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Longitudinal Monitoring of Extended-Spectrum-Beta-Lactamase/AmpC-Producing *Escherichia coli* at German Broiler Chicken Fattening Farms

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Antimicrobial resistance of *Escherichia coli* to modern beta-lactam antibiotics due to the production of extended-spectrum beta-lactamases (ESBL) and/or plasmid-mediated AmpC beta-lactamases (AmpC) represents an emerging and increasing resistance problem that dramatically limits therapeutic options in both human and veterinary medicine. The presence of ESBL/AmpC genes in commensal *E. coli* from food-producing animals like broilers may pose a human health hazard. However, there are no data available concerning the prevalence of ESBL/AmpC-producing *E. coli* in German broiler flocks using selective methods. In this longitudinal study, samples were taken from seven conventional broiler fattening farms at three different times within one fattening period. Various samples originating from the animals as well as from their direct environment in the barn were investigated for the occurrence of ESBL/AmpC-producing *E. coli*. Average detection levels of 51, 75, and 76% in animal samples collected during the three samplings in the course of the fattening period demonstrate a colonization of even 1-day-old chicks, as well as a continuous significant ($P < 0.001$) increase in prevalence thereafter. The detection frequencies in housing environmental samples were relatively high, with an increase over time, and ranged between 54.2 and 100%. A total of 359 *E. coli* isolates were characterized by PCR and partly via the disc diffusion method. This study shows that prevalence of ESBL/AmpC-producing *E. coli* increases during the fattening period of the broiler flocks examined. Both colonized day-old chicks and contaminated farm environments could represent significant sources of ESBL/AmpC-producing *E. coli* in German broiler fattening farms.

Antimicrobial resistance is a major problem in both human and veterinary medicine. One mechanism of resistance which has recently gained more and more importance is the production of extended-spectrum beta-lactamases (ESBL) and plasmid-encoded cephamycinases (pAmpC). These enzymes inactivate many beta-lactam antibiotics, including modern extended-spectrum cephalosporins, by hydrolyzing their beta-lactam ring. Currently, CTX-M beta-lactamases represent the most widespread and still increasing ESBL type in humans and in animals (1–7). Among the AmpC beta-lactamases, CMY represents by far the most frequent beta-lactamase in livestock in Europe (3, 4, 8–10).

ESBL producers transfer resistance to oxyiminocephalosporins and often express a multidrug-resistant phenotype (6, 11, 12), resulting in dramatically limited therapeutic options. Additionally, plasmid-mediated AmpC beta-lactamases and, even more alarming, carbapenemases are leading to a worsening of the situation in both human and veterinary medicine (13). Although the resistant bacteria were initially observed in human samples, an increase in the detection of ESBL/AmpC-producing *E. coli* in animals, such as pigs (9, 14), horses (15), cattle, dogs, cats (4, 15), fish (16), and, particularly, broiler chickens, has been reported worldwide (3, 8, 12, 14). The gastrointestinal tract of healthy broilers may be an important reservoir for these beta-lactamase-producing bacteria (11). The relevance for German food production is reflected by the finding in a German study that 43.9% of chicken meat samples tested positive for ESBL-producing *Enterobacteriaceae*, predominantly *E. coli*, in a convenience sample (17). This represents an issue of public health due to a possible route for transmission to consumers, which was shown in different international studies (18, 19). So far, data on neither the prevalence of ESBL/AmpC-producing *E. coli* in German broiler fattening flocks nor the changes in the prevalence in the course of the fattening period are available. Moreover, little is known about the distribution and spread of these multidrug-resistant bacteria within broiler flocks. However, apart from the gastrointestinal tract of healthy broilers, the barn's environment itself serves as a reservoir for *E. coli* with zoonotic potential (20). Therefore, in this study, carried out within the consort project RESET (http://www.reset-verbund.de/), the changes in the prevalence of ESBL/AmpC-producing *E. coli* isolates in chicken as well as their occurrence within the barn's environment were investigated in the course of a fattening period in seven conventional broiler fattening flocks in Germany.

MATERIALS AND METHODS

Sampled farms. Broiler fattening farms in Germany were preselected by initial testing of pooled feces samples for the occurrence of ESBL/AmpC-producing *E. coli*. All of the screened broiler farms ($n = 16$) were suspected to be positive for these microorganisms based on phenotypic data.

Out of these, seven conventional broiler fattening farms, dispersed throughout Germany (northwest, northeast, and east), were selected for long-term investigations of the occurrence of ESBL/AmpC-producing *E. coli*. Besides the declaration of consent of the farmers, criteria for farm

Received 15 March 2013 Accepted 30 May 2013
Published ahead of print 7 June 2013
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.00856-13.

doi:10.1128/AEM.00856-13

TABLE 1 Detection frequencies of ESBL/AmpC-producing *E. coli* obtained from cloacal swabs from seven German broiler fattening farms

Sampling[b]	% ESBL/AmpC-positive samples (total no.) for farm[a]:							
	All	1	2	3	4	5	6	7
First	51 (140)	85 ($n = 20$)	95 (20); ENR	40 (20); AMX, INN	60 (20)	55 (20); ENR	0 (20); ENR	20 (20); ENR
Second	75 (140)	100 (20)	60 (20)	60 (20); PEN, COL	85 (20); COL	75 (20)	95 (20)	50 (20); ENR
Third	76 (140)	100 (20)	85 (20)	85 (20)	75 (20)	25 (20)	100 (20)	68 (20)

[a] Numbers in parentheses are numbers of samples from all farms at each sampling. Between-sample antimicrobial treatments, if any, are listed after the numbers of samples. COL, colistin; ENR, enrofloxacine; AMX, amoxicillin; INN, neomycin; PEN, benzylpenicillin.
[b] First sampling, about the 1st to 2nd day of life; second sampling, about the 14th to 18th day of life; third sampling, about the 26th to 35th day of life.

selection were a positive result for phenotypically suspected ESBL/AmpC-producing *E. coli* based on growth on MacConkey agar (CM 0115; Oxoid, Wesel, Germany) supplemented with 1 mg/liter cefotaxime (D-6429; Applichen GmbH, Darmstadt, Germany) (termed MC$^+$ agar) and species identification by matrix-assisted laser desorption ionization-time of flight (Bruker Daltonik GmbH, Bremen, Germany) in initial tests of pooled feces samples. On each farm, a single representative barn was chosen for sample collection. Each broiler flock was investigated at three time points (3 to 36 h after restocking and 14 to 18 and 26 to 35 days after being housed) during one fattening period. On all farms, a standardized questionnaire was used to obtain information concerning general management and hygiene, including the use of antibiotics for the flock under investigation. Unit sizes ranged from 48,000 to 360,000 animals per farm, 2 to 15 animal houses per farm, and 20,000 to 82,000 animals per house. All farms employed an all-in, all-out management scheme for the individual animal houses. The fattening period for the examined flocks ranged between 35 and 42 days. In three out of the seven animal houses, preharvesting of a few animals was established at the third sampling. On farm 1, no antibiotics were used during the entire fattening period. On all other farms, antibiotics were applied at least once during the fattening period. The antibiotic treatments were carried out due to pericardial and yolk sac infections, *E. coli* enteritis/infections, and/or mixed infections. The antibiotics used were mainly enrofloxacin and colistin. Enrofloxacin was used on farms 2, 5, and 6 during the first 3 days of fattening and also at the end of the fattening period on farm 7. On farms 3 and 4, colistin was used at the beginning of the fattening period, whereas on farm 4 the substance was also applied for 2 days at the end of the fattening period. Amoxicillin, neomycin, and benzylpenicillin were used on farm 3 only (Table 1).

Sampling at the broiler farms. During each sampling, 20 broilers were sampled randomly by collecting cloacal swabs (dry thin cotton swab; Nerbe Plus GmbH, Winsen/Luhe, Germany). Environmental samples, such as pooled samples of approximately 200 g feces and 200 g litter, as well as approximately 2 g pooled dust samples, were collected from at least 10 different spots on each sampling date. Moreover, four environmental swabs from water and feeding troughs, walls, and scale/radiators were collected using dry cotton swabs (Sarstedt AG & Co. KG, Nümbrecht, Germany) moistened with phosphate-buffered saline (PBS). With each environmental swab, material was collected from 10 different places evenly distributed inside the barn, swabbing a surface of approximately 15 cm^2 each. Additionally, a pair of boot swabs (Finnimport, Hamburg, Germany) was taken at each sampling date by walking the whole length of the investigated barn.

Laboratory analysis. All samples were processed within 24 h of sampling, at which time they were cooled to 4°C.

Animal and environmental samples. All samples were cultured on MC$^+$ agar without preenrichment and after preenrichment in Luria-Bertani (LB) broth (Merk KGaA, Darmstadt, Germany) (21). For culture without preenrichment, cloacal swabs as well as environmental swabs were streaked directly on MC$^+$ agar; for all other samples, 100 μl of an initial suspension was plated. To prepare the initial suspension, 225 ml LB broth was added to 25 g of pooled feces samples, 25 g of litter samples, and one pair of boot swabs, respectively, and homogenized using a stomacher (260 rpm for 2 min). For dust, 0.1 g of the dust sample was dissolved in 10 ml PBS plus 0.01% Tween 20 and shaken for approximately 30 min.

To determine quantitative data concerning ESBL/AmpC-producing *E. coli* and overall *E. coli* numbers in the pooled feces and dust samples, respectively, 100 μl of the initial suspension and a 1:10 dilution were streaked onto three MC$^+$ agar plates as well as onto three Gassner agar plates (CM431; Oxoid GmbH, Wesel, Germany). All agar plates were incubated overnight at 37°C under aerobic conditions.

For preenrichment, 1 ml of the initial dilution of the dust, feces, litter, and sock samples was transferred into 9 ml LB broth, while cloacal swabs and environmental swabs were directly transferred into 9 ml LB broth. The LB broths were incubated overnight at 37°C under aerobic conditions. After the preenrichment step, a loopful of all samples was streaked onto MC$^+$ agar and incubated again overnight at 37°C under aerobic conditions.

Confirmation and characterization of ESBL/AmpC β-lactamases in *E. coli*. For each sample, the species was identified with a randomly chosen suspected *E. coli* colony using MALDI-TOF (Bruker Daltonik GmbH, Bremen), and the occurrence of ESBL/AmpC was confirmed by PCR. To this end, the presence of the extended-spectrum beta-lactamase genes $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} and the AmpC beta-lactamase gene bla_{CMY} were examined as described previously (22–25) or using protocols kindly provided by the ESBL SafefoodEra Consortium (EU-SAFEFOODERA project 08176). The bla_{CMY} gene was chosen because it is the most frequently detected AmpC beta-lactamase gene in livestock in Europe (3, 4, 8–10). All isolates yielding positive results in bla_{TEM} and/or bla_{SHV} PCRs were subsequently sequenced by using SHV primers as described by Weill et al. (24) or by using TEM primers as described by Olesen et al. (26) in order to unequivocally confirm the presence of extended-spectrum beta-lactamases in these samples (24, 26). The nucleotide sequences were analyzed with BioNumerics software (version 6.6). The BLAST program of NCBI (http://blast.ncbi.nlm.nih.gov/) was used for database comparison.

Antimicrobial susceptibility testing for ESBL/AmpC detection. All isolates tested negative by PCR ($n = 7$), and all isolates harboring only the $bla_{\text{TEM-1}}$ gene ($n = 45$) were subjected to susceptibility testing by the disc diffusion method (27). The antibiotics tested were cefotaxime (CTX; 30 μg), amoxicillin-clavulanic acid (AMC; 30 μg), cefepime (FEP; 30 μg), cefoxitin (FOX; 30 μg), and cefotaxime-clavulanic acid (CTX-Clav; 30 and 10 μg, respectively). An inhibition zone diameter (IZD) of ≤26 mm for both CTX and CTX-Clav in combination with an IZD of ≤18 mm for FOX and AMC was considered phenotypic proof of AmpC production. An increase of more than 5 mm in the IZD for CTX-Clav versus that for CTX alone was considered phenotypic proof of ESBL production (28, 29) (Table 2).

Statistical analyses. Statistical analyses were performed by using SPSS (version 13; Chicago, IL). To explore the data, classical chi-square tests were used, omitting any adjustment for multiple testing and considering statistical significance if $P < 0.05$. For descriptive statistical analysis of quantitative bacterial counts, measures of location were used to describe the outcome. Explorative comparison of groups was performed using the Wilcoxon test procedure.

RESULTS

Animal samples and samples of animals' environments. ESBL/AmpC-producing *E. coli* organisms were detected in all investi-

TABLE 2 Detection frequencies of ESBL/AmpC-producing *E. coli* (mean values) in samples from the housing environments of all investigated farms

Source of ESBL/AmpC-positive samples (three samplings each)	% ESBL/AmpC-positive samples (total no.) for all farms/all samplings	Detection frequency for farm[a]:						
		1	2	3	4	5	6	7
Pooled feces	100 (20)	+++	+++	+++	+++	+++	o++	+++
Litter	95.2 (18)	+++	+++	+++	+++	+++	−++	+++
Boot swabs	90.4 (21)	+++	+++	+++	+++	−++	−++	+++
Pooled dust	71.4 (21)	+++	+++	+++	−++	+−+	−++	+−+
Environmental swabs	54.2 (83)	+++	+++	+++	+++	+++	−−+	+++

[a] Each sign represents one sampling date; +, positive for ESBL/AmpC-producing *E. coli*; −, negative for ESBL/AmpC-producing *E. coli*; o, sample not taken.

gated broiler fattening farms. Except for farm 6, where no ESBL/AmpC-producing *E. coli* could be detected during the first sampling, these pathogens were found at all times of investigation on all farms. At least one sample per barn and sampling tested positive for ESBL/AmpC-producing *E. coli* even without the preenrichment step, again with the exception of farm 6. Without preenrichment, the detection frequencies of ESBL/AmpC-producing *E. coli* in individual animal samples were 32% ($n = 140$) for the first, 59% ($n = 140$) for the second, and 64% ($n = 140$) for the third sampling. Including the preenrichment step, the detection frequencies increased from 51% ($n = 140$) for the first sampling up to 75 and 76% ($n = 140$) for the second and third samplings, respectively (Table 1). Moreover, the detection frequency of ESBL/AmpC-producing *E. coli* in animal samples (cloacal swabs) increased significantly ($P < 0.001$) from the first sampling (3 to 36 h after housing of the hatched broilers) to the second sampling (about 14 to 18 days of life). High detection frequencies of ESBL/AmpC-producing *E. coli* were also found in all samples of the animals' environments (Table 2). All pooled feces samples (100%; $n = 20$), 95.2% of the pooled litter samples ($n = 21$), and 90.4% of the boot swabs ($n = 21$) tested positive. Even 71.4% of the pooled dust samples ($n = 21$) and 54.2% of the different environmental swabs, including walls, scales, water, and feeding troughs ($n = 83$), revealed positive results for ESBL/AmpC-producing *E. coli*. Moreover, the detection frequency of ESBL/AmpC-producing *E. coli* in all environmental samples together also increased significantly ($P < 0.05$) from the first to the second sampling. Detection frequencies for litter and boot swabs increased from 86 and 71%, respectively, at the first samplings to 100% at samplings two and three. The detection frequencies of ESBL/AmpC-producing *E. coli* in pooled dust samples were 57% for the first, 71% for the second, and 86% for the third sampling. For the environmental swabs, there was an increase in the detection frequencies from 37 to 61% during the fattening period.

The geometric mean bacterial count (CFU/g) of ESBL/AmpC-presumptive *E. coli* was 1.25E+06 CFU/g for pooled feces samples and 4.00E+03 CFU/g for dust samples (Table 3). The amount of *E. coli* obtained from Gassner agar was 1.57E+06 CFU/g in pooled feces and 5.87E+03 CFU/g in dust samples. In all evaluable fecal and dust samples, the average proportion of ESBL/AmpC-producing *E. coli* out of the total amount of *E. coli* was 17.41% (values ranged between 0.023 and 92.41% for the single investigated samples) and 8.62% for dust samples, respectively (values ranged between 0.38 and 22.1% for the single investigated samples). The average number of ESBL/AmpC-suspected *E. coli* organisms obtained from pooled feces and pooled dust samples did not increase during the fattening period ($P = 0.775$ and 0.104 for pooled feces and pooled dust, respectively, by the Wilcoxon test).

Characterization of beta-lactamases in *E. coli*. The occurrence of ESBL/AmpC beta-lactamase genes was confirmed for one

TABLE 3 Number of ESBL/AmpC-producing *E. coli* (CFU/g) obtained from MC$^+$ agar and Gassner agar from pooled feces and pooled dust samples

Sample type and statistic	No. of ESBL/AmpC-producing *E. coli* (CFU/g) obtained with:					
	MC$^+$ agar			Gassner agar		
	1st sampling[a] ($n = 6$)	2nd sampling ($n = 7$)	3rd sampling ($n = 7$)	1st sampling ($n = 4$)	2nd sampling[b] ($n = 6$)	3rd sampling[c] ($n = 6$)
Pooled feces						
Minimum	7.13E+04	2.80E+04	3.97E+03	2.82E+05	8.00E+05	3.00E+05
Median	1.48E+06	1.69E+06	1.79E+06	6.39E+05	2.07E+06	7.30E+05
Mean	1.60E+07	4.48E+05	6.90E+06	1.01E+06	1.25E+08	1.22E+06
Geometric mean	2.00E+06	1.35E+06	7.65E+05	7.20E+05	5.24E+06	7.86E+05
Maximum	8.50E+07	1.86E+06	2.36E+07	2.49E+06	7.34E+08	4.00E+06
Pooled dust						
Minimum	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.36E+03	3.30E+02
Median	0.00E+00	8.33E+03	1.00E+03	0.00E+00	2.60E+04	5.30E+03
Mean	6.29E+03	5.81E+03	3.90E+04	1.50E+02	2.52E+04	4.72E+04
Geometric mean	4.40E+04	5.04E+03	2.22E+03	6.00E+02	1.20E+04	6.04E+03
Maximum	4.40E+04	1.26E+04	1.67E+04	6.00E+02	4.83E+04	2.12E+05

[a] For the pooled dust sampling, $n = 7$.
[b] For the pooled dust sampling, $n = 3$.
[c] For the pooled dust sampling, $n = 5$.

TABLE 4 Proven ESBL/AmpC producers (using PCR and ESBL/AmpC genes) from the investigated German broiler fattening farms[b]

Detected gene(s)	ESBL/AmpC producer status[a]	No. of *E. coli* isolates	% *E. coli* isolates ($n = 359$)
CMY	+	78	21.73
SHV-12	+	47	13.12
CTX-M	+	38	10.59
TEM-52	+	26	7.24
TEM-1	(−)	45	12.54
CMYand TEM-1	+	69	19.22
SHV-12 and TEM-1	+	33	9.12
CTX-M and CMY	+	6	1.67
CTX-M and TEM-1	+	4	1.11
TEM-52 and SHV-12	+	2	0.56
TEM-52 and CMY	+	1	0.28
SHV-12 and CTX-M	+	1	0.28
CTX-M, SHV-12, and TEM-1	+	1	0.28
SHV-12, CMY, and TEM-1	+	1	0.28
None[c]	(−)	7	1.95
Proven ESBL/AmpC producer		307	85.51

[a] +, proven to be an ESBL/AmpC producer; (−), still suspected of being an ESBL/AmpC producer.
[b] Three hundred fifty-nine *E. coli isolates* suspected of being ESBL/AmpC producers were examined.
[c] Negative in all PCRs.

E. coli isolate chosen randomly from each presumptive positive sample. In total, 359 suspected ESBL/AmpC-producing *E. coli* isolates were analyzed by PCR and, if necessary, sequenced to examine the presence of the beta-lactamase genes $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} and the AmpC beta-lactamase gene bla_{CMY}. Therefore, ESBL and/or plasmidic class C beta-lactamase genes (bla_{CMY}) were confirmed in 307 (85.5%) *E. coli* isolates. Details concerning all characterized genes and gene combinations are shown in Table 4.

On farms 1 and 2, mostly only bla_{CMY} genes and combinations of bla_{CMY} and $bla_{\text{TEM-1}}$ genes were detected in both animal samples (cloacal swabs) and samples from the animals' environment during all three samplings. Additionally, on farm 2, colonies carrying $bla_{\text{TEM-1}}$ genes only were frequently detected in environmental samples, and *E. coli* colonies harboring $bla_{\text{CTX-M}}$ genes only were obtained from single animals during the third sampling. In contrast, $bla_{\text{SHV-12}}$ was the dominant gene found in samples of animals on farms 3 and 4. Sporadically, single $bla_{\text{TEM-52}}$ genes as well as $bla_{\text{TEM-1}}$ genes, in combination with a bla_{CMY} gene, were detected in animal samples from the first sampling. Environmental samples also mainly harbored single $bla_{\text{SHV-12}}$ genes or $bla_{\text{TEM-1}}$ genes during the entire fattening period. Farm 5 showed single bla_{CMY} genes exclusively at the first sampling in all kinds of samples. In the course of the fattening period, other genes, such as the $bla_{\text{TEM-1}}$ gene, alone or in combination with bla_{CMY}, as well as single $bla_{\text{TEM-52}}$ genes, were also found. On farm 6, which was completely negative for ESBL/AmpC-producing *E. coli* during the first sampling, $bla_{\text{SHV-12}}$ alone and/or in combinations with $bla_{\text{TEM-1}}$ genes and $bla_{\text{CTX-M}}$ genes were detected in *E. coli* colonies from all samples during the later samplings. Isolates collected on farm 7 at the first sampling harbored single $bla_{\text{SHV-12}}$ genes and single bla_{CMY} genes in all samples, sometimes in combination with $bla_{\text{TEM-1}}$ genes. In the course of the fattening period, mainly isolates harboring single $bla_{\text{CTX-M}}$ genes and isolates harboring a combination of bla_{CMY} and $bla_{\text{TEM-1}}$ genes were detected in both kinds of samples (see Table S1 in the supplemental material).

Antimicrobial susceptibility testing. All isolates harboring only the $bla_{\text{TEM-1}}$ gene ($n = 45$), as well as those that tested negative via PCR ($n = 7$), were subjected to susceptibility testing by the disc diffusion method. ESBL/AmpC production was confirmed in 93.3% of the *E. coli* isolates with a single $bla_{\text{TEM-1}}$ gene. Three isolates (6.67%) could not be confirmed as ESBL/AmpC producers. The isolates that tested negative for $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, and bla_{CMY} by PCR were positive for ESBL (6/7) and AmpC (1/7) production by the disc diffusion method.

DISCUSSION

The results show a high occurrence of ESBL/AmpC-producing *E. coli* in German broiler flocks. Another main finding is its occurrence even in 1-day-old broilers, a result that has not been shown previously for German broilers. Additionally, ESBL/AmpC-producing *E. coli* could be found in samples of the animals' environment during the first sampling. The high detection frequencies shortly after the arrival of the broiler chicks raise the issue of the entry of these resistant microorganisms into the farms. In this regard, Mevius et al. (30) showed that the risk of introducing ESBL/AmpC-producing *E. coli* in the broiler production chain occurs two steps before by buying positive chicks for the restocking of grandparent's flocks. They showed a detection frequency of ESBL-producing *E. coli* between 22.5 and 44% in 1-day-old grandparent chicks. In the same study, the prevalence was 3.3% in 1-day-old broiler chicks, which is much lower than the value of 51% determined in our study. Thus, detailed investigation of possible vertical transmission routes of ESBL/AmpC-producing *E. coli* from hatching eggs to the day-old chicks is of fundamental importance and should be considered in further studies. In contrast to Mevius et al. (30), a study presented by Hiroi et al. (31) assumed contaminated barns as a consequence of insufficient cleaning and disinfection as a cause for high incidences of ESBL/AmpC-producing *E. coli* in broiler farms. The positive findings for various environmental samples at the first sampling date in our study support this hypothesis. Moreover, we showed a significant increase in the detection of ESBL/AmpC-producing *E. coli* from the first to the second samplings in animal as well as in environmental samples. In one farm there was no detection at all at the first sampling but very high detection rates of 95 and 100% during the following samplings. This leads to the suggestion of an enrichment of ESBL/AmpC-producing *E. coli* in the course of a fattening period in animals and/or in their environment.

Costa et al. (11) described the high concentration of animals in conventional flocks, such as the farms investigated in our study, as a possible cause of the high concentration of these pathogens in the animal house, which also facilitates/enhances the transmission of ESBL/AmpC-producing *E. coli* among animals. Additionally, our results show that feces, litter, and even dust may act as transmission sources of ESBL/AmpC-producing *E. coli* within a broiler barn; therefore, the organism may be on a farm and in different barns due to the use of the same equipment, shoes, or clothes. The fact that ESBL/AmpC-producing *E. coli* represents a high proportion out of all *E. coli* organisms in pooled feces and dust samples shows the high relevance of environmental samples as a source of transmission.

Another factor that may have influenced the high detection frequencies in our investigated farms is the use of antibiotics. As

only farm 3 used beta-lactam antibiotics, coselection under antibiotic pressure has to be discussed as a possible cause for the enrichment of ESBL/AmpC-producing *E. coli* on broiler fattening farms (3, 11, 32–34). In our study, 6 out of 7 ESBL/AmpC-positive broiler fattening flocks were treated with antibiotics, mainly enrofloxacin and colistin. The assumed coselection in our obtained ESBL/AmpC-producing isolates will be investigated in this ongoing project. In contrast, Hiroi et al. (31) demonstrated that non-beta-lactam antibiotics do not promote the development of ESBL producers at broilers in each case. Those authors suggested initial contamination of barns due to insufficient cleaning and disinfection as major causes for the high incidence of ESBL/AmpC-producing *E. coli* on broiler farms. In our study, even in the flock without any antibiotic treatment, ESBL/AmpC-producing *E. coli* were detected at a level of 100% in individual animal samples at the end of the fattening period. The high detection frequency (85%) in animal samples from this farm at the beginning of the fattening period suggests entry via hatched chicks or contaminated barn environments. As all investigated barns were from farms that tested positive for ESBL/AmpC-producing *E. coli* in the initial investigation, coselection may not be necessary for spread of ESBL/AmpC-producing *E. coli* within the farms in such high-prevalence situations.

Nevertheless, the influence of antibiotic treatment on ESBL prevalence seems likely; however, due to the small number of untreated farms in our study, this presumption could be not statistically proved. Further investigations about the role of different factors are necessary.

The high detection frequencies of ESBL-producing *E. coli* in this study are comparable to those of reports of other European investigations carried out at the farm or slaughterhouse level (1, 3, 10, 11, 34–36). In addition to these one-time samplings, our long-term investigations show increased detection frequencies of ESBL/AmpC-producing *E. coli* on most of the investigated farms during the fattening period. Even the barn without any findings of ESBL/AmpC-producing *E. coli* at the first sampling showed high detection frequencies, from 95 to 100%, in individual animal samples. Consequently, the time points of samplings also could influence the prevalence in screening studies.

Nevertheless, the spread of ESBL/AmpC-producing *E. coli* in healthy chickens and inside broiler barns is recognized worldwide, although differences in prevalence in different farms and countries are obvious (4). The wide spread of ESBL/AmpC-producing *E. coli* in food animals, especially in broilers, represents an issue of food safety. To this end, a recent German study detected ESBL-producing *Enterobacteriaceae*, predominantly *E. coli*, in 43.9% of 399 poultry meat samples. However, plasmid-mediated AmpC-producing microorganisms were not detected due to the experimental setup (17). In addition, Egea et al. (37) reported a contamination of raw poultry meat with ESBL-producing *E. coli* of 93.3%. Cohen et al. (18) also reported that 94% of chicken meat samples harbored at least one *E. coli* isolate with an ESBL phenotype. Viewed in context with our results, this is very alarming due to hypothesized transmission to humans via the food chain (38, 39).

We demonstrated that, including the results of PCR and disc diffusion testing, 99.2% of our investigated isolates could be confirmed as ESBL/AmpC producers, which is alarming.

Interestingly, on farms 3 and 4, mainly $bla_{\text{SHV-12}}$ and $bla_{\text{TEM-52}}$ genes were detected in all samples during the entire fattening period. These two individual barns belong to the same large fattening company, which leads to the suggestion that each fattening chain, including hatcheries, grandparent, and parent flocks, harbors a specific ESBL/AmpC gene pool. Further studies investigating the whole broiler production chain in detail are necessary to confirm this hypothesis. The first reported instance of a *Salmonella* isolate encoding carbapenemases (VIM-1) originated from poultry on one of the farms (G1). This isolate also encoded AmpC (40).

E. coli isolates in our study mainly harbored bla_{CMY} genes, often in combination with $bla_{\text{TEM-1}}$. Additionally, $bla_{\text{SHV-12}}$, genes of the $bla_{\text{CTX-M}}$ group, and (sporadically) $bla_{\text{TEM-52}}$ were detected. This is in line with the first detection of ESBL/AmpC-producing *E. coli* strains in healthy chickens harboring the resistance genes $bla_{\text{CTX-M}}$, $bla_{\text{SHV-12}}$, and bla_{CMY} (8) and is comparable to findings in numerous other studies on healthy broilers (9, 12, 14, 41).

Recently, a German study on ESBL-producing *E. coli* in chicken meat samples most frequently found the genes $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$, and $bla_{\text{CTX-M-1}}$ (17). The detection of the same ESBL genes in German broiler fattening farms as well as in chicken meat samples suggests a possible risk of transmission between animals and humans, which is in agreement with a study by Leverstein-van Hall et al. (42). Another study also showed identical ESBL genes, $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-52}}$, in poultry meat and human rectal swabs, which confirms possible transmission (19). Cortes et al. (9) even suggested that ESBL/AmpC-producing isolates, recovered from poultry, can have a higher pathogenicity for humans. However, there is no data on the exact risk of transmission of these pathogens between animals and humans.

ACKNOWLEDGMENTS

This work was financially supported by the Federal Ministry of Education and Research (funding number 01Kl1013C) as part of the joint research consortium RESET (http://www.reset-verbund.de).

We gratefully thank N. Roschanski, I. Rodríguez, and J. Fischer for scientific advice. All cooperating farmers and veterinarians, as well as the excellent technical assistance of H. Jansen, M. Thieck, S. Sellenthin, and K. Fiedler (Institute for Animal Hygiene and Environmental Health), are gratefully acknowledged.

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