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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