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Diversity in prevalence and characteristics of ESBL/pAmpC producing *E. coli* in food in Germany

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ABSTRACT

The spread of extended-spectrum β-lactamases (ESBLs) in *Escherichia coli* is a major public health issue and ESBL-producing bacteria are frequently reported in livestock. For the assessment of the role of the foodborne transmission pathway in Germany, detailed data on the prevalence and characteristics of isolates of food origin are necessary. The objective of this study was to describe the prevalence of cefotaxime resistant *E. coli* as well as ESBL/pAmpC-producing *E. coli* and their characteristics in foods in Germany.

Out of 2256 food samples, the highest prevalence of cefotaxime resistant *E. coli* was observed in chicken meat (74.9%), followed by turkey meat (40.1%). Prevalence in beef, pork and minced meat was considerably lower (4.2–15.3%). Whereas 18.0% of the raw milk samples, collected at farm level were positive, this was true only for few cheese samples (1.3%). In one out of 399 vegetable samples a cefotaxime-resistant *E. coli* was isolated.

ESBL resistance genes of the CTX-M-group (10.1% of all samples) were most frequently detected, followed by genes of the pAmpC (2.6%), SHV (2.0%) and TEM (0.8%) families. Distribution of ESBL/AmpC-encoding *E. coli* resistance genes and *E. coli* phylogroups was significantly different between the chicken related food samples and all other food items.

Our study results reflect that consumers might get exposed to ESBL/pAmpC-producing *E. coli* through several food chains. These results together with those collected at primary production and in the human population in other studies will allow more detailed analysis of the foodborne pathways, considering transmission from livestock populations to food at retail and to consumers in Germany.

1. Introduction

The production of extended-spectrum β-lactamases (ESBLs) is the worldwide most important mechanism conferring resistance to 3rd generation cephalosporins in *Escherichia (E.) coli* and a major public health issue (EFSA, 2011; ECDC, 2017). Both the increase in combined resistance to multiple antimicrobial groups, as well as the high proportion of ESBL-producing *E. coli*, is especially worrying, as this limits treatment options for patients suffering from infections caused by these pathogens (ECDC, 2017). In recent years, it has been widely recognized that the dissemination of ESBL-producing bacteria is not restricted to the medical/healthcare system and represents a growing problem involving food safety and environmental exposure. Knowledge about the prevalence of resistant bacteria in primary production is an important element needed to estimate the transmission along the food chain and the exposure of the human population (Valentin et al., 2014).

ESBL-producing isolates are frequently reported from samples of livestock origin in Germany. Recent studies have shown frequent colonization of poultry (Kola et al., 2012; Laube et al., 2014), cattle (Schmid et al., 2013; Hille et al., 2017), pigs (Von Salviati et al., 2014) as well as other domestic and wild animal species (EFSA, 2011; Ewers et al., 2012) with ESBL-producing bacteria.

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Regarding food, currently available studies show that there are significant differences in the prevalence of ESBL *E. coli* in meat of different animal origin. Whereas in chicken highest prevalence rates (3.3–100%) are reported, detection rates in beef and pork (0.7–25%) were considerably lower. Recently, specific monitoring of pork and beef at retail at EU-level showed that on average 9.7% of the pork samples and 7.1% of the beef samples were carrying ESBL/AmpC-producing *E. coli* (EFSA and ECDC, 2017).

Based on indirect evidence by detecting e.g. similar clones, plasmids or sequence types, it is assumed that foodborne exposure has an impact on the probability of human colonization and/or infection caused by ESBL-producing bacteria (Valentin et al., 2014; EFSA, 2011). Also, direct transmission from livestock to humans has been shown. The same ESBL genes, plasmids or even *E. coli* isolates could be detected in animals and the farmers taking care for them (Dierikx et al., 2013; Fischer et al., 2017).

Most recent studies highlight the need to compare isolates from different origins to assess the relevance of the foodborne pathway for human infection (Dorado-García et al., 2017; Ojer-Usoz et al., 2017; Börjesson et al., 2016). A recent large-scale study in Sweden found that clonal spread of cephalosporin-resistant *E. coli* from food and farm animals to human was unlikely. There was limited dissemination of ESBL or plasmidic (p) AmpC-genes and the plasmids carrying such genes from foods and farm animals to either healthy humans or patients (Börjesson et al., 2016). Similarly, in a pooled analysis performed in the Netherlands the molecular relatedness of ESBL/pAmpC-producing *E. coli* from humans, animals, food and the environment was investigated. The results did not demonstrate a close epidemiological linkage of ESBL/pAmpC genes and plasmid replicon types between livestock farms and people in the general population (Dorado-García et al., 2017).

For assessing the role of the foodborne transmission pathway in Germany, detailed data on the major characteristics of isolates of food origin are necessary. The objective of this study was to describe the prevalence of cefotaxime resistant *E. coli* as well as ESBL/pAmpC-producing *E. coli* and their characteristics in foods in Germany.

2. Methods

2.1. Sampling

Four federal states from different parts of Germany representing 38% of the German population were involved in the study. Food samples of different origins (poultry $n = 426$, cattle $n = 933$, swine $n = 498$, vegetables $n = 399$) and matrices (meat, minced meat, raw milk, cheese, vegetables) were taken by official food inspectors in food processing plants and retail shops during their routine visits in the years 2012 and 2013. Raw milk samples were collected at the farm level. Desired sample sizes by food category was 50 samples in each of three federal states and 100 samples in one federal state as additional resources were available there. Overall, 2256 food samples were collected. The type of food matrix, the type of sampling site and the date of sampling were recorded.

2.2. Detection method and characterization

Samples were sent the same day to the laboratory and investigated in the regional veterinary investigation centers following a standardized protocol (Irrgang et al., 2017). Of each sample 25 g were prepared using a stomacher and incubated overnight for 18–24 h at 37 °C in Lysogeny Broth, followed by cultivation on MacConkey-Agar supplemented with 1 µg/ml cefotaxime for 18–24 h at 37 °C. Suspicious *E. coli* isolates were confirmed by biochemical tests or MALDI-TOF. Only one phenotypically resistant *E. coli* isolate per sample was sent to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR) for further confirmation and characterization. There, in all isolates cefotaxime-resistance was confirmed by broth microdilution method according to CLSI guidelines (CLSI, 2012). For phenotypic confirmation of ESBL and plasmidic AmpC (pAmpC) phenotype the antimicrobial panel in concordance to the decision 2013/652/EU of the European Union was used with microtiter plates from TREK Diagnostic Systems (Thermo Fisher Scientific, Schwerte, Germany). ESBL/pAmpC-encoding genes were identified by PCR amplification and sequencing as previously described (Rodriguez et al., 2009). The location of the AmpC gene on a plasmid was determined by S1-nuclease PFGE with subsequent southern blot hybridization using a AmpC specific PCR-probe (Rodriguez et al., 2009). Only ESBL specific and pAmpC-resistance genes were considered in the analysis and *E. coli* phylogenetic groups were determined (Doumith et al., 2012).

2.3. Descriptive analysis

The food type, business type and analysing institute (as surrogate for the region) were included in the analyses. We differentiated the following food types: “vegetables”, “milk”, “cheese”, “chicken” (meat and raw meat products), “turkey” (meat and raw meat products), “pork” (meat and raw meat products), “pork-minced meat”, “beef” (meat and raw meat products) and “beef-minced meat”. Further, we assigned the type of business either to “production”, which included butcher shops, dairies and dairy farms, and business type “retail”, including retail, supermarkets, farmer's markets, direct marketer, restaurants and canteens. The analysing institutes were “A”, “B”, “C” and “D” which also represent four different federal states in Germany as each institution investigated the samples originating from their region.

Milk and cheese samples were not available from all analysing institutes, and the samples of vegetables were all but one negative. Therefore, data from those three food types were shown in a descriptive way but were excluded from the detailed statistical analysis to avoid missing values in the dataset.

2.4. Statistical analysis

Detailed analysis included those samples where information was complete. Eighteen cefotaxime-resistant *E. coli* isolates from samples detected at regional level were not available for characterization at the NRL-AR and excluded from the statistical analysis as their ESBL status could not be confirmed. Those samples did not differ in their distribution of food type ($\chi^2 = 3.714$, $p = 0.781$) or business type ($\chi^2 = 2.713$, p-value = 0.137) from the analyzed samples. However, there was a significant effect regarding the analysing institute ($\chi^2 = 14.157$, p-value = 0.002), as not-further-analyzed-samples occurred in three of the four institutes (χ^2-tests with simulated p-values based on 2000 replicates).

In the detailed statistical analysis we tested the dependence of the occurrence of ESBL/pAmpC producing *E. coli* in food samples on the possible categorical influencing factors “food type”, “analysing institute” and “business type”. To test which factors influence the occurrence of ESBL/pAmpC producing *E. coli* in food we used a generalized linear model with the binomial response factor (number of samples positive for ESBL/pAmpC producing *E. coli* vs. number of samples negative for each subcategory) and tested for the possible effects of the above mentioned factors as well as their interaction terms. Based on this global model we generated all possible models with fewer terms and used the AICc (Akaike, 1974) as a selection criterion to determine the model which fitted the data best. For this final model we applied for the significant factors Tukey's post-hoc test to analyze which factor levels differentiate. To estimate model fit we used McFadden's pseudo R^2 (McFadden, 1974), which is defined as $R^2 = 1 - \log(\textit{likelihood model})/\log(\textit{likelihood null-model})$.

The ESBL/pAmpC producing *E. coli* positive samples were analyzed in detail in the statistical model by verifying separately for each sample the presence of genes encoding β-lactamases CTX-M, TEM, SHV and/or pAmpC. Further, the phylogenetic group of the isolates was considered

in the analysis.

To test which factors influence the occurrence of the β-lactamase CTX-M family we conducted the same analysis as mentioned above for the occurrence of ESBL/pAmpC producing *E. coli*. For this analysis we assumed that all samples negative for ESBL/pAmpC producing *E. coli* were also negative for CTX-M. It was not possible to build a model for the other ESBL-groups as most occurred only at a low frequency and were not present in all of the possible food types.

In addition we wanted to illustrate how the composition of β-lactamases differed between food type, analysing institute, business type and phylogenetic group. To account for differences in sample sizes, we converted the values for each category to represent the number of detected β-lactamases per 100 samples. To quantify the difference between categories we calculated the Bray–Curtis-dissimilarity (BC) (Bray and Curtis, 1957). The BC is bounded between 0 and 1, where 0 means the two categories have the same composition, and 1 indicates the two categories do not share any of the β-lactamases.

In a second step we applied a permutational multivariate analysis of variance (PERMANOVA, formerly "nonparametric MANOVA") (Anderson, 2001) to analyze whether food-type, analysing institute, business-type and phylogenetic group had a significant effect on the composition of the β-lactamases. For this analysis we generated a distance matrix by calculating the difference between each of the individual samples using the Sørensen dissimilarity (Sørensen, 1948). The Sørensen dissimilarity is similar to the Bray–Curtis-dissimilarity, but suitable for binary data (as for the single samples we have only the presence/absence data of each β-lactamase). This distance matrix was used as the dependent variable in the PERMANOVA. The overall sum of the distances represents the total amount of variance and can be partitioned into the variability between groups and the variability within groups. The analysis follows thus the principle of an ANOVA. The *p*-values were obtained by using permutation test with 10,000 iterations. In the case of a significant factor we applied a post hoc test by using pairwise comparisons of each possible pair of levels. To compensate for the effect of multiple comparisons we give the Bonferroni-corrected *p*-values. As the PERMANOVA does not provide any "effect size", to present some quantitative value for the differences, we present the values of BC together with the PERMANOVA, although the BC is not a result of the PERMANOVA and the given BC values are not corrected for other possible influential factors.

To analyze how the distribution of the phylogenetic group is influenced by food type, analysing institute, business type and β-lactamases we used the same methods as for the analysis of the composition of β-lactamases, namely we calculated the BC and conducted a PERMANOVA. However, while several different β-lactamases can be found in one sample, each sample is assigned to only one phylogenetic group.

All statistical analyses were conducted using the statistical software R version 3.3.2 (R Development Core Team, 2016). Additional packages were used for model selection ("MuMIn" (Barton, 2016)), for the generation of distance matrices and the PERMANOVA ("vegan" (Oksanen et al., 2017)), and for the illustration of the distance matrices ("corrplot" (Wei and Simko, 2016)).

3. Results

3.1. 3rd generation cephalosporin resistance

Overall, out of 2256 food samples tested 399 (17.7%) samples gave a positive result for cefotaxime-resistant *E. coli* (Table 1). The highest prevalence was observed in chicken meat (74.9%), followed by turkey meat (40.1%). Compared to the poultry derived samples, prevalence in beef, pork and minced meat was considerably lower (4.2–15.3%). Whereas 18.0% of the raw milk samples, collected at farm level were positive, this was true only for two cheese samples (1.3%), both made from raw milk. From one out of 399 vegetable samples, a cucumber, a cefotaxime-resistant *E. coli* was isolated.

3.2. ESBL/pAmpC-producing E. coli – full dataset

In the analysis of ESBL/pAmpC-producing *E. coli*, 2238 samples could be included. Eighteen samples were excluded as results of isolate characterization were missing for detailed analysis (Table 1). From these 2238 samples, 17.0% ($n = 381$) were found to be cefotaxime-resistant in the first screening, however, the ESBL/pAmpC-positive status was confirmed for 15.2% ($n = 341$) of the samples only (Table 1).

ESBL resistance genes of the CTX-M-group ($n = 226$; 10.1% of all samples) were most frequently detected, followed by genes of the pAmpC ($n = 58$; 2.6%), SHV ($n = 44$; 2.0%) and TEM ($n = 19$; 0.8%) families (Table 2). Descriptive analysis showed that distribution of ESBL/pAmpC-encoding resistance genes and phylogenetic groups was different between the chicken related food samples and other food items (details not shown).

3.3. ESBL/pAmpC-producing E. coli – subset of dataset

From the full dataset ($N = 2238$) with detailed information on the ESBL/pAmpC-type, 803 samples from milk, cheese or vegetables were excluded from the statistical analysis due to data limitations. In the remaining data set ($N = 1435$) 21.2% ($n = 304$) of the samples were found to be ESBL/pAmpC-positive.

As shown by the full model ($R^2_{\text{McFadden}} = 0.747$), the proportion of ESBL/pAmpC positive samples differed between food types and business type, however, it depended on the food type whether more positive samples were found in production or retail businesses (Table S1). As shown in Fig. 1, most ESBL/pAmpC positive samples originated from chicken, followed by turkey. Further, we found a trend regarding the interaction terms including the investigating institutes, indicating that the region of sampling contributes to explaining the variance in the occurrence of ESBL/pAmpC positive food samples (Table S1).

The final model ($R^2_{\text{McFadden}} = 0.668$) included only the additive effects of food type and analysing institute (reflecting the region). Both factors had a significant influence on the proportion of ESBL/pAmpC positive samples ($N = 1435$; Analysis of Deviance; food type: $\chi^2 = 357.50$, df = 5, $p < 0.001$; region reflected by analysing institute: $\chi^2 = 20.25$, df = 3, $p < 0.001$).

The proportion of ESBL/pAmpC positive samples found in chicken (71.9%) was significantly higher ($p < 0.001$) than in all other food types (Fig. 1). The second highest proportion was found for turkey meat (30.5%), which was again significantly higher ($p < 0.001$) than for the remaining food types. The contamination rate with ESBL/pAmpC in pork ($p = 0.018$) and pork-minced meat ($p = 0.002$) was higher than for beef. However, there was no difference between minced meat originated from pork or beef ($p = 0.585$).

The proportion of ESBL/pAmpC positive food samples differed also according to analysing institute. In 29.8% of the samples analyzed by institute D the ESBL/pAmpC screening was positive, which was significantly higher ($p < 0.05$) than for the other institutes (Fig. S1).

3.4. Analysis by β-lactamase group

We found that 191 (13.3%) of the samples contained a β-lactamase of the CTX-M group. The global model for CTX-M ($R^2_{\text{McFadden}} = 0.524$) revealed that its occurrence depended on the analysing institute and the interaction term of food type and business type (Table S1). However, for some food types, prevalence of CTX-M was higher from the production samples and in others from the retail samples. The best more parsimonious model ($R^2_{\text{McFadden}} = 0.404$) included the same terms as the final model for the occurrence of ESBL/pAmpC, namely the additive effects of food type and analysing institute. Both factors had a significant influence on the proportion of CTX-M positive samples ($N = 1435$; Analysis of Deviance; food type: $\chi^2 = 83.07$, df = 5, $p < 0.001$; analysing institute: $\chi^2 = 34.47$, df = 3, $p < 0.001$).

Table 1
Number and proportion of food samples positive for cefotaxime-resistant *E. coli* and ESBL/pAmpC-producing *E. coli*.

Matrix	Cefotaxime-resistant *E.coli* Number of positive samples			ESBL/pAmpC-*E.coli* Number of positive samples		
	N=	Pos (%)	95%CI	*N*=	Pos (%)	95%CI
Vegetables	399	1 (0.3)	[0.0–1.4]	399	1 (0.3)	[0.0–1.4]
Raw milk	255	46 (18.0)	[13.5–23.3]	253	35 (13.8)	[9.8–18.7]
Cheese	151	2 (1.3)	[0.2–4.7]	151	1 (0.7)	[0.0–3.6]
Chicken	199	149 (74.9)	[68.3–80.7]	192	138 (71.9)	[64.9–78.1]
Turkey	227	91 (40.1)	[33.7–46.8]	223	68 (30.5)	[24.5–37.0]
Pork – meat	283	36 (12.7)	[9.1–17.2]	282	34 (12.1)	[8.5–16.4]
Pork – minced meat	215	33 (15.3)	[10.8–20.9]	214	29 (13.6)	[9.3–18.9]
Beef – meat	284	12 (4.2)	[2.2–7.3]	284	12 (4.2)	[2.2–7.3]
Beef – minced meat	243	29 (11.9)	[8.1–16.7]	240	23 (9.6)	[6.2–14.0]
Total	2256	399 (17.7)		2238	341 (15.2)	

Table 2
Number and proportion of ESBL-/pAmpC resistance gene groups in *E. coli* from the food samples (N = 2238).

Matrix	ESBL/ pAmpC Number of positive samples	CTX-M	TEM	SHV	CMY
	Pos (%)	Pos (%)	Pos (%)	Pos (%)	Pos (%)
Vegetables	1 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Raw milk	35 (13.8)	33 (13.0)	1 (0.4)	0 (0.0)	1 (0.4)
Cheese	1 (0.7)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Chicken	138 (71.9)	46 (24.0)	12 (6.3)	34 (17.7)	50 (26.0)
Turkey	68 (30.5)	58 (26.0)	0 (0.0)	6 (2.7)	4 (1.8)
Pork – meat	34 (12.1)	32 (11.3)	2 (0.7)	1 (0.4)	1 (0.4)
Pork – minced meat	29 (13.6)	23 (10.7	2 (0.9)	2 (0.9)	2 (0.9)
Beef – meat	12 (4.2)	11 (3.9)	0 (0.0)	1 (0.4)	0 (0.0)
Beef – minced meat	23 (9.6)	21 (8.8)	2 (0.8)	0 (0.0)	0 (0.0)
Total	341 (15.2)	226 (10.1)	19 (0.8)	44 (2.0)	58 (2.6)

Prevalence of CTX-M group was highest in turkey (26.0%) and chicken (24.0%) and lowest in beef (3.9%) (Fig. 2). CTX-M was more often found in turkey than in all other food categories ($p < 0.005$), except for chicken ($p = 0.972$). The proportion of CTX-M positive samples was significantly ($p < 0.05$) higher in chicken than in the other food types, except for turkey. Further, the proportion of CTX-M positive samples was higher in pork ($p = 0.043$) and pork-minced meat ($p = 0.012$) than in beef. The analysing institute D found significantly ($p < 0.001$) higher proportions of CTX-M positive food samples than the other institutes (Fig. S2).

Overall, nine different CTX-M-types were found (Table S2). Most of the CTX-M positive samples belonged to CTX-M-1 (80.6%), which was the only one found in all food types, further CTX-M-15 (7.3%), and CTX-M-14 (3.7%). Turkey samples were the most diverse, as eight of the nine CTX-M-types were found in this food category.

Out of all samples, 57 (4.0%) tested positive for the CIT-type pAmpC enzyme CMY-2. It was mainly found in chicken samples, where it occurred in 26.0% of the samples (Fig. 2). Further, it was found in turkey (1.8%), pork-minced meat (0.9%), and in pork (0.4%). Most samples positive for pAmpC were detected by the analysing institute A (8.5%), while the other institutes found this β-lactamase at lower frequencies (Fig. S2).

Out of all samples, 44 (3.1%) were identified as SHV positive. Again, its frequency was highest in chicken, where 17.7% of the samples were found SHV positive (Fig. 2). Further, 2.7% of the turkey samples were found contaminated with SHV, as well as 0.9% of the pork-minced meat, 0.04% of the pork, and 0.04% of the beef samples. SHV was present at a higher rate in samples analyzed by D (5.0%) and C (4.4%) than in those analyzed by B (2.3%) and A (1.4%) (Fig. S2). In all

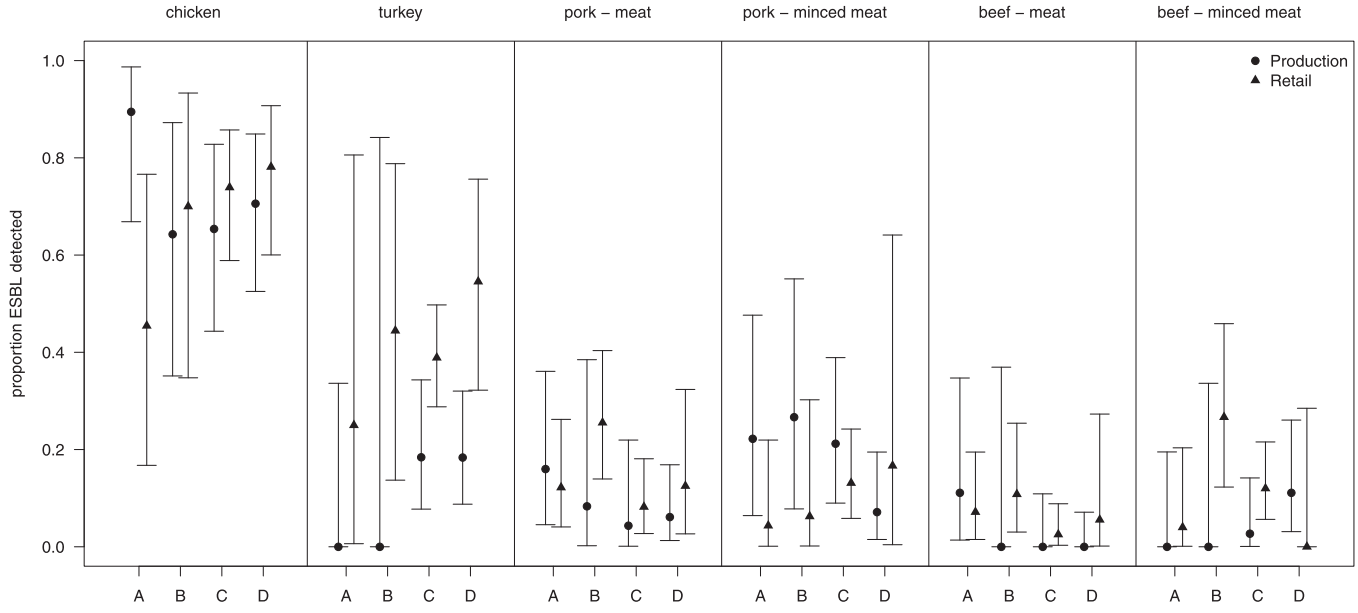

Fig. 1. Proportion of ESBL positive food samples (± 95% CI) according to food type, analysing institute (A: N = 295; B: N = 483; C: N = 284; D: N = 251) and business type.

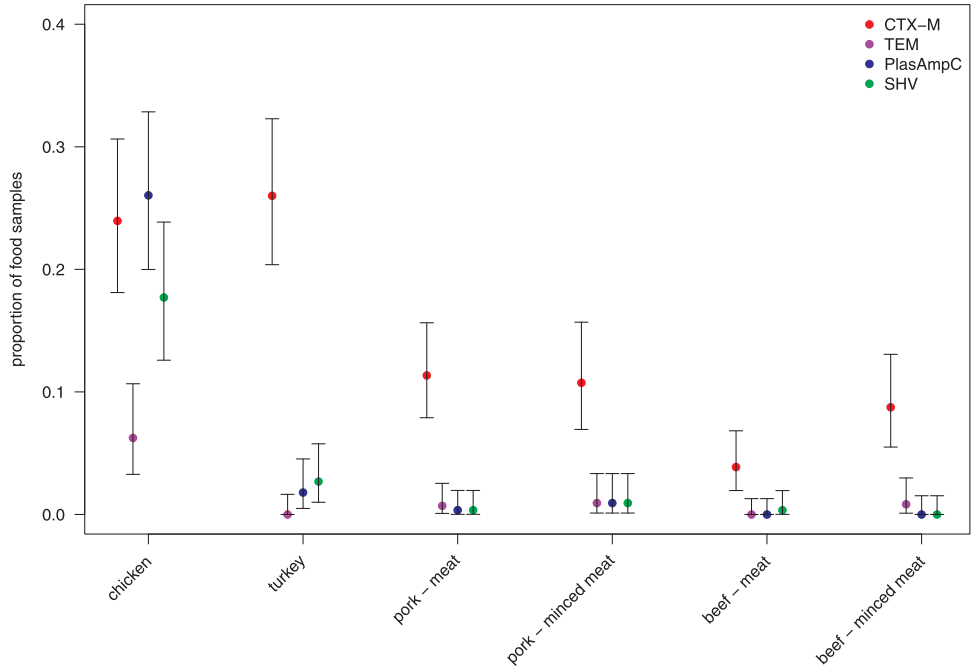

Fig. 2. Proportion of CTX-M, TEM, Plasmidic AmpC, and SHV positive food samples (± 95% CI) according to food type.

but two cases the samples were positive for SHV-12, only two chicken samples were identified as SHV-2 positive.

Of the occurring β-lactamases in this study ESBL specific TEM was the rarest, as it was found only in 18 (1.3%) of the food samples. The highest proportion of TEM positive samples was found in chicken (6.3%). In pork, pork minced meat as well as in beef minced meat, TEM positive samples were also found, in each of those food types twice (Fig. 2). TEM was present at a higher rate in samples analyzed by institute C (2.2%) than in those analyzed by B, C (1.1% each) and A (0.7%) (Fig. S2).

We found three different ESBL positive β-lactamase encoding gene (*bla*) bla_{TEM}-variants in our analysis. Of the isolates tested positive for TEM, 88.9% belonged to type $bla_{\text{TEM-52}}$, further one sample each was positive for $bla_{\text{TEM-20}}$ and $bla_{\text{TEM-52c}}$, both found in chicken samples.

β-Lactamases without an ESBL phenotype were found in samples identified as cefotaxim-resistant in the first screening, but whose ESBL/pAmpC status was not confirmed in the second analysis. Those β-lactamases were found in 1.8% of the samples ($N = 26$) and were mostly found in turkey (7.6%) and chicken (2.1%) samples. They included variants of $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$, or were suspected to show overexpression of the chromosomal AmpC gene. Further non-ESBL gene variants ($bla_{\text{TEM-1a}}$, $bla_{\text{TEM-1b}}$, $bla_{\text{TEM-1c}}$, $bla_{\text{TEM-135}}$) were found in samples in addition to other ESBL types.

In most cases (98.4%) only one ESBL variant was found in a sample. In four cases (1.3%) two different ESBL variants were found, and three different variants were found only once (0.3%). The composition of the β-lactamases differed according to food type, analysing institute and phylogenetic group (Table S3). The highest difference in the composition was found for the food-types chicken and beef meat (BC = 0.589). Generally, as shown in Fig. 3a, chicken samples differed considerably from the other food types. This was also confirmed by the post-hoc analysis, were the composition of the chicken samples was significantly different compared to all other food types ($p < 0.005$), while no other significant differences between food types were found.

The composition of β-lactamases differed between the analysing institutes as well (Fig. 3b), as D differed from A (BC = 0.387, $p < 0.001$), from B (BC = 0.192, $p = 0.027$) and from C (BC = 0.272, $p = 0.007$). The β-lactamase-composition from the samples analyzed by A differed from C (BC = 0.272, $p = 0.028$).

3.5. Phylogenetic group

Furthermore, we found differences depending on the phylogenetic group (Fig. 3d), as phylogenetic group D differed ($p < 0.001$) from B1 (BC = 0.397) and A (BC = 0.387). As shown in Fig. S3, CTX-M was found at high rates in phylogenetic group A and B1, while phylogenetic group B2 and D contained higher proportions of pAmpC. Phylogenetic group A ($n = 111$, 37%) was dominating, followed by phylogenetic groups B1 ($n = 96$, 32%) and D ($n = 84$, 28%). Only 13 isolates (4%) belonged to phylogenetic group B2. The phylogenetic group of the samples differed between food types (Table S4). The post-hoc analysis revealed that this effect was due to a significant difference in the phylogenetic group between chicken and samples obtained from turkey (BC = 0.316, $p = 0.001$), pork (BC = 0.463, $p = 0.001$), minced pork (BC = 0.418, $p = 0.003$) and minced beef (BC = 0.391, $p = 0.046$). This difference was mainly due to the high proportion of chicken samples belonging to phylogenetic group D compared the other food types (Fig. S4) (Fig. 4).

4. Discussion

Our results confirmed previous studies where highest prevalence for ESBL-producing *E. coli* was observed in poultry derived foods and the lowest in vegetables. Similar to our results, prevalence in beef and pork samples was lower than in chicken samples (Randall et al., 2017; Ojer-Usoz et al., 2013; Pehlivanlar Önen et al., 2015). In another study performed in Turkey where also milk and cheese samples were considered, prevalence in chicken (24%) was lower, but prevalence for ESBL-producing *E. coli* in raw milk samples (18%) and raw cow milk cheese samples (4%) was similar to our study results (Tekiner & Özpınar, 2016).

A high contamination level of German chicken meat was reported already previously (Kola et al., 2012; Belmar Campos et al., 2014) and confirmed again in the National monitoring program in 2016 (BVL, 2017). As regards prevalence of ESBL/pAmpC *E. coli* in turkey meat, in the German monitoring program, conducted in 2016, very similar results to our study, and a lower prevalence in turkey meat compared to broiler meat was shown (BVL, 2017). Randall et al. (2011) also described earlier a low prevalence in turkeys in the UK.

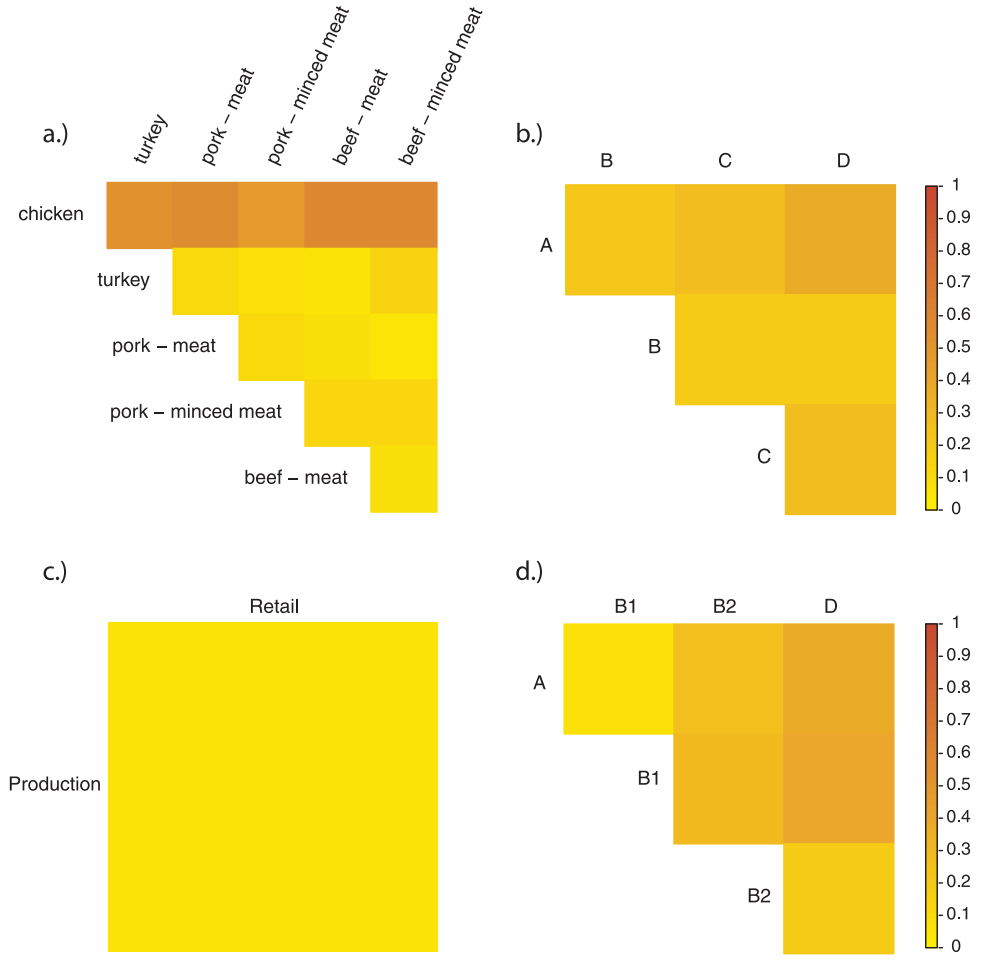

Fig. 3. Representation of differences in β-lactamases composition for (a) food-type, (b) analysing institute, (c) business-type, and (d) phylogenetic group using the Bray–Curtis dissimilarity. A value of 0 means the two categories have the same composition, and 1 indicates the two categories do not share any of the β-lactamases.

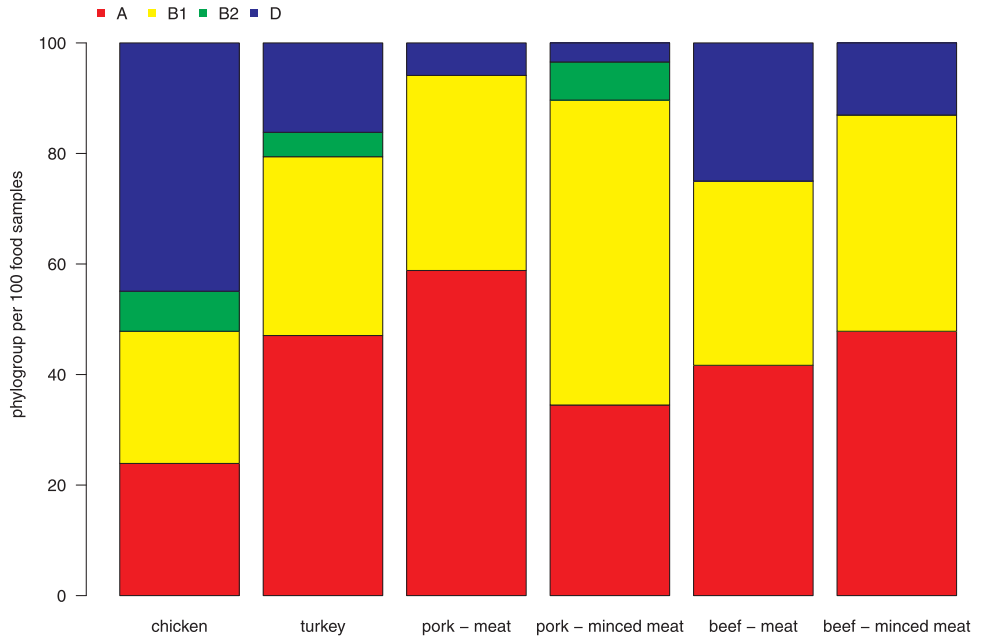

Fig. 4. Distribution of the phylogenetic groups according to food-type.

Several studies investigating pork and/or beef showed the presence of ESBL/AmpC–*E. coli*, usually at low rates and, as shown also in our study, a higher prevalence in pork compared to beef (Randall et al., 2017; EFSA and ECDC, 2017).

Data available on the prevalence of ESBL/pAmpC–*E. coli* in minced meat are diverse. Whereas a study in the city of Graz, Austria also showed higher detection rates in minced pork and minced beef in comparison to the raw meat, in another study conducted in Switzerland none of the minced meat samples contained ESBL-producing strains (Petternel et al., 2014; Geser et al., 2012). The higher bacterial load in minced meat is frequently attributed to the additional handling procedures and the mixing of meat from several batches (Khalafalla et al., 1993).

Our study results for raw milk samples and cheese samples are in line with the high prevalence observed in German dairy cattle (Schmid et al., 2013) and may reflect that there is quite some contamination from dairy cattle to the raw milk, collected at the farm, during milking, handling or storage of milk. Whereas similar results had been reported in a Turkish study (Tekiner and Özpınar, 2016), two studies conducted in Switzerland documented quite low levels in milk and cheese (Marti et al., 2016; Geser et al., 2012).

In the crop production sector, products might get contaminated through application of manure (animal origin) or sewage sludge (human origin) to the soil or through application of treated or untreated waste water that is used for irrigation of crops (Hartmann et al., 2012). Recent studies indicate that fresh vegetables constitute a source of ESBL-producers and represent a possible route for the dissemination of resistance genes via the consumer in the community (Raphael et al., 2011; Reuland et al., 2014; Schwaiger et al., 2011; Ben Said et al., 2015), but prevalence might be very low (van Hoek et al., 2015; Randall et al., 2017) as shown in our study. Contrasting results were reported in a study in Switzerland, where 15.4% of the vegetables imported from the Dominican Republic, India, Thailand and Vietnam were positive for ESBL-producing *E. coli* (Zurfluh et al., 2015). These results may reflect differences in the production, transporting and handling conditions of vegetables in these countries.

Our results did not hint toward a systematic impact of the business type of sampling on the prevalence rate. But our prevalence rates in foods are quite different to the results from previous studies on the prevalence of cefotaxime-resistant and ESBL/pAmpC producing *E. coli* in livestock populations in Germany most probably reflecting hygienic measures taken to avoid contamination of carcasses during harvest. In all (100%) broiler flocks (Hering et al., 2016), in most of the dairy and beef cattle farms (85% and 70% respectively) (Hille et al., 2017; Schmid et al., 2013) and in 85% of fattening pig farms (Hering et al., 2014), cefotaxime-resistant *E. coli* were observed.

Differences in the regional distribution of ESBL/pAmpC-encoding resistance genes and phylogenetic groups in *E. coli* were also highlighted in studies where domestic and imported foods were analyzed as well as when comparing results from different countries (Egervärn et al., 2014; Agersø et al., 2012).

4.1. Distribution of resistance genes by food type

Our study confirms that the distribution of ESBL/pAmpC resistance genes among the individual food items was very much in line with those described in the German livestock populations (Valentin et al., 2014).

Dominating ESBL-genes in chicken meat in our study are in line with two regional studies in Germany (Kola et al., 2012; Belmar Campos et al., 2014) and those commonly found in poultry isolates from the Netherlands, Belgium, France and England, but also in human isolates from Germany and the Netherlands (EFSA, 2011). Several studies confirmed that CMY-2 and CTX-M-1 dominated in European chicken meat and that SHV could also regularly be detected (Egervärn et al., 2014; Pehlivanlar Önen et al., 2015).

Predominance of CTX-M genes in pork and beef samples is in line with reports from other studies, where also the CTX-M-group was dominating (EFSA, 2011). In contrast to our findings, the study in the European Union during 2015 showed that beside ESBL positive *E. coli* dominated in fattening pigs (31.9%) also AmpC positive *E. coli* was present in 9.7% of the samples and both types in 1.5% of the pigs. In calves similarly, ESBL were more frequent (36.8%) but also AmpC (4.8%) and both types (2.0%) were reported (EFSA and ECDC, 2017). This pattern is true for most but not all countries. Similarly, in pork and beef both ESBL (7.0 and 5.0%) and AmpC type (2.3% and 1.8%) β-lactamases as well as a combination of both (0.4% and 0.3%) were reported (EFSA and ECDC, 2017).

In the study in Vietnam, patterns in beef and pork were quite different. Whereas in pork, enzymes belonging to the CTX-M-9 group were dominating, in beef those of the CIT-group were most frequent, followed by CTX-M-9 group (Nguyen et al., 2016). In Taiwan and China, similarly CTX-M-1 and CTX-M-9 groups dominated in beef and pork (Chen et al., 2017; Li et al., 2016). In all these studies, enzymes of the SHV-group were rarely reported.

In our study, the single isolate found in vegetables carried a CTX-M enzyme. In a study on imported vegetables, CTX-M group 1 and CTX-M group 9 ESBLs dominated (Zurfluh et al., 2015).

As previously shown for dairy cattle, CTX-M-group also clearly dominated in our milk and cheese isolates. This is in line with other study results (Marti et al., 2016). In the German cattle population, CTX-M and among them CTX-M-1 was the most frequently found group (Schmid et al., 2013).

4.2. Distribution of resistance genes by gene group

In our study, we found that 191 (13.3%) of the samples contained **CTX-M**, which was significantly influenced by food type and the region of sampling. Furthermore, the significant differences in prevalence rates for the individual food matrices were also true if only CTX-M findings were considered. In a recent study, characterization of the CTX-M-15-producing *E. coli* isolated within this study showed that highly similar isolates and mobile genetic elements are circulating in livestock animals in Germany (Irrgang et al., 2017). Furthermore, detailed investigation of the CTX-M-1 producing *E. coli* isolated within this study evidenced that the majority of the isolates harbored the $bla_{\text{CTX-M-1}}$ gene on IncI1 plasmids. Overall no spread of single clonal lineages of CTX-M-1 producing *E. coli* could be shown (Irrgang et al., 2018).

CMY-2-producing *E. coli*, the predominant pAmpC in the poultry population and mainly found in chicken meat, is only rarely detected in human isolates and points toward low transmission rates (Valenza et al., 2014; Jorgensen et al., 2010). In contrast to our findings, the recent EU wide monitoring in 2015 showed that in a few countries AmpC phenotype *E. coli* exceeded ESBL phenotype *E. coli* in pork or beef (EFSA and ECDC, 2017). Nextgeneration sequencing-based analysis showed distinct CMY-2-producing *E. coli* clonal lineages (ST131 and ST38) in humans, livestock animals and foodstuff. A direct clonal relationship of isolates from food animals and humans was only noticeable for a few cases, hinting toward plasmid-mediated spread, especially via IncI1 and IncK plasmids, playing an important role for emergence and transmission of $bla_{\text{CMY-2}}$ between animals and humans (Pietsch et al., 2018).

Similar to other studies, frequency of SHV type enzymes, predominantly SHV-12, was highest in chicken, followed by turkey meat. In the other meat samples, SHV genes were only sporadically detected (Ewers et al., 2012; Sheikh et al., 2012; Pehlivanlar Önen et al., 2015; Nguyen et al., 2016).

ESBL specific TEM, predominantly TEM-52, was the rarest in our food samples. Similar to other studies, the highest proportion of TEM positive samples was found in chicken whereas there were only a few findings in pork, minced pork and minced beef (Kola et al., 2012).

4.3. Contributing factors

There are several potential sources of bacteria on meat, including the animals from which the meat was derived, cross-contaminating from other products, machinery and the environment, as well as those workers who are producing and handling the meat product which can contribute to the observed differences in prevalence as well as isolate characteristics (EFSA and ECDC, 2017). In our study, we could not confirm a clear difference between the prevalence rates of food items collected at production places compared to those collected at retail. This may be considered as a hint that most of the samples were national production, and both sampling places are reflecting similar situations. As regards the contribution of the investigating institutes to explaining the variance in the occurrence of ESBL/pAmpC positive food samples, the most probable explanation is the impact of different production and trading chains, but data are lacking to investigate this further. Nevertheless, although protocols had been harmonised in advance, differences in the detection methods cannot be completely ruled out.

4.4. Limitations of the study

In our study, for some positive samples detailed results of confirmation and characterization were missing as the isolate was not available. This introduces some bias into the data, but it could be shown that it didn't have a major impact. Furthermore, as expected, not all phenotypic cefotaxime-resistant *E. coli* could finally be confirmed to be ESBL or pAmpC producers, as also other mechanisms including β-lactamases and overexpression of the chromosomal AmpC gene may cause this phenotype. Our findings are in line with published studies which showed that a high proportion (~90%) of cefotaxime-resistant *E. coli* strains could be confirmed by PCR to carry ESBL or pAmpC genes (Laube et al., 2014). Compared to other studies, we tried to be very precise in identification of true ESBLs and pAmpCs.

One of the major limitations of the study is that collection of detailed data on the brand and history of the products (places of harvest, production and processing) was not possible. Sampling was organized via official food inspectors, which ensured a high level of standardization of the procedures. Furthermore, we included only four federal states, both facts might have its limitation in collecting a true representative sample for the territory of Germany. Furthermore, primary isolation was conducted by four different regional laboratories, which were trained in the detection method and successfully passed ring trials. Nevertheless, collection of a true random sample by one person and investigating all samples in one laboratory might have reduced variability observed. But as similar results regarding prevalence and subtypes have been observed in other studies, and this study was much more comprehensive to others, this is not considered a major limitation.

5. Conclusions

Our study results reflect that consumers might get exposed to ESBL/pAmpC-producing *E. coli* through several food chains. These results together with those collected at primary production and in the human population in other studies will allow more detailed analysis of the foodborne pathways, considering transmission from livestock populations to food at retail and to consumers. Further detailed studies are needed to give a better insight into the factors contributing to their prevalence, distribution and relevance as a foodborne threat.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vetmic.2019.03.025.

References

Agersø, Y., Aarestrup, F.M., Pedersen, K., Seyfarth, A.M., Struve, T., Hasman, H., 2012. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. J. Antimicrob. Chemother. 67, 582–588.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Control 19, 716–723.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.

Barton, K., 2016. MuMIn: Multi-Model Inference. R Package Version 1.15, pp. 6.

Belmar Campos, C., Fenner, I., Wiese, N., Lensing, C., Christner, M., Rohde, H., Aepfelbacher, M., Fenner, T., Hentschke, M., 2014. Prevalence and genotypes of extended spectrum beta-lactamases in Enterobacteriaceae isolated from human stool and chicken meat in Hamburg, Germany. Int. J. Med. Microbiol. 304, 678–684.

Ben Said, L., Jouini, A., Klibi, N., Dziri, R., Alonso, C.A., Boudabous, A., Ben Slama, K., Torres, C., 2015. Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. Int. J. Food Microbiol. 203, 86–92.

Börjesson, S., Ny, S., Egervärn, M., Bergström, J., Rosengren, Å., Englund, S., Löfmark, S., Byfors, S., 2016. Limited dissemination of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing *Escherichia coli* from food and farm animals, Sweden. Emerg. Infect. Dis. 22, 634–640.

Bray, J.R., Curtis, J.T., 1957. An ordination of upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349.

BVL-Report, 2017. Berichte zur Lebensmittelsicherheit. . Zoonosen-Monitoring 2016. https://www.bvl.bund.de/SharedDocs/Downloads/01_Lebensmittel/04_Zoonosen_Monitoring/Zoonosen_Monitoring_Bericht_2016.html?.nn=1401286.

Chen, C.M., Ke, S.C., Li, C.R., Wu, Y.C., Chen, T.H., Lai, C.H., Wu, X.X., Wu, L.T., 2017. High diversity of antimicrobial resistance genes. Class 1 integrons, and genotypes of multidrug-resistant *Escherichia coli* in beef carcasses. Microb. Drug Resist. 23, 915–924.

CLSI M07-A9, 2012. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9 (ISBN 1-56238-784-7 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., Mevius, D., 2013. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J. Antimicrob. Chemother. 68, 60–67.

Dorado-García, A., Smid, J.H., van Pelt, W., Bonten, M.J.M., Fluit, A.C., van den Bunt, G., Wagenaar, J.A., Hordijk, J., Dierikx, C.M., Veldman, K.T., de Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T.J.G.M., Velthuis, A.G., Heuvelink, A., Gonggrijp, M.A., van Duijkeren, E., van Hoek, A.H.A.M., de Roda Husman, A.M., Blaak, H., Havelaar, A.H., Mevius, D.J., Heederik, D.J.J., 2017. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. J. Antimicrob. Chemother. 18 (Epub ahead of print).

Doumith, M., Day, M.J., Hope, R., Wain, J., Woodford, N., 2012. Improved multiplex PCR strategy for rapid assignment of the four major *Escherichia coli* phylogenetic groups. J. Clin. Microbiol. 50, 3108–3110.

ECDC (European Centre for Disease Prevention and Control), 2017. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net), Stockholm.

EFSA, 2011. Panel on Biological Hazards (BIOHAZ) Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA J. 9, 2322.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017. The European Union summary report on

antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. EFSA J. 15, 4694.
Egervärn, M., Börjesson, S., Byfors, S., Finn, M., Kaipe, C., Englund, S., Lindblad, M., 2014. *Escherichia coli* with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and Salmonella on meat imported into Sweden. Int. J. Food Microbiol. 171, 8–14.
Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin. Microbiol. Infect. 18, 646–655.
Fischer, J., Hille, K., Ruddat, I., Mellmann, A., Köck, R., Kreienbrock, L., 2017. Simultaneous occurrence of MRSA and ESBL-producing Enterobacteriaceae on pig farms and in nasal and stool samples from farmers. Vet. Microbiol. 200, 107–113.
Geser, N., Stephan, R., Hächler, H., 2012. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet. Res. 8, 21.
Hartmann, A., Locatelli, A., Amoureux, L., Depret, G., Jolivet, C., Gueneau, E., Neuwirth, C., 2012. Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy Region). Front. Microbiol. 3, 83.
Hering, J., Frömke, C., von Münchhausen, C., Hartmann, M., Schneider, B., Friese, A., Rösler, U., Kreienbrock, L., Hille, K., 2016. Cefotaxime-resistant *Escherichia coli* in broiler farms – a cross-sectional investigation in Germany. Prev. Vet. Med. 125, 154–157.
Hering, J., Hille, K., Frömke, C., von Münchhausen, C., Hartmann, M., Schneider, B., Friese, A., Roesler, U., Merle, R., Kreienbrock, L., 2014. Prevalence and potential risk factors for the occurrence of cefotaxime resistant *Escherichia coli* in German fattening pig farms – a cross-sectional study. Prev. Vet. Med. 116, 129–137.
Hille, K., Ruddat, I., Schmid, A., Hering, J., Hartmann, M., von Münchhausen, C., Schneider, B., Messelhäusser, U., Friese, A., Mansfeld, R., Käsbohrer, A., Hörmansdorfer, S., Roesler, U., Kreienbrock, L., 2017. Cefotaxime-resistant *E. coli* in dairy and beef cattle farms-Joint analyses of two cross-sectional investigations in Germany. Prev. Vet. Med. 142, 39–45.
Irrgang, A., Falgenhauer, L., Fischer, J., Ghosh, H., Guiral, E., Guerra, B., Schmoger, S., Imirzalioglu, C., Chakraborty, T., Hammerl, J.A., Käsbohrer, A., 2017. CTX-M-15-producing *E. coli* isolates from food products in Germany are mainly associated with an IncF-type plasmid and belong to two predominant clonal *E. coli* lineages. Front. Microbiol. 8, 2318.
Irrgang, A., Hammerl, J.A., Falgenhauer, L., Guiral, E., Schmoger, S., Imirzalioglu, C., Fischer, J., Guerra, B., Chakraborty, T., Käsbohrer, A., 2018. Diversity of CTX-M-1 producing *E. coli* from German food samples and the spread of the ESBL gene due to an IncI1 ST3 plasmid. Vet. Microbiol. 221, 98–104.
Jorgensen, R.L., Nielsen, J.B., Friis-Moller, A., Fjeldsoe-Nielsen, H., Schonning, K., 2010. Prevalence and molecular characterization of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. J. Antimicrob. Chemother. 65, 460–464.
Khalafalla, F., Gergis, A.F., el-Sherif, A., 1993. Effect of freezing and mincing technique on microbial load of minced meat. Nahrung 37, 422–427.
Kola, A., Kohler, C., Pfeifer, Y., Schwab, F., Kuhn, K., Schulz, K., Balau, V., Breitbach, K., Bast, A., Witte, W., Gastmeier, P., Steinmetz, I., 2012. High prevalence of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany. J. Antimicrob. Chemother. 67, 2631–2634.
Laube, H., Friese, A., von Salviati, C., Guerra, B., Rösler, U., 2014. Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. Vet. Microbiol. 172, 519–527.
Li, L., Ye, L., Yu, L., Zhou, C., Meng, H., 2016. Characterization of extended spectrum β-lactamase producing enterobacteria and methicillin-resistant *Staphylococcus aureus* isolated from raw pork and cooked pork products in South China. J. Food Sci. 81, M1773–M1777.
Marti, R., Muniesa, M., Schmid, M., Ahrens, C.H., Naskova, J., Hummerjohann, J., 2016. Short communication: heat-resistant *Escherichia coli* as potential persistent reservoir of extended-spectrum β-lactamases and Shiga toxin-encoding phages in dairy. J. Dairy Sci. 99, 8622–8632.
McFadden, D., 1974. Conditional Logit Analysis of Qualitative Choice Behavior. Academic Press, New York, pp. 105–142.
Nguyen, P., Nguyen, T.A., Le, T.H., Tran, N.M., Ngo, T.P., Dang, V.C., Kawai, T., Kanki, M., Kawahara, R., Jinnai, M., Yonogi, S., Hirai, Y., Yamamoto, Y., Kumeda, Y., 2016. Dissemination of extended-spectrum β-lactamase- and AmpC β-lactamase-producing *Escherichia coli* within the food distribution system of Ho Chi Minh City, Vietnam. Biomed. Res. Int. 2016, 8182096.
Ojer-Usoz, E., González, D., Vitas, A.I., Leiva, J., García-Jalón, I., Febles-Casquero, A., Escolano Mde, L., 2013. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in meat products sold in Navarra, Spain. Meat Sci. 93, 316–321.
Ojer-Usoz, E., González, D., Vitas, A.I., 2017. Clonal diversity of ESBL-producing *Escherichia coli* isolated from environmental. Human and food samples. Int. J. Environ. Res. Public Health 14 (7).
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, H.M.H., Szoecs, E., Wagner, H., 2017. Vegan: Community Ecology Package. R Package Version 2.4-2.
Pehlivanlar Önen, S., Aslantaş, Ö., Şebnem Yılmaz, E., Kürekci, C., 2015. Prevalence of β-lactamase producing *Escherichia coli* from retail meat in Turkey. J. Food Sci. 80 (9) M2023-9.
Petternel, C., Galler, H., Zarfel, G., Luxner, J., Haas, D., Grisold, A.J., Reinthaler, F.F., Feierl, G., 2014. Isolation and characterization of multidrug-resistant bacteria from minced meat in Austria. Food Microbiol. 44, 41–46.
Pietsch, M., Irrgang, A., Roschanski, N., Brenner Michael, G., Hamprecht, A., Rieber, H., Käsbohrer, A., Schwarz, S., Rösler, U., Kreienbrock, L., Pfeifer, Y., Fuchs, S., Werner, G., RESET Study Group, 2018. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. BMC Genom. 19, 601.
Development Core Team R, 2016. R: A Language and Environment for Statistical Computing, Version 3.3.2. R Foundation for Statistical Computing, Vienna, Austria.
Randall, L.P., Lodge, M.P., Elviss, N.C., Lemma, F.L., Hopkins, K.L., Teale, C.J., Woodford, N., 2017. Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant *Escherichia coli*. Int. J. Food Microbiol. 241, 283–290.
Randall, L.P., Clouting, C., Horton, R.A., Coldham, N.G., Wu, G., Clifton-Hadley, F.A., Davies, R.H., Teale, C.J., 2011. Prevalence of *Escherichia coli* carrying extended-spectrum beta-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J. Antimicrob. Chemother. 66, 86–95.
Raphael, E., Wong, L.K., Riley, L.W., 2011. Extended-spectrum betalactamase gene sequences in gram-negative saprophytes on retail organic and nonorganic spinach. Appl. Environ. Microbiol. 77, 1601–1607.
Reuland, E.A., Al Naiemi, N., Raadsen, S.A., Savelkoul, P.H., Kluytmans, J.A., Vandenbroucke-Grauls, C.M., 2014. Prevalence of ESBL-producing Enterobacteriaceae in raw vegetables. Eur. J. Clin. Microbiol. Infect. Dis. 33, 1843–1846.
Rodriguez, I., Barownick, W., Helmuth, R., Mendoza, M.C., Rodici o, M.R., Schroeter, A., Guerra, B., 2009. Extended-spectrum beta-lactamases and AmpC beta-lactamases in ceftiofur-resistant Salmonella enterica isolates from food and livestock obtained in Germany during 2003–07. J. Antimicrob. Chemother. 64, 301–309.
Schmid, A., Hörmansdorfer, S., Messelhäusser, U., Käsbohrer, A., Sauter-Louis, C., Mansfeld, R., 2013. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* on Bavarian dairy and beef cattle farms. Appl. Environ. Microbiol. 79, 3027–3032.
Schwaiger, K., Helmke, K., Hölzel, C.S., Bauer, J., 2011. Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. supermarket). Int. J. Food Microbiol. 148, 191–196.
Sheikh, A.A., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Boerlin, P., Reid-Smith, R., Aslam, M., 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* isolated from retail meat purchased in Alberta, Canada. Foodborne Pathog. Dis. 9, 625–631.
Sørensen, T., 1948. A method of establishing groups of equal amplitudes in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. Kongelige Danske Videnskabernes Selskab 5, 1–34.
Tekiner, İ.H., Özpınar, H., 2016. Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. Braz. J. Microbiol. 47, 444–451.
Valentin, L., Sharp, H., Hille, K., Seibt, U., Fischer, J., Pfeifer, Y., Michael, G.B., Nickel, S., Schmiedel, J., Falgenhauer, L., Friese, A., Bauerfeind, R., Roesler, U., Imirzalioglu, C., Chakraborty, T., Helmuth, R., Valenza, G., Werner, G., Schwarz, S., Guerra, B., Appel, B., Kreienbrock, L., Käsbohrer, A., 2014. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. Int. J. Med. Microbiol. 304, 805–816.
Valenza, G., Nickel, S., Pfeifer, Y., Eller, C., Krupa, E., Lehner-Reindl, V., Höller, C., 2014. Extended-spectrum-beta-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. Antimicrob. Agents Chemother. 58, 1228–1230.
van Hoek, A.H., Veenman, C., van Overbeek, W.M., Lynch, G., de Roda Husman, A.M., Blaak, H., 2015. Prevalence and characterization of ESBL- and AmpC-producing Enterobacteriaceae on retail vegetables. Int. J. Food Microbiol. 204, 1–8.
Von Salviati, C., Friese, A., Roschanski, N., Laube, H., Guerra, B., Käsbohrer, A., Kreienbrock, L., Roesler, U., 2014. Extended-spectrum beta-lactamases (ESBL)/AmpC beta-lactamases-producing *Escherichia coli* in German fattening pig farms: a longitudinal study. Berl. Munch. Tierarztl. Wochenschr. 127, 412–419.
Wei, T., Simko, V., 2016. Corrplot: Visualization of a Correlation Matrix. R Package Version 0, pp. 77.
Zurfluh, K., Nüesch-Inderbinen, M., Morach, M., Zihler Berner, A., Hächler, H., Stephan, R., 2015. Extended-spectrum-β-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. Appl. Environ. Microbiol. 81, 3115–3120.