
| 10161 | Scalding water 1[a] | 10 ml water | | | | |
| 10162 | Scalding water 1[a] | 10 ml water | | | | |
| 10163 | Defeathering machine 1[a] | Swab sample | | | | |
| 10164 | Defeathering machine 1[a] | Swab sample | | | | |

[a] Strains were isolated from samples which were taken before the processing of the respective broiler fattening flock.

been demonstrated. However, detailed molecular investigations using high resolution typing techniques like next generation sequencing on possible sources for the cross-contamination of chicken carcasses with ESBL-producing Enterobacteriaceae during processing were not reported. Knowledge on these contamination sources is an important factor for the development and application of intervention measures against the spread of ESBL-producers during processing in the slaughterhouse. Furthermore, the dissemination of these resistant bacteria into the environment needs to be prevented as they also contribute to transmissions of antibiotic resistance characteristics (Egervarn et al., 2017; Mahfouz et al., 2018; Martin et al., 2017; Njage and Buys, 2015; Smet et al., 2011).

In our pilot scale study, we investigated ESBL-producing *K. pneumoniae* and ESBL-producing *E. coli* strains concerning possible cross-contamination events during the processing of two different broiler flocks (flocks F and G) in the same slaughterhouse between September 2015 and March 2016 (von Tippelskirch et al., 2018).

*K. pneumoniae* isolates were derived from the slaughterhouse environment, from chicken carcasses and retail meat, respectively, of flock F which was negative for ESBL-producing *K. pneumoniae* during the fattening period (Daehre et al., 2018). ESBL-producing *E. coli* isolates were collected during the processing of flock G which was previously tested ESBL free during the whole fattening period (Daehre et al., 2018). A whole genome sequencing based bioinformatic approach was applied on selected ESBL-producing isolates and the phylogenetic relationship between strains from the slaughterhouse environment (before and during processing of the respective flock) as well as strains from chicken carcasses and retail meat was determined.

## 2. Material and methods

### 2.1. Sampling

Two different German broiler chicken flocks were investigated in September 2015 (flock F) and March 2016 (flock G) (von Tippelskirch et al., 2018) concerning cross-contamination with ESBL-producing Enterobacteriaceae during processing in one slaughter house. Both flocks were first sampled on the respective fattening farms using 40 cloacal swabs one day prior to transportation to the slaughter facility to determine their current ESBL-status as published previously (Daehre et al., 2018).

Samplings at the slaughterhouse comprised for each flock 25 carcasses and the corresponding bundles of internal organs which were taken in parallel after the evisceration at the point if the official meat inspection as well as 25 filet samples collected 1–2 h after slaughter. All samples were collected into sterile bags. In addition, various environmental samples were taken directly prior to the processing of flock F and G, respectively, and also during their processing. Both flocks were not the first ones slaughtered at the respective day. Environmental samples comprised the scalding water as well as environmental swabs (Copan, Brescia, Italy) from the washer, the hooks of the carcasses and the hooks of the internal organs in the evisceration room and from the defeathering machine as well as the transport crates after cleaning and disinfection in the slaughter room. Environmental swabs were taken just prior to the sampled flock (one sample each, except transport crates) and during processing of the respective flocks (two samples each).

### 2.2. Bacterial strain isolation

Ten ml of the scalding water, 5 g of the cecum content as well as 25 g of breast skin and fillet, respectively, were used for further analyses as already described (von Tippelskirch et al., 2018). In brief, samples were diluted 1:10 in with Luria-Bertani broth (LB) (Merck) and mixed in a stomacher (BagMixer 400, Interscience, St Nom la Bretèche, France). Quantitative and qualitative analyses concerning ESBL-producing Enterobacteriaceae were done using MacConkey Agar No.3 (Oxoid, Wesel, Germany) containing 1 mg/L cefotaxime (AppliChem, Darmstadt, Germany). From each sample up to 10 suspicious colonies (two colonies of each morphology type) were selected for further analyses. Molecular species confirmation was done using MALDI-TOF MS (Microflex LT and Biotyper database, Bruker Daltonics, Bremen, Germany), phylogroup Multiplex-PCR in case of *E. coli* with modifications according to Projahn et al. (2017), real-time Multiplex PCR for the determination of predominant beta-lactamase genes (Roschanski et al., 2014) as well as sequencing (Projahn et al., 2017).

Isolates for further whole genome sequence analysis (Table 1) were selected from the whole strain collection of the original study (von Tippelskirch et al., 2018) based on their ESBL resistance conferring gene and in case of *E. coli* also based on their phylogroup (same resistance gene, same phylogroup). If possible, of each positive sample type two samples with two isolates each (intra-sample variability) were

chosen. Whole genome sequence based phylogenetic analyses was performed for seven ESBL-producing *K. pneumoniae* (flock F) and 14 E. *coli* strains (flock G), respectively, which were isolated from broiler carcass samples (skin, filet) and environmental samples from the slaughterhouse (scalding water, swab samples from the defeathering machine) collected before and during the processing of the two different broiler flocks (Table 1).

#### 2.2.1. ESBL-K. pneumoniae transmission (flock F)

For the investigation of cross-contaminations during processing, seven *K. pneumoniae* isolates were selected from the strain collection of the original study of which one was derived from a swab sample of the defeathering machine, which was taken before the processing of flock F. The other isolates were derived from the defeathering machine and skin samples after the processing of flock F (two isolates each, Table 1). All *K. pneumoniae* isolates harbored the ESBL gene $bla_{SHV-2}$ (Table 1). Previous investigations of broiler chickens of flock F during the whole fattening period using cloacal swabs revealed no colonization with ESBL-producing *K. pneumoniae* strains (Daehre et al., 2018).

#### 2.2.2. ESBL-E. coli transmission (flock G)

From the flock G investigations, 14 E. *coli* isolates of phylogroup A/C harboring a $bla_{CTX-M-15}$ gene were selected from seven different samples (two isolates each), of which one scalding water sample and one swab sample of the defeathering machine were taken before the processing of flock G. The other isolates were derived from the defeathering machine, skin and filet samples after the processing of flock G (Table 1). Broiler chickens of this flock were previously tested ESBL-negative three times during the whole fattening period using cloacal swabs [15].

### 2.3. Whole genome sequencing

Whole genomic DNA was isolated using the MasterPure™ DNA purification kit (Epicentre, Illumina) according to the manufacturer's instructions. Sequencing was performed using Illumina MiSeq paired-end sequencing (GATC, Konstanz, Germany). After quality control using the NGS QC toolkit (Patel and Jain, 2012)the high-quality reads were assembled *de novo* into contiguous sequences using the CLC Genomics Workbench 8.0 (CLC bio, Aarhus, Denmark).

### 2.4. Phylogenetic analyses

*In silico* multi-locus sequence typing (MLST) based on the contiguous whole genomic sequences was performed by the CGE Bacterial Analysis Pipeline (Thomsen et al., 2016). Furthermore, a "core-genome" including all common sequences (housekeeping genes, accessory and non-coding sequences) of the seven *K. pneumoniae* and the 14 E. *coli* isolates, respectively, was calculated using the multi-aligner program parsnp (Harvest suite 1.0)(Treangen et al., 2014). Phylogenetic trees were constructed using the neighbor-joining algorithm (1000 bootstraps) based on the number of SNP differences determined by MEGA 7.0 (Kumar et al., 2016).

### 2.5. Virulence-associated genes

Whole genome contigs of the seven *K. pneumoniae* strains were analyzed concerning the presence of the following virulence-associated genes (VAGs) using an automated in-house BLAST algorithm (parameters: > 90% identity, > 90% coverage): *allS, cnf-1, entB, fimH, hly, iroN, irp-1/-2, iutA, k2A, kpn, magA, mrkD, rpmA, uge, wabG, wcaG, ycfM, ybtS, fyuA, traT, wzi* and the *cps* gene cluster. Presence of genes and identity were cross-checked using the NCBI BLASTn suite (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

All *E. coli* isolates were checked for the presence of 89 VAGs which comprised genes that are considered to be associated with the adhesion of bacteria (n = 34), invasion (n = 4), iron uptake (n = 12), protection (n = 6), toxins (n = 12) and other functions (n = 2) (extraintestinal pathogenicity - ExVAGs) as well as 19 genes which are associated with an intestinal pathogenicity (InVAGs) of *E. coli* strains (Suppl. Table).

## 3. Results

Both flocks F and G were negative concerning ESBL-producing Enterobacteriaceae using 40 cloacal swabs, respectively, just prior to transportation to the slaughterhouse facility as published by Daehre et al. (2018). Samplings in the slaughterhouse before and during processing of these flocks revealed 42% (37/88) ESBL-positive samples for flock F and 31,8% (28/88) for flock G as described by von Tippelkirch et al. (2018). From the positive samples seven ESBL-*K. pneumoniae* isolates from flock F and 14 ESBL-*E. coli* isolates from flock G were selected for whole genome analyses to investigate possible cross-contamination during the processing.

### 3.1. ESBL-K. pneumoniae transmission

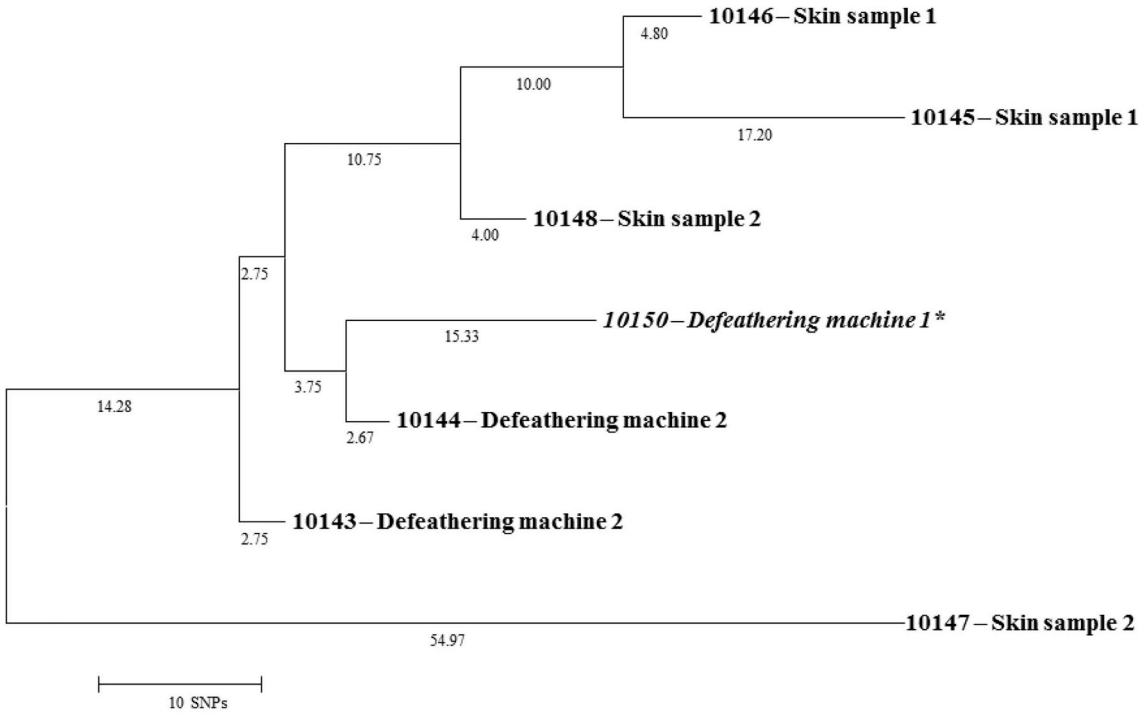
All seven *K. pneumoniae* isolates were determined as ST-2762. Phylogenetic analyses revealed only one cluster which also included the isolate from the defeathering machine taken before the processing of flock F (Fig. 1). The number of SNP differences between this isolate and all other isolates ranged between 18 and 90 SNPs (max. 17 SNPs $Mbp^{-1}$). The overall number of SNP differences in the phylogenetic tree of the *K. pneumoniae* isolates was calculated as 18 to 111 SNPs (max. 20 SNPs $Mbp^{-1}$). The intra-sample variability of the isolates of the two skin samples and the swab sample from the defeathering machine were determined as 22 SNPs, 87 SNPs and 13 SNPs respectively. Further analyses of the presence of certain VAGs revealed positive results for *entB, mrkD, rpmA, uge, wabG* and *ycfM* (> 90% identity) as well as *kpn* and *wzi* with identities of 87.9% and 87.7%, respectively.

### 3.2. ESBL-E. coli transmission

All *E. coli* isolates from flock G were found to be of ST-361. Phylogenetic analyses resulted in one large cluster with one smaller sub-cluster. The sub-cluster consisted of the four isolates from one skin sample and the defeathering machine taken before the processing of flock G (Fig. 2). The numbers of SNP differences between these four isolates ranged between 14 and 39 SNPs (max. 8 SNPs $Mbp^{-1}$) whereas the SNP differences between all the other isolates including the ones from the scalding water sample, taken before the processing of flock G, were calculated as up to 107 SNPs (22 SNPs $Mbp^{-1}$). The intra-sample variability between the two respective isolates of the same sample ranged between 19 and 84 SNPs. The *E. coli* isolates determined from samples of flock G were also investigated concerning the occurrence of VAGs. In none of the isolates InVAGs were detected but in total 18 of the 89 ExVAGs comprising all six functional categories could be identified in all isolates (Table 2).

## 4. Discussion and conclusion

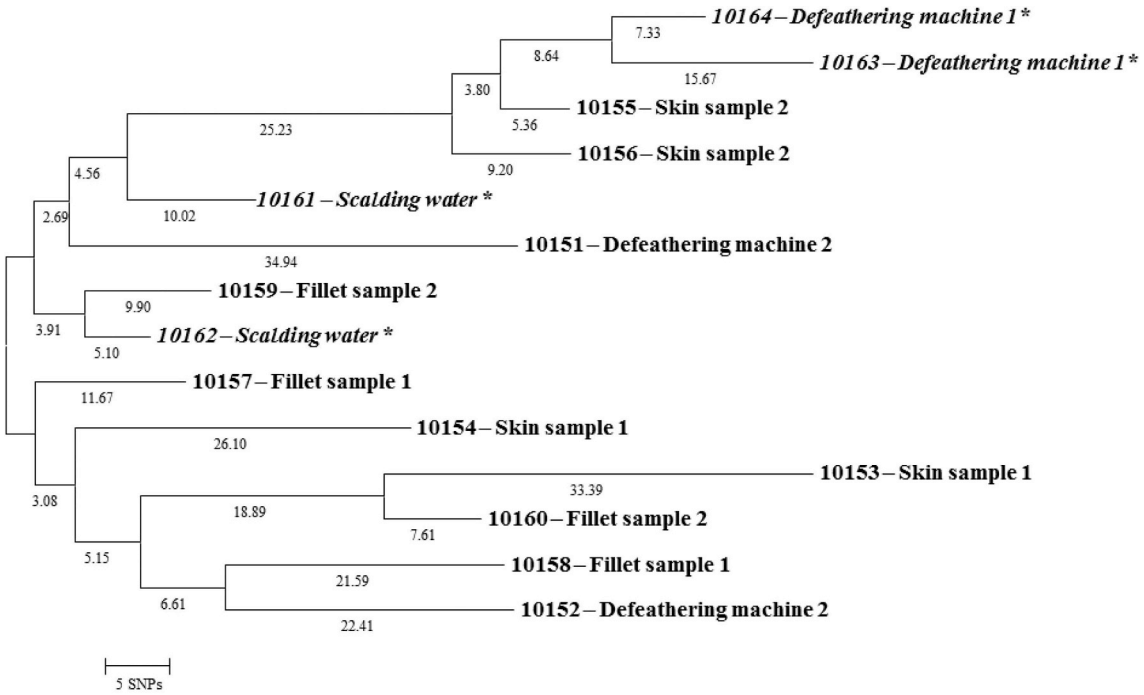
ESBL-producing Enterobacteriaceae are widely found at the different stages of the broiler production chain and certain transmission routes of these resistant bacteria could be identified (Daehre et al., 2018; Nilsson et al., 2014; Petersen et al., 2006; Projahn et al., 2017). In this study we investigated the contamination of broiler carcasses and chicken meat during processing in the slaughterhouse with *K. pneumoniae* harboring a $bla_{SHV-2}$ resistance gene and *E. coli* strains producing a CTX-M-15 beta-lactamase. The transmission potential of ESBL-producing Enterobacteriaceae from broiler chickens and chicken meat to humans is has been discussed (Belmar Campos et al., 2014; Carmo et al., 2014; Evers et al., 2017; Kluytmans et al., 2013; Leverstein-van Hall et al., 2011). However, ESBL-producing *K. pneumoniae* were only




* Swab sample from the defeathering machine was taken before the processing of fattening flock F

**Fig. 1.** Phylogenetic tree of *K. pneumoniae* isolates (flock F). Neighbor joining tree was constructed using parsnp (Harvest suite) and MEGA7 with 1000 bootstraps based on the number of SNP differences.

rarely detected in food producing animals (Hiroi et al., 2012; Kim et al., 2005; Overdevest et al., 2014) but multi-drug resistant strains were associated with severe infections (Fraenkel-Wandel et al., 2016) and clinical outbreaks (Halaby et al., 2016; Kaiser et al., 2018; Kassis-Chikhani et al., 2010). Even though only few studies report the carriage of a $bla_{SHV-2}$ resistance gene (Coque et al., 2002; Crowley et al., 2002),




* Sample from scalding water and swab sample from the defeathering machine were taken before the processing of fattening flock G

**Fig. 2.** Phylogenetic tree of *E. coli* isolates (flock G). Neighbor joining tree was constructed using parsnp (Harvest suite) and MEGA7 with 1000 bootstraps based on the number of SNP differences.

**Table 2**
Virulence-associated genes (VAGs) determined in the genome of all ST-361 E. *coli* isolates of slaughterhouse samples of flock G.

| Category | VAGs | Proposed function |
|---|---|---|
| Adhesion | *crl* | curli fibre gene |
| | *csgA* | curli major subunit |
| | *fimC* | fimbrial chaperone protein |
| | *fimH* | minor component of type 1 fimbriae |
| | *matA (ecpR)* | transcriptional regulator |
| | *feoA/B* | ferrous iron transporter, protein A/B |
| Iron Uptake | *iroN* | iron outer membrane receptor |
| | *iucD* | L-lysine 6-monooxygenase |
| | *sitA* | structural injection transglycosylase |
| | *sitC* | structural injection transglycosylase |
| Invasion | *iss* | increased serum survival |
| | *cvi* | colicin V immunity protein |
| Protection | *ompA* | outermembrane protein |
| | *traT* | conjugal transfer surface exclusion protein |
| | *astA (East-1)* | arginine succinyltransferase |
| Toxins | *cvaC* | colicin V synthesis protein |
| | *hlyE (clyA)* | hemolysin E |
| other | *malX* | maltose and glucose-specific enzyme |

clonal dissemination of multi-drug resistant *K. pneumoniae* strains has been observed (Borgmann et al., 2018; Damjanova et al., 2011; Harada et al., 2016). The detection of ESBL-producing *K. pneumoniae* in fattening farms and the slaughterhouse indicates a potential for a zoonotic transmission via the food-borne route (Davis and Price, 2016). In our study, we show that the skin of the broiler carcasses of flock F was contaminated by ESBL-producing *K. pneumoniae* strains during the defeathering process due to the contamination of the defeathering machine from other broiler flocks processed earlier this day. Broiler chickens of flock F were negative concerning *K. pneumoniae* during the fattening period (Daehre et al., 2018) and also no resistant *K. pneumoniae* was found in the caecum after evisceration (von Tippelskirch et al., 2018). However, as *K. pneumoniae* harboring a $bla_{SHV-2}$ resistance gene were detected in a swab sample from the defeathering machine before the processing of flock F and then also on skin samples of the carcasses after defeathering, a cross-contamination during this defeathering process is most likely. It was found that the *K. pneumoniae* isolate from the defeathering machine and the isolates from the skin samples differed in not more than 90 SNPs and, therefore, may indicate a highly clonal relationship. This is in concordance with previous findings of transmission investigations of *K. pneumoniae* strains in clonal clinical outbreaks (Jiang et al., 2015; Perez-Vazquez et al., 2016; Sonda et al., 2018). Jiang et al. (2015) found SNP differences of nine to 131 SNPs between *K. pneumoniae* isolates representing possible transmission events. Perez-Vazquez et al. (2016) found a difference of 42 SNPs at maximum during their outbreak investigations. However, they removed SNPs in mobile genetic elements, phages and repetitive sequences from their analyses which was not done in our study. Sonda et al. (2018) reported a SNP difference of 57 between outbreak strains and a maximum of 42893 SNPs between non-related *K. pneumoniae* strains. Therefore, we can conclude that the very low numbers of SNP differences suggests the contamination of the carcasses during defeathering although further detailed investigations of every single step in the processing line were not conducted in the original study (von Tippelskirch et al., 2018). Analyses of the presence of 22 VAGs in the genome of the *K. pneumoniae* isolates resulted in the detection of eight of these VAGs (36%). Certain genes which are involved in pathogenicity of *K. pneumoniae* strains like *entB* (enterobactin, siderophore), *mrkD* (type 3 fimbriae, adhesion), *uge* (UDP galacturonate 4-epimerase, LPS biosynthesis), *wabG* (glucuronic acid transferase, capsule) and *ycfM* (outer membrane lipoprotein) are frequently detected in *K. pneumoniae* isolates (Candan and Aksoz, 2015; El Fertas-Aissani et al., 2013). In contrast, the *rpmA* gene (regulator of capsular polysaccharide synthesis) is more often shown to be present in *K. pneumoniae* strains with a hypervirulent phenotype (hvKP) (Alcantar-Curiel and Giron, 2015; Li et al., 2014). These hvKP strains are mainly isolated from severe infections like liver abscess, urinary tract infections or bacteremia (Cubero et al., 2016; Decre et al., 2011; Jung et al., 2013; Lin et al., 2010). This indicates that the slaughterhouse environment may be a possible source of contamination of chicken carcasses and for the exposition of the slaughterhouse stuff to potential clinical relevant ESBL-producing *K. pneumoniae* strains. This should be taken into consideration for further studies on intervention measures against Enterobacteriaceae that are resistant to beta-lactam antibiotics.

*E. coli* strains producing CTX-M-15 beta-lactamases are mainly isolated from human samples and companion animals but were only rarely found in poultry (Ewers et al., 2012). During the investigation of the broiler carcasses of flock G (von Tippelskirch et al., 2018) *E. coli* strains harboring a $bla_{CTX-M-15}$ gene were isolated from skin and filet samples as well as from the slaughterhouse environment (scalding water, defeathering machine) before the processing of flock G. A high phylogenetic relationship could be observed between these isolates based on the number of SNP differences (max. 22 SNPs $Mbp^{-1}$) indicating a contamination of the skin and the filet samples during scalding and defeathering. In a previous study we found that clonal ESBL-*E. coli* strains collected from broiler chicken during a whole fattening period of five weeks differ in up to 68 SNPs (Projahn et al., 2018). Whole genomic analyses concerning outbreaks of clonal enterohemorrhagic *E. coli* were they determined 5 to 27 SNPs in difference in the core genome between outbreak strains (Berenger et al., 2015; de Been et al., 2014; Rusconi et al., 2016). These numbers are slightly lower than the numbers detected in our analyses; however, the authors used reference genomes for SNP mapping, used coding sequences for orthologous proteins only or excluded phages, IS elements, or plasmid regions which was not the case in our analyses. The phylogenetic tree of our strains led to the assumption of a multi-cross-contamination event as a small subcluster could be identified comprising the isolates of the swab sample from the defeathering machine taken before the processing of flock G as well as isolates of one skin sample. These findings are even more alarming as the broiler chickens of flock G were previously tested ESBL-negative during the whole broiler fattening period (Daehre et al., 2018) and that the chicken meat of this flock only got contaminated most likely due to the processing standards in the slaughterhouse. Further analyses of VAGs in the genome of the *E. coli* isolates revealed high numbers of those genes (n = 18). However, based on the occurrence of certain VAGs they cannot be classified as extraintestinal-pathogenic *E. coli* (ExPEC) or avian-pathogenic *E. coli* (APEC) stains (Ewers et al., 2005; Johnson et al., 2003, 2008; Skyberg et al., 2003) and therefore a risk for human infections seems to be low. These findings also indicate that a variety of VAGs are not only found in pathogenic *E. coli* strains but seemed to be more widespread among *E. coli* populations. This is in concordance with other studies were also a certain number of VAGs was detected in commensal *E. coli* strains from poultry (Mendonca et al., 2016; Paixao et al., 2016). It was also found that *E. coli* isolates were able to produce curli fibers (data not shown) which are an important factor in biofilm production (Barnhart and Chapman, 2006; Carter et al., 2016). Biofilms in slaughterhouse facilities are especially problematic as these structures provide enhanced survival against cleaning and disinfection procedures and therefore lead to the risk of persistence and contamination in the food production (Flemming and Wingender, 2010; Park and Chen, 2015).

Taken together, our analyses of ESBL-producing Enterobacteriaceae presents first time evidence of a contamination and/or cross-contamination of broiler carcasses during scalding and defeathering with resistant bacteria due to a contaminated slaughterhouse environment based on whole genome sequence analysis. There might be other possibilities for cross-contamination with ESBL-producing Enterobacteriaceae during the processing of broiler chickens which need to be investigated in further studies. The detected cross-contamination in our pilot study also happened to carcasses of an originally

ESBL-free broiler flock indicating that it might be possible that only one contaminated broiler flock in the processing line could lead to the contamination of all following broiler carcasses. This scenario was previously shown for contaminations with *Campylobacter* sp. (Colles et al., 2010; Johnsen et al., 2006)but not for ESBL-producing Enterobacteriaceae. It clearly shows the need not only for intervention measures on farm level but also for effective interventions against cross-contamination with ESBL-producing Enterobacteriaceae in the slaughterhouse. Also bacterial factors like VAGs and biofilm formation should be considered in upcoming studies concerning the development of interventions. Even though the impact of chicken meat on the transmission of ESBL-producing Enterobacteriaceae to humans is considered, a dissemination of the resistant bacteria into the environment and the households should be prevented as these bacteria can also act as reservoirs for further transmission of resistance characteristics.

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## Appendix A. Supplementary data

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