

Low airborne tenacity and spread of ESBL-/AmpC-producing *Escherichia coli* from fertilized soil by wind erosion

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Summary

ESBL-/AmpC-producing *Escherichia coli* from organic fertilizers were previously detected on soil surfaces of arable land and might be emitted by wind erosion. To investigate this potential environmental transmission path, we exposed ESBL-/AmpC-positive chicken litter, incorporated in agricultural soils, to different wind velocities in a wind tunnel and took air samples for microbiological analysis. No data exist concerning the airborne tenacity of ESBL-/AmpC-producing *E. coli*. Therefore, we explored the tenacity of two ESBL/AmpC *E. coli* strains and *E. coli* K12 in aerosol chamber experiments at different environmental conditions. In the wind tunnel, ESBL/AmpC-producing *E. coli* were detected in none of the air

samples ($n = 66$). Non-resistant *E. coli* were qualitatively detected in 40.7% of air samples taken at wind velocities exceeding 7.3 m s^{-1} . Significantly increased emission of total viable bacteria with increasing wind velocity was observed. In the aerosol chamber trials, recovery rates of airborne *E. coli* ranged from 0.003% to 2.8%, indicating a low airborne tenacity. Concluding, an emission of ESBL/AmpC *E. coli* by wind erosion in relevant concentrations appears unlikely because of the low concentration in chicken litter compared with non-resistant *E. coli* and their low airborne tenacity, proven in the aerosol chamber trials.

Introduction

Enterobacteriaceae including *Escherichia coli* (*E. coli*) can have the capability to produce ESBL- (extended-spectrum beta-lactamase) and AmpC-enzymes. These enzymes hydrolyze the beta-lactam ring of most beta-lactam antibiotics, including extended-spectrum cephalosporins belonging to the third, fourth and fifth generation. Genes encoding for the production of ESBL- and AmpC-enzymes are found in pathogenic and commensal Enterobacteriaceae (Day *et al.*, 2016). Among resistant bacteria, ESBL-/AmpC-producing Enterobacteriaceae are of outstanding importance and categorized as critical priority concerning the development of new drugs by the World Health Organization (WHO, 2017).

The presence of antimicrobial resistance in the environment represents an increasing ‘One Health’ problem. There is evidence of a transmission of ESBL-producing *Escherichia coli* (*E. coli*) and their mobile genetic elements encoding for antimicrobial resistance between humans, animals and the environment (Smet *et al.*, 2010; Leverstein-van Hall *et al.*, 2011). ESBL-producing *E. coli* are frequently detected in broiler barns, with a prevalence of up to 100% (Dierikx *et al.*, 2010; Laube *et al.*, 2013; Blaak *et al.*, 2015; Hering *et al.*, 2016; Daehre *et al.*, 2017).

Besides the potential transfer of ESBL-producing *E. coli* from chickens to humans via direct contact (Dierikx

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et al., 2013; Huijbers et al., 2014) or contaminated meat (Leverstein-van Hall et al., 2011; Pietsch et al., 2018), ESBL-producing *E. coli* can spread in the farm environment and, thus, may act as a source for antimicrobial resistance. Apart from the spread through vectors such as flies (Blaak et al., 2014; von Salviati et al., 2015), another possibility of an environmental dissemination of ESBL-*E. coli* is the airborne route, which has been suggested from municipal sewage (Korzeniewska and Harnisz, 2013) and animal husbandries (Laube et al., 2014; Gao et al., 2015).

Applying ESBL-positive organic fertilizers to arable land presents a further important mode of transmission to the environment. For instance, ESBL-producing *E. coli* were detected in fertilized soil in studies carried out in Germany (Friese et al., 2013) and China (Zheng et al., 2017). In a French study ESBL-producing *E. coli* were even detected up to 1 year after the soil was fertilized with cow manure (Hartmann et al., 2012). Fields amended with ESBL-positive organic fertilizers might be affected by wind erosion, leading to bioaerosol formation (Jones and Harrison, 2004) and a possible airborne spread of these resistant bacteria. The microorganisms gain a lot of advantages from being transported together with soil particles, which ensure their survival during transport (Acosta-Martínez et al., 2015). The aim of this study was, therefore, to assess the relevance of airborne transmission of ESBL-producing *E. coli* by wind erosion after organic fertilization of fields. We performed three wind erosion trials with ESBL-positive chicken litter in a wind tunnel. In this context, the tenacity of resistant bacteria including ESBL-producing *E. coli* in the aerosolized state is of high interest and the current data situation is poor (Environment Agency, 2019). Therefore, we carried out a series of aerosol chamber experiments in order to investigate the airborne tenacity of two poultry-associated ESBL-/AmpC-producing *E. coli* strains and the non-resistant laboratory strain *E. coli* K12 under different conditions. This enabled a more in-depth interpretation of the results of the wind tunnel trial.

Results

Results from the wind tunnel trials

Concentrations of ESBL-/AmpC-producing and non-resistant *E. coli* in the litter and soil-litter mixtures. The results of the quantitative and qualitative analysis of ESBL-producing and non-resistant *E. coli* in the chicken litter and soil-litter mixtures for all trials are displayed in Table 1.

The molecular characteristics of the *E. coli* isolate taken in the three wind tunnel trials are summarized in Table S1 (supplementary material).

Wind velocity, relative humidity (RH) and temperature measured in the wind tunnel trials and detection of ESBL-/AmpC-producing and non-resistant *E. coli* in the air samples. The wind velocity measured for the different levels of wind tunnel engine power and in different distances to the soil-litter mixture is depicted in Table 2. The wind velocity increased with increasing wind tunnel engine power and increasing distance to the soil-litter mixture because friction decreases with increasing distance to soil. Marginal deviations were observed in the wind velocity measurements between the three trials caused by changes in atmospheric conditions outside the tunnel, like air temperature and atmospheric pressure. For that reason, the arithmetic mean of the wind velocity of all three trials is depicted. A distinct variation of the environmental conditions (relative humidity (RH) and temperature) was observed between the three wind tunnel trials. The highest mean temperature was measured in the first trial at 25.7°C. The temperature was the lowest for the second trial with 16.1°C. The RH was highest for the first trial with 56% and lowest for the third wind tunnel trial with 29.7% (Table 2).

Sixty-six air samples were analysed throughout the three trials at different wind velocities (11 AGI-30 and 11 Coriolis μ air samples per trial). Neither ESBL/AmpC-producing nor non-resistant *E. coli* were detected in the AGI-30 samples. In the Coriolis μ samples, only non-resistant *E. coli* were detected, albeit in levels below the detection levels of the quantitative assay (Table 2). Statistical analysis using logistic regression revealed that an increased wind velocity significantly correlated with a qualitative detection of *E. coli* in the air samples ($p = 0.022$).

Total viable bacterial count, PM_{10} and $PM_{2.5}$ emitted per m^2 of soil-litter mixture, soil aggregate size and collection efficiency of the AGI-30 and Coriolis μ air sampler. The geometric mean of total viable bacterial count emitted per m^2 of soil-litter mix for each wind tunnel trial is depicted in Fig. 1. Figure 1A shows the data for the AGI-30, Fig. 1B shows the data for the Coriolis μ air sampler. A similar emission was observed when comparing the three trials concerning the total viable count emitted at each wind velocity. However, with increasing wind velocity, a significant increase concerning the emission of total viable bacteria was observed in some trials for both air samplers. As all three trials showed a similar emission of total viable bacteria per m^2 , a pooled analysis was performed for both air sampling devices. The data are shown in the supplementary material in Fig. S1 for the AGI-30 (a) and the Coriolis μ air sampler (b). Here it becomes evident that there was a significantly increased emission between 5.6 and 7.3 $m s^{-1}$ and also between 7.3 and 9.8 $m s^{-1}$ but not between 9.8 and 10.6 $m s^{-1}$. Particle emission in

Table 1. Quantitative and qualitative detection of ESBL-/AmpC-producing and non-resistant *E. coli* in the chicken litter directly before and after mixing with soil for the three wind tunnel trials.

ESBL-/AmpC-producing <i>E. coli</i>				
	Trial 1	Trial 2		Trial 3
Litter (directly before mixing)	Below detection limit (+)	Barn A: 3.2×10^5 cfu g ⁻¹	Barn B: 4.2×10^5 cfu g ⁻¹	1.2×10^4 cfu g ⁻¹
Soil-litter mixture	Below detection limit (+/-)	2.9×10^3 cfu g ⁻¹		Below detection limit (+)
Non-resistant <i>E. coli</i>				
	Trial 1	Trial 2		Trial 3
Litter (directly before mixing)	Not determined (+)	Barn A: 2.0×10^7 cfu g ⁻¹	Barn B: 9.5×10^6 cfu g ⁻¹	8.0×10^5 cfu g ⁻¹
Soil-litter mixture	Not determined (+)	2.4×10^4 cfu g ⁻¹		8.2×10^2 cfu g ⁻¹

The result of the qualitative analysis is displayed in brackets when samples were not quantifiable.

Table 2. Qualitative detection of non-resistant *E. coli* in the Coriolis μ air samples at different wind velocities and environmental conditions measured for the three wind tunnel trials.

Wind tunnel engine power (%)	\bar{x} wind velocity 5 cm (m s ⁻¹)	\bar{x} wind velocity 30 cm (m s ⁻¹)	\bar{x} wind velocity 60 cm (m s ⁻¹)	\bar{x} wind velocity suspension chamber (m s ⁻¹)	Trial 1 T: 25.7°C RH: 56%	Trial 2 T: 16.1°C RH: 54.4%	Trial 3 T: 23.1°C RH: 29.7%
0	0	0	0	0	-	-	-
40	5.6	7.8	8.1	3.3	-	-	-
60	7.3	10.6	10.9	5.1	-	-	-
80	9.8	14.5	15.4	6.6	+	+	-
100	10.6	15.4	16.1	6.9	+	+	-

(+) *E. coli* qualitatively detectable (-) *E. coli* qualitatively undetectable; T: Temperature; RH: Relative humidity.

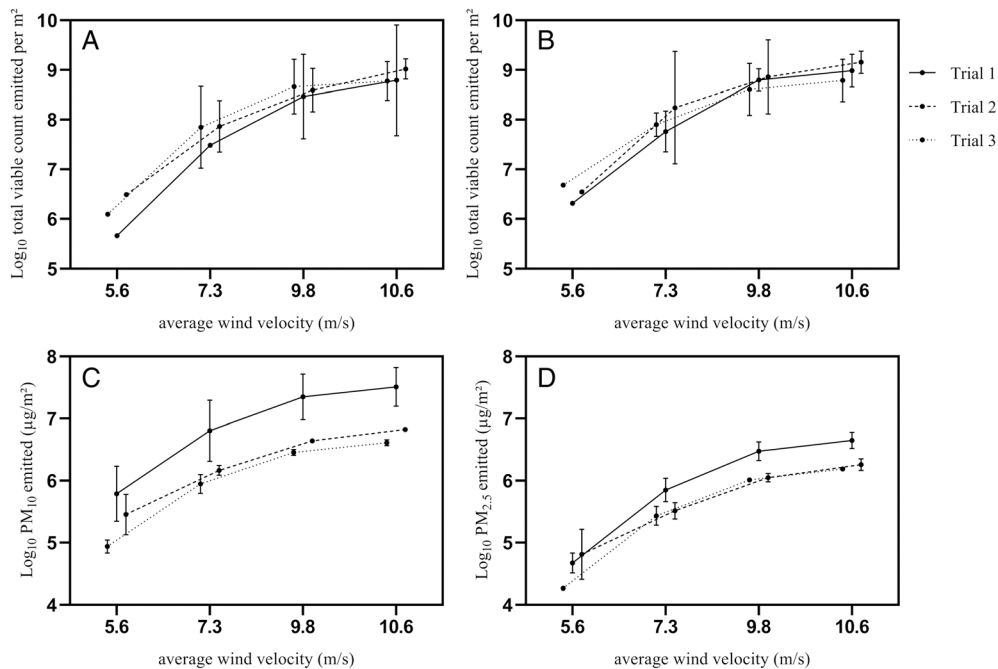


Fig. 1. LOG₁₀ total viable count emitted per m² of soil for each level of wind velocity determined with the all-glass-impinger 30 (A) and the Coriolis μ (B) for the three wind tunnel trials. PM₁₀ (C) and PM_{2.5} (D) emitted per m² of soil in μ g for the three wind tunnel trials. The error bars indicate the upper and lower 95% confidence intervals.

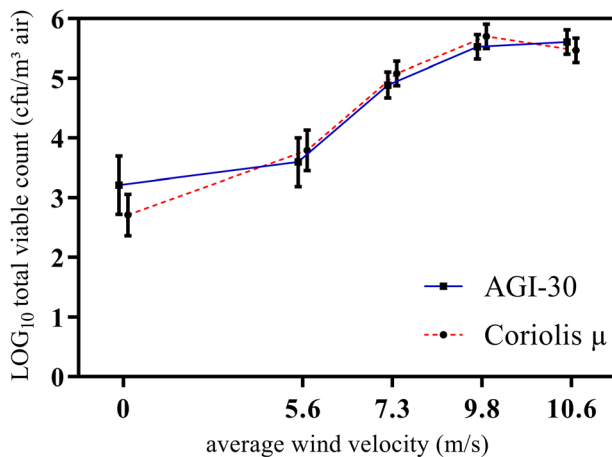


Fig. 2. Influence of the air sampling device used on total viable count detected per m³ of air in the wind tunnel trials at different wind velocities. The error bars indicate the upper and lower 95% confidence intervals.

μg m⁻² of soil-litter mixture is additionally depicted in Fig. 1 for PM₁₀ (c) and PM_{2.5} (d). At wind velocities of 7.3, 9.8 and 10.6 m s⁻¹, significantly increased PM emissions were observed for the first trial. Soil-litter mixture aggregates were the smallest in the first trial with only 33.8% of the particle mass having a diameter of >500 μm. For the second and third wind tunnel trial, aggregates were coarser with 45.2% and 49.4% of the particle mass having a diameter of >500 μm respectively.

Statistical modelling was used to investigate the influence of the air sampling device on the total viable bacterial count detected per m³ of air in the wind tunnel trials. The three wind tunnel trials were not stratified for the analysis. The data, including the upper and lower 95% confidence intervals, are visualized in Fig. 2. Overall and between the different wind velocities, no significant differences were observed between the collection efficiency of the air sampling devices and confidence intervals show low maximum deviations.

Results from the aerosol chamber trials

Concentration of *E. coli* in the bacterial suspensions, concentration and recovery rates of aerosolized *E. coli*

The mean concentration in the bacterial suspensions for aerosolization was 9.8×10^8 cfu ml⁻¹ for *E. coli* K12 (range: 2.6×10^8 to 3.8×10^9 cfu ml⁻¹), 9.0×10^8 cfu ml⁻¹ for *E. coli* R56 (range: 2.7×10^8 to 3.4×10^9 cfu ml⁻¹) and 2.0×10^9 cfu ml⁻¹ for *E. coli* G-148-1 (range: 4.1×10^8 to 3.9×10^9 cfu ml⁻¹).

The airborne concentrations of the three *E. coli* strains detected in the air samples at the different AGI-30 impinger heights are depicted in Fig. 3.

In the trials without organic soiling, the geometric mean concentration was lowest at 30% RH for all strains. The highest mean concentration in the trial without organic soiling was detected at 70% RH for *E. coli* K12 and *E. coli* R56 and at 50% RH for *E. coli* G-148-1. However, corresponding recovery rates were highest at 70% RH for all three strains.

In the trials with organic soiling, the highest airborne concentrations were detected at 30% RH for all three *E. coli* strains. The lowest concentrations were detected at 70% RH for all strains. The airborne concentrations and the recovery rates for all *E. coli* strains under different conditions are depicted in Table 3.

Differences in the recovery rates of the three *E. coli* strains

For all experimental replicates under different conditions, the recovery rate of *E. coli* K12 from the aerosol was 0.16%. For the ESBL-producing *E. coli* R56, the recovery rate was 0.76% and for the AmpC-producing *E. coli* strain G-148-1, it was 0.79%.

The results of the repeated measures negative binomial model revealed that the recovery rate or the tenacity in the airborne status of the AmpC-producing *E. coli* strain G-148-1 was 4.1-fold higher compared with the non-resistant strain *E. coli* K12 (p -value = 0.016), taking all experimental replications under different conditions into consideration. For the ESBL-producing strain *E. coli* R56, the recovery rate was 3.5-fold higher compared with *E. coli* K12 (p = 0.041). The recovery rate of *E. coli* G-148-1 was 1.2-fold higher compared with *E. coli* R56 (p = 0.64).

Influence of RH and organic soiling on the recovery rate of airborne *E. coli*

The influence of organic soiling on the recovery rate of *E. coli* was analysed in the repeated measures negative binomial model for the different RH. Since these factors had a similar effect on all strains, we did not stratify the three strains for the analysis. The calculated cfu of *E. coli* per m³, including the upper and lower 95% confidence intervals, are visualized in Fig. 4. At an RH of 30%, the recovery rate of *E. coli* from the aerosol is 41.3-fold higher when adding 10 g L⁻¹ yeast extract and bovine serum albumin (BSA) (p < 0.001). At 50% RH, the recovery rate was only marginally (1.1-fold) higher with organic soiling (p = 0.91). At 70% RH, the addition of organic substances had an adverse effect. The recovery rate of aerosolized *E. coli* significantly decreased by 13.3-fold compared with the experiments where no organic substances were added (p = 0.003).

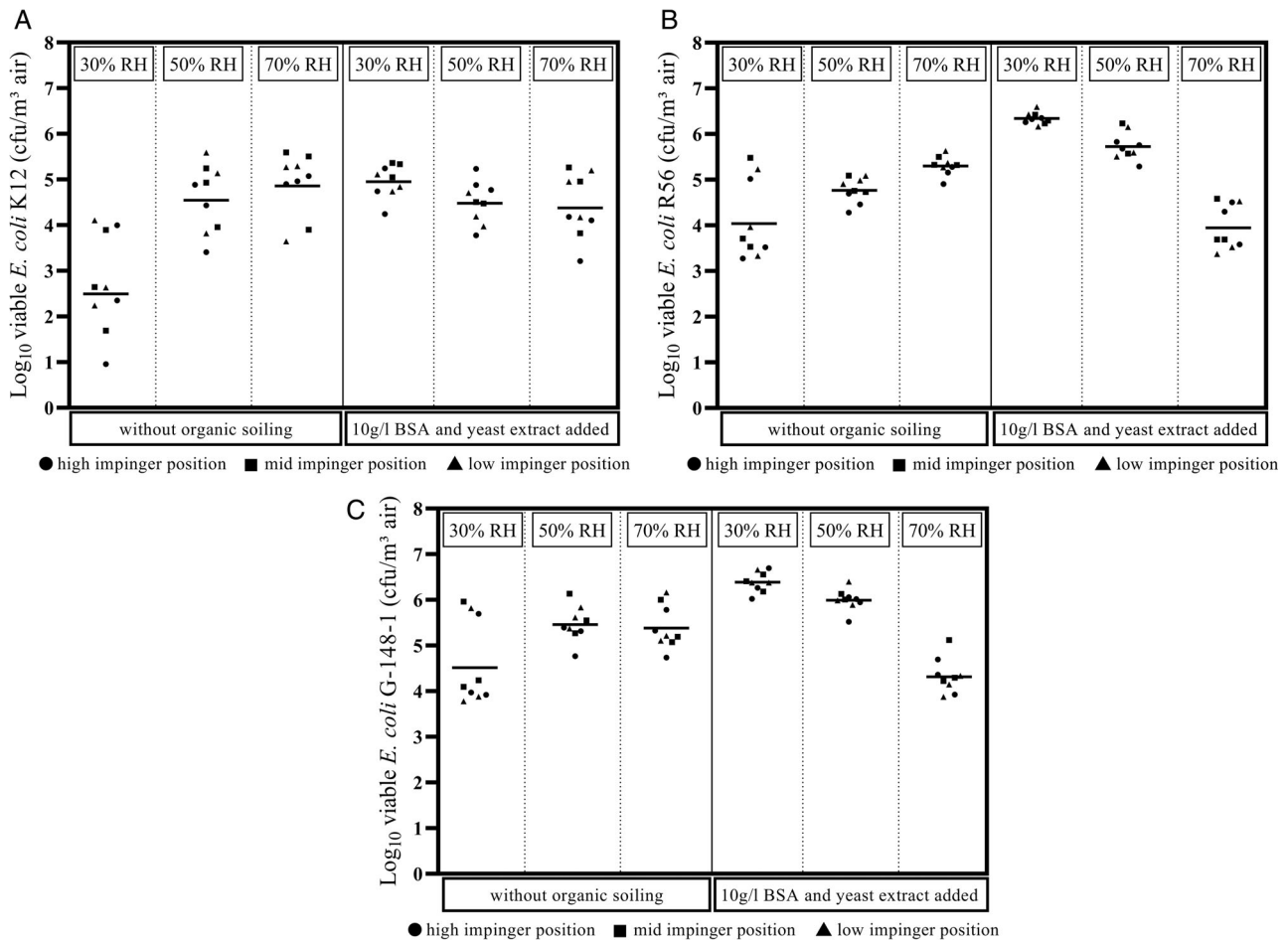


Fig. 3. LOG₁₀ airborne concentration of *E. coli* K12 (A), *E. coli* R56 (B) and *E. coli* G-148-1 (C) in cfu m⁻³ air for 30%, 50% and 70% RH, with and without the addition of 10 g/L BSA and yeast extract. The horizontal line indicates the geometric mean. The different symbols indicate the sampling height of the AGI-30 impingers.

Table 3. *E. coli* concentration detected per m³ of air for different strains and conditions, including the respective recovery rates.

<i>E. coli</i> strain	RH (%)	Organic soiling	cfu m ⁻³	Recovery rate (%)
<i>E. coli</i> K12	30	None	5.2 × 10 ²	0.003
	50	None	2.8 × 10 ⁴	0.28
	70	None	7.7 × 10 ⁴	0.3
	30	10 g L ⁻¹ yeast extract+BSA	8.45 × 10 ⁴	0.26
	50	10 g L ⁻¹ yeast extract+BSA	2.8 × 10 ⁴	0.05
	70	10 g L ⁻¹ yeast extract+BSA	2.0 × 10 ⁴	0.06
<i>E. coli</i> R56	30	None	1.1 × 10 ⁴	0.11
	50	None	5.9 × 10 ⁴	0.14
	70	None	2.0 × 10 ⁵	0.7
	30	10 g L ⁻¹ yeast extract+BSA	2.2 × 10 ⁶	2.66
	50	10 g L ⁻¹ yeast extract+BSA	5.4 × 10 ⁵	0.93
	70	10 g L ⁻¹ yeast extract+BSA	7.6 × 10 ³	0.01
<i>E. coli</i> G-148-1	30	None	3.8 × 10 ⁴	0.12
	50	None	2.9 × 10 ⁵	0.26
	70	None	2.5 × 10 ⁵	0.42
	30	10 g L ⁻¹ yeast extract+BSA	2.4 × 10 ⁶	2.8
	50	10 g L ⁻¹ yeast extract+BSA	9.8 × 10 ⁵	1.17
	70	10 g L ⁻¹ yeast extract+BSA	1.9 × 10 ⁴	0.03

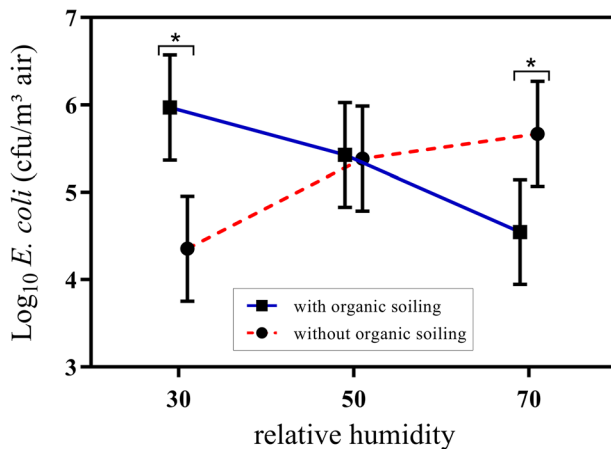


Fig. 4. Influence of organic soiling on the recovery rate of *E. coli* for the different RH, considering all three *E. coli* strains tested. The error bars indicate the upper and lower 95% confidence intervals.

Discussion

This study aimed to assess the relevance of an airborne spread of ESBL/AmpC-producing *E. coli* from soil fertilized with chicken litter to the environment by wind erosion.

The high total viable bacterial counts detected in the air samples indicate notable bio-aerosol formation, especially at high wind velocities. Wind erosion occurs when wind velocities exceed 6 m s^{-1} over dry soil (Zobeck and Pelt, 2015). This is consistent with the significantly increased average total viable bacterial count ($7.1 \times 10^7 \text{ cfu m}^{-2}$) detected at a wind velocity of 7.3 m s^{-1} in 5 cm distance to the soil-litter mix compared with the bacterial count detected at 5.6 m s^{-1} ($2.0 \times 10^6 \text{ cfu m}^{-2}$). Additionally, only for wind velocities exceeding 7 m s^{-1} , non-resistant *E. coli* were detected in the Coriolis μ air samples. However, we did not detect ESBL/AmpC-producing *E. coli* in all air samples taken in the wind tunnel trials. Within each wind velocity, no significant difference was observed in the total viable count emitted for the three wind tunnel trials, which indicates a good predictability of total viable bacteria emitted from fertilized loamy sand soil for certain wind velocities. Although the emitted total viable count was similar for all trials, a significantly increased $\text{PM}_{2.5}$ and PM_{10} emission was observed for the first wind tunnel trial. This increased fine dust emission was likely linked to the finer soil structure with smaller aggregates in the first wind tunnel trial, compared with the subsequent trials. The similar amount of total viable bacteria emitted in all three trials might be explained by assuming that larger particles, which were present in the second and third trials, might carry more total viable bacteria (Clauß, 2015). A further explanation for the similar bacterial emission for the three trials may

be that the largest fraction of bacteria collected in the air samples was derived from the chicken litter and not from the soil, as recently suggested by Thiel *et al.* (2020), especially considering that the same amount of litter was used for all three trials. Based on our results on bacterial and particle emission, it can be assumed that fine dust and total viable count emission correlate. However, predicting wind-driven bacterial emissions from fertilized soil on the basis of particle emission is difficult, because it is influenced by many factors like soil type, soil structure, bacterial concentrations and composition of fertilizer and environmental conditions.

Data on airborne ESBL/AmpC-producing *E. coli* in the environment are scarce and few studies investigating this issue were conducted to date. Laube *et al.* (2014) detected ESBL-/AmpC-producing *E. coli* in 7.5% (3/40) of air samples taken from the exhaust air of chicken barns, but a quantification was not possible for any of the samples. While Blaak *et al.* (2015) detected ESBL-producing *E. coli* in 7.7% of air samples taken inside broiler barns, all air samples taken in the vicinity of the barns were negative for ESBL-producing *E. coli*, which might indicate a low environmental tenacity of poultry-associated ESBL-*E. coli* in the airborne state. In a study by Korzeniewska and Harnisz (2013), 23.8% of air samples collected at a wastewater treatment plant tested positive for phenotypic ESBL-producing *E. coli*. In that study, a significantly positive correlation between the wind velocity and the number of ESBL-positive air samples was shown.

In our study, aerosol chamber trials were performed to gain additional knowledge on the tenacity of ESBL-/AmpC-producing *E. coli* compared with non-resistant *E. coli* in the airborne state, which has never been investigated systematically to date. However, experimental studies on the airborne tenacity of non-resistant *E. coli* date back several decades (Poon, 1966; Benbough, 1967; Cox, 1968; Wathes *et al.*, 1986).

The airborne survival of bacteria under experimental conditions is influenced by various factors, including the bacterial strain, the composition of the culture and suspension fluid, the growth conditions, processing conditions of the bacterial cultures and the atmosphere into which the bacteria are released (Wathes *et al.*, 1986). There are further important environmental factors, including the RH, oxygen concentration, temperature, ozone concentration, UV-radiation and air pollutants, which influence the tenacity of microorganisms in the aerosolized state (Zhao *et al.*, 2014). The existence of overlapping lethal mechanisms for aerosolized bacteria renders the exact diagnosis of which mechanisms and conditions cause bacterial death difficult (Benbough, 1967).

Several studies have investigated the influence of temperature on the survival of *E. coli* in the aerosolized state.

Hoeksma *et al.* (2014) investigated the survival of the *E. coli* strain DSM-1936 between 10°C and 30°C and was able to show that after the initial decay, the bacteria survived the longest at 30°C. In contrast, Wathes *et al.* (1986) reported approximately four times higher death rates at 30°C compared with 15°C for a nalidixic acid-resistant strain of *E. coli* (serotype O149). A possible explanation might be that the influence of temperature on the survival of airborne *E. coli* is strain-specific. Zhao *et al.* (2014) stated that, in general, the decay of microorganisms is faster at higher ambient temperatures. In our aerosol chamber experiments, a uniform temperature of 24°C was used for all experiments. The temperature in the wind tunnel trials varied between 16.1°C in the first trial and 25.7°C in the third trial. We did not observe a correlation between the temperature and the number of air samples positive for non-resistant *E. coli* or the total viable count in the wind tunnel trials.

Concerning the RH, we showed a significantly higher recovery rate of different *E. coli* strains in the aerosol chamber experiments without organic soiling at an RH of 50% and 70% compared with 30% RH. This finding is in line with a series of studies that showed decreased survival rates of aerosolized *E. coli* at low RH values. Wathes *et al.* (1986) reported half-life times of 3 min for low RH (<50%) and 14 min in humid conditions for *E. coli* aerosolized from phosphate-buffered saline (PBS) at 30°C. According to Poon (1966), the death rate of aerosolized *E. coli* is directly proportional to the rate of water evaporation and the water evaporation increases with decreasing environmental RH. Hoeksma *et al.* (2014) observed a fast biological decay of *E. coli* during the first 30 s after the aerosolization and a much smaller decay in the following 30 min, which he explains by a sudden cooling down effect caused by the evaporation of water. It is hypothesized that after aerosolization in dry conditions, water in the newly formed bacterial droplet will evaporate in milliseconds, thus altering the temperature, solute content and other environmental conditions of the droplet, which might promote biological decay (Liu *et al.*, 2017). Additionally, under dry conditions, the toxicity of oxygen by the formation of free oxygen radicals which damage flavin-linked bacterial enzymes is increased (Benbough, 1967) compared with humid conditions, under which oxygen-induced free radicals are present to a lesser extent (HECKLY *et al.*, 1963).

At 30% RH, bacterial suspensions with organic soiling showed a significantly increased recovery rate compared with suspensions without organic soiling. The explanation might be that airborne microorganisms are protected from external influences by particles coagulated within the viable bacterial particles, thus protecting bacterial proteins and membrane phospholipids, which appear to be targets for humidity and temperature-induced bacterial inactivity (Zhao, 2011).

In the trials with organic soiling, the lowest concentrations of aerosolized *E. coli* were detected in the aerosol at 70% RH (4.5×10^4 cfu m⁻³ air). This is unexpected because a high RH and the addition of organic substances both seemed beneficial for the survival of airborne bacteria. According to Marthi *et al.* (1990), a high RH might lead to clumping of cells, potentially increasing the odds of cell survival. This clumping effect may be enhanced by organic soiling in the *E. coli* suspensions. Viable *E. coli* may therefore have quickly deposited in large aggregates on the floor of the aerosol chamber and were undetectable in the air samples. Quantification of the deposited *E. coli* fraction might have been of particular interest. A limitation of this study is that we did not generate valid information about the particle size in the aerosol chamber trials, which may have supported this hypothesis.

In the wind tunnel trials, the RH was similar for the first and second wind tunnel trials with 56% and 54.4%. In the third wind tunnel trial, the RH was considerably lower, with 29.7%. Non-resistant *E. coli* were detected in 50% (5/10) of the Coriolis μ air samples in the first trial and 60% (6/10) in the second trial but in none of the air samples taken in the third wind tunnel trial. The lower environmental RH in the third wind tunnel trial might have led to inactivation by desiccation of *E. coli* adhering to soil or litter particles. This hypothesis seems inconsistent with the results of the aerosol chamber trial, where the highest survival rates under the presence of organic soiling were found at 30% RH. This deviation might be explained by the fact that we aerosolized the *E. coli* strains in the aerosol chamber trials from bacterial suspensions. Zhao (2011) stated that the biological decay of microorganisms aerosolized from dry sources might differ from the decay in wet aerosolization. He, therefore, recommended using dry aerosolization for microorganisms released from dry sources like faeces or litter. However, Hoeksma *et al.* (2014) pointed out that *E. coli* rarely survives the procedure for preparing dry aerosols.

The recovery rate for poultry-associated ESBL-/AmpC-producing *E. coli* strains R56 and G-148-1 was significantly higher than the recovery rate of the laboratory strain *E. coli* K12 under all experimental conditions. This is in accordance with findings by Marshall *et al.* (1988), who observed a prolonged survival of aerosolized *E. coli* of wild-type strains compared with the laboratory strain *E. coli* K12. The reasons for the significant differences in the recovery rates between the *E. coli* strains are still unclear. Additional research is warranted. To estimate the influence of ESBL-/AmpC-plasmid carriage on the tenacity of *E. coli* during aerosolization, a comparison of the recovery rates of ESBL/AmpC *E. coli* strains and their plasmid-cured variants would be of interest, as Ranjan *et al.* (2018) have demonstrated that the carriage of

certain ESBL-plasmids was beneficial regarding competition fitness *in vitro*. Little is known about the molecular mechanisms and bacterial stress response in aerosolized bacteria. In a recent study by Ng *et al.* (2018), comparative transcriptome analysis was used to gain knowledge on gene expression in *E. coli* following aerosolization. Results indicate that *E. coli* responds to environmental stimuli in the air very quickly by changing the transcriptional signature. During aerosolization, 11 stress-responsive genes and 13 stimulus-responsive genes were regulated. It might be hypothesized that wild-type strains can change their transcriptional signature faster compared with laboratory strains in reaction to environmental stimuli.

Estimation of the potential of an airborne environmental spread of ESBL-/AmpC-producing E. coli by wind erosion

In the wind tunnel trials, there were considerable differences in the concentrations of ESBL-producing *E. coli* in the chicken litter between the trials, reflecting realistic variations in the colonization of broiler flocks with these bacteria (Daehre *et al.*, 2017). While in the first trial, ESBL-*E. coli* were only qualitatively detectable in the chicken litter, in the second wind tunnel trial, chicken litter with a high concentration (3.2×10^5 cfu g⁻¹ litter in barn A and 4.2×10^5 cfu g⁻¹ in barn B) of ESBL-producing *E. coli* was used for aerosolization. Blaak *et al.* (2014) reported a similar average concentration (5.3×10^5 cfu g⁻¹) of ESBL-producing *E. coli* in fresh chicken litter. The chicken litter used in the wind tunnel trials naturally contained ESBL/AmpC-producing *E. coli*, which were already adapted to the existing environmental conditions. This is beneficial because bacterial stress is minimized (Wesche *et al.*, 2009) and may lead to a survival advantage over artificially added bacteria. The concentration of ESBL/AmpC-producing *E. coli* in the chicken litter and the mixing ratio of the soil-litter mixture reflected practical conditions, which ensures a good transferability of the results to field conditions.

The two air samplers used in the wind tunnel trial have different properties. The airspeed at the inlet of the AGI-30 is 4 m s^{-1} , for the Coriolis μ it is 25 m s^{-1} . Therefore, air sampling was always super-isokinetic with the Coriolis μ , while it was only super-isokinetic for the AGI-30 at the lowest wind speed, where a mean of 3.3 m s^{-1} was measured in the suspensions chamber and sub-isokinetic for higher wind speeds (Table 2). Sub-isokinetic sampling is not ideal and has higher measurement errors compared with isokinetic or super-isokinetic sampling (Friedlander, 1977). Additionally, both air samplers have different particle cut-offs. The AGI-30 has a cut-off of $0.3 \mu\text{m}$ (Yao and Mainelis, 2006). Particles with a diameter above $15 \mu\text{m}$ are also not sampled in the AGI-30 collection fluid,

because they are collected at the tube wall by inertial force (Lindsley *et al.*, 2017). The Coriolis μ has a cut-off size of $0.5 \mu\text{m}$ at an operation flow of 300 L min^{-1} , which means that particles of $0.5 \mu\text{m}$ are sampled at 50% efficiency and larger particles are sampled at higher efficiency (Mbareche *et al.*, 2018). Because of their different cut-offs, it was unexpected that both air sampling systems showed similar collection efficiencies. However, Clauß *et al.* (2013) stated that most airborne microorganisms are bound to particles with a size of $5\text{--}10 \mu\text{m}$. For this size fraction, both air samplers have a high collection efficiency, which might explain the similar results concerning the total viable count detected per m³ of air. Additionally, the detection limits of both air samplers differ. In our experimental setup, the quantitative detection limit was approximately 8.5×10^2 cfu m⁻³ for the AGI-30 air samples and 1.2×10^1 cfu m⁻³ for the Coriolis μ air samples. The qualitative detection limit was 8.0×10^1 cfu m⁻³ for the AGI-30 samples and only approximately 1 cfu m^{-3} for the Coriolis μ air samples. Despite the very low qualitative detection limit of the Coriolis μ , no ESBL-/AmpC-producing *E. coli* were detected in the air samples taken in the wind tunnel trials. The reasoning for this appears to be multifactorial. ESBL-producing *E. coli* are rapidly inactivated in chicken litter. In a study recently published by Siller *et al.* (2020), ESBL-producing *E. coli* concentrations decreased from an average of 3.4×10^5 cfu g⁻¹ in fresh chicken litter below the detection limit after storage periods of 36 h in the summer and 72 h in the winter. To limit this inactivation in the litter used in the wind tunnel trials, the timespan between litter collection and aerosolization was minimized. A high concentration of ESBL-producing *E. coli* in the litter or fertilized soil seems to be essential in order to detect ESBL-/AmpC-producing *E. coli* in air samples. Chinivasagam *et al.* (2009) were able to show a direct link between levels of non-resistant *E. coli* in chicken litter and airborne *E. coli* concentrations in chicken barns. At concentrations of 10^8 cfu g⁻¹ *E. coli* in the litter, airborne concentrations in the barns ranged from 10^2 to 10^5 cfu m⁻³. In our study, despite the fact that ESBL/AmpC-positive litter was used in all trials, after mixing and diluting the litter with soil, quantitative detection of ESBL-producing *E. coli* in the soil-litter mixture was only possible in the second wind tunnel trial. Presumably, the vast majority of ESBL/AmpC *E. coli* present on the soil surface in the wind tunnel trials was subsequently inactivated during aerosolization. This hypothesis is strongly supported by the results of the aerosol chamber trials, which confirmed a low tenacity of aerosolized ESBL-/AmpC-producing *E. coli* with an average reduction of 2.25 LOG₁₀.

The detection of high total viable bacterial counts and low amounts of *E. coli* in air samples taken in the wind tunnel might be explained by the fact that *E. coli* is a

Gram-negative bacterium. Gram-negative bacteria account for a small proportion of airborne bacteria (Zucker *et al.*, 2000). In poultry houses, Bakutis *et al.* (2004) reported a proportion of 2.6% of Gram-negative bacteria of the total bacterial count. Zhao *et al.* (2014) suspected that airborne Gram-negative bacteria are less frequently detected in air samples of livestock production systems, because they might be more vulnerable to environmental stress such as oxidation, radiation, and dehydration, presumably due to their thinner cell walls.

Additionally, it has to be considered that the ESBL/AmpC-*E. coli* producing subpopulation represents only a small proportion of the total *E. coli* population. This could explain why ESBL/AmpC-producing *E. coli* have remained below the detection limit in the air samples while non-resistant *E. coli* were qualitatively detected. In the second wind tunnel trial, ESBL-*E. coli* represented 1.6% of the entire *E. coli* population in the chicken litter for barn A and 4.4% for barn B. In the third wind tunnel trial, 1.5% of *E. coli* were ESBL-producing. Friese *et al.* (2019) recently reported an according proportion of 1.1% ESBL-producing *E. coli* in turkey rearing flocks.

Considering all factors discussed, we conclude that an airborne spread of ESBL/AmpC-producing *E. coli* in the environment by wind erosion seems unlikely. However, because non-resistant *E. coli* were detected in the air samples, we suspect that ESBL-/AmpC-producing *E. coli* may have been present below the detection limit; therefore, a potential airborne spread of extremely low quantities of ESBL/AmpC-producing *E. coli* cannot be excluded. In two out of three wind tunnel trials, soil-litter mixtures with a low concentration of ESBL/AmpC-producing *E. coli* were used. If litter with a significantly higher concentration of ESBL-*E. coli* would be applied to arable land, the potential of emission might be increased. However, under practical conditions, it seems unlikely that ESBL-*E. coli* from chicken litter are applied to arable land in relevant concentrations because the largest proportion of ESBL-producing *E. coli* in chicken litter is quickly inactivated when transported from barns to arable land (Thiel *et al.*, 2020). Additionally, short-term storage (5 days) of chicken litter was proven to inactivate ESBL-*E. coli* effectively (Siller *et al.*, 2020) and a recent lab-scale study by Thomas *et al.* (2019) confirmed the extinction of ESBL-*E. coli* in chicken litter within 2 h at temperatures exceeding 55°C. Additionally, the present study confirmed a low airborne tenacity for non-resistant and ESBL-/AmpC-producing *E. coli*. An airborne spread of ESBL/AmpC-producing *E. coli* from organic fertilizers from farm animals other than chickens seems even more improbable because ESBL/AmpC-producing *E. coli* are detected in broiler farms in the highest quantities and with a prevalence of up to 100% (Dierikx *et al.*, 2010; Laube

et al., 2013; Hering *et al.*, 2016). Additionally, in the largest comparative study to date, by far higher concentrations of airborne bacteria were detected in poultry barns with 2.7×10^6 cfu m⁻³ air compared with 1.3×10^5 cfu m⁻³ in pig barns and 2×10^4 cfu m⁻³ in cattle buildings (Seedorf *et al.*, 1998), which might be an indication for a pronounced potential of chicken litter, which typically has a high dry matter content, to form bioaerosols.

If emission of extremely low quantities of ESBL-/AmpC-producing *E. coli* to the environment via wind erosion occurs, the risk of colonization in humans and animals remains unclear. Dungan (2010) stated that information on the infectivity of aerosolized enteric pathogens is scarce. However, it was recently shown by Robé *et al.* (2019) that an oral infection dose as low as 10¹ cfu ESBL-*E. coli* has led to persistent colonization of broiler chicks. This suggests that even very low quantities of these bacteria emitted to the environment might lead to health implications and thus highlight the crucial role of further research in the domain of airborne environmental antimicrobial resistance.

Experimental procedures

Wind tunnel trials

Characteristics of the wind tunnel. Detailed technical descriptions of the wind tunnel located at the ZALF have been published by Funk (2000) and Funk *et al.* (2008, 2019). A modified sketch of the wind tunnel illustrating the most important technical features and the experimental setup is included in Fig. S2 in the supplementary material.

The wind tunnel has a total length of 25 m and is of the Eiffel-type (push-type), meaning the air circulation is not a closed system. The airflow is generated by two axial ventilators, which are on top of each other at the beginning of the measuring section. The wind velocity is continuously adjustable up to 18 m s⁻¹. Rectifiers reduce turbulences and the profile former creates a logarithmic wind profile before the air enters the measuring section. The measuring section of the wind tunnel has a length of 7 m and a height and width of 0.7 m, equating a cross-sectional area of 0.49 m². It is accessible from one side and can be closed with acrylic glass plates. The floor space on which the soil-litter mixture was deposited measures 4.9 m². Aerosolized particles are blown to the suspension chamber, where the air sampling devices were positioned. An exhaust air channel is located in the ceiling of the suspension chamber. The cross-sectional area of the suspension chamber is 12 times larger compared with the measuring section, resulting in a reduction of the

average wind velocity to an eighth of the original wind velocity.

Experimental design. To obtain ESBL-/AmpC-positive chicken litter for the wind tunnel trials, an initial screening was carried out to identify ESBL/AmpC-positive broiler barns. In total, 35 barns of two different broiler farms were tested by taking boot swab samples, pooled faeces and litter samples. All samples were qualitatively and quantitatively analysed for ESBL-/AmpC-producing *E. coli* (please see below for laboratory protocols). Litter from the barns with the highest ESBL/AmpC-producing *E. coli* concentration was chosen for further investigations in the wind tunnel trials. The concentration is shown in Table 1. In the first wind tunnel trial, the result of the qualitative analysis of the soil-litter mixture concerning AmpC-producing *E. coli* is uncertain, because only a few small colonies, which phenotypically resembled *E. coli*, were detected on the MC+ plates. These small colonies were transferred to a new set of plates and incubated overnight. The next day, no visible growth of *E. coli* was observed. In the second wind tunnel trial, litter from two barns was mixed because the concentration of ESBL-*E. coli* in the screening was similarly high.

For each trial, approximately 10 kg of chicken litter from the selected barns was collected in sterile plastic bags and stored in polystyrene boxes with ice packs until use. Trial 1 took place 1 day after litter collection; the chicken litter was stored overnight at 4°C. Trials 2 and 3 took place on the same day as litter collection. Two samples were stored for microbiological analyses: One directly after litter collection and one upon arrival in the wind tunnel.

The wind tunnel trials were carried out at the Leibniz Centre for Agricultural Landscape Research (ZALF). A concrete mixer was used to mix 3 kg of chicken litter with 250 dm³ of loamy sandy soil taken from a trial plot of land. This ratio was chosen based on the German legislation, Paragraph 6 (4) of the Düngeverordnung (German Fertilizer Ordinance) which limits total nitrogen to 170 kg per hectare and data from the Leibniz-Institute of Vegetable and Ornamental Crops (2017), which estimates the average total nitrogen of fresh chicken litter from Brandenburg at 17.5 kg per metric ton. The grain size of the soil was measured by wet sieving (2000–63 µm) and the sedimentation method (<63 µm; DIN ISO 11277, 2002). This resulted in the following soil particle composition: Sand (2000–63 µm): 69%; Silt (63–2 µm): 25%; Clay (<2 µm) 6%. Derived from the particle composition, the soil can be classified as a 'loamy sand' and is therefore susceptible to wind erosion.

In the second wind tunnel trial, chicken litter from two barns was mixed at a ratio of 1:1, because these barns showed similarly high concentrations of ESBL-producing

E. coli in the screenings. To ensure that the soil was negative for ESBL/AmpC-producing and non-resistant *E. coli*, soil samples for microbiological analysis were taken before mixing. After the mixing procedure, the soil-litter mixture was sampled again for microbiological analysis.

The wind tunnel was then filled with a 5 cm layer of soil-litter mixture and exposed to different wind velocities (5.6, 7.3, 9.8 and 10.6 m s⁻¹ in 5 cm distance to the soil surface on average) for a time span of 10 min each. The wind velocity was monitored at a height of 5, 30 and 60 cm in the wind tunnel section using a hot wire anemometer (Lambrecht Thermal Anemometer 642, Lambrecht, Goettingen, Germany). The RH, temperature and PM₁₀, as well as the PM_{2.5} concentration [µg m⁻³], were measured using an Environmental Dust Monitor (EDM 164, GRIMM-Aerosol Technik, Ainring, Germany).

There was no exchange of the soil-litter mixture between wind velocities and the soil surface remained unaffected for each replicate of the wind tunnel trials. Therefore, total viable bacterial counts and particle concentrations were calculated by gradually adding up the total viable count and particles for each wind velocity, since a proportion of particles were already aerosolized at lower wind velocities and are therefore missing at higher wind velocity measurements, as described by Funk *et al.* (2019). Particle concentrations and total viable counts measured per m³ of air were multiplied with the air volume (in m³), which passed the wind tunnel for each wind velocity and divided by 4.9 (floor space in m²) to calculate the emission of particles and total viable bacteria per m² of soil-litter mixture and therefore considering the dilution factor at increasing wind velocity. The experiments were carried out in triplicate for each trial. After one cycle, the soil was turned and mixed to create a fresh surface. Air samples were taken at the terminus of the wind tunnel using all-glass impingers 30 (AGI-30; Neubert Glas GbR, Geschwenda, Germany, VDI Norm 4252-3) and a Coriolis µ cyclone air sampler (Bertin Instruments, Montigny-le-Bretonneux, France). The impingers were filled with 30 ml of PBS (Oxoid, Wesel, Germany) and connected to a vacuum pump with a plastic tube. The airflow was monitored using a rotameter. It was approximately 12.5 L min⁻¹. Coriolis µ cones were filled with 15 ml PBS. The airflow for the Coriolis µ was set to 300 L min⁻¹. Sampling times for the AGI-30 impinger and Coriolis µ air samples were 10 min for each level of wind velocity. Impingers and Coriolis cones were stored at cool temperatures before and after air sampling. The air sampling devices were positioned 1.5 m above the ground, to imitate the height at which average humans respire. In total, 11 air samples were taken with the AGI-30 impingers and Coriolis µ per wind tunnel trial: Directly after the wind tunnel was filled with soil-litter

mixture, a check plot sample was taken using impingement and cyclone sampling when the wind tunnel was turned off. At 5.6 m s^{-1} , only one air sample was taken for each trial. At 7.3 , 9.8 and 10.6 m s^{-1} , three air samples were taken with each air sampling device per trial.

Laboratory analyses. All boot swabs, pooled faeces, litter, soil and air samples from the screenings of the barns and the wind tunnel trials were analysed qualitatively and quantitatively for ESBL/AmpC-producing and non-resistant *E. coli* as described by Siller *et al.* (2020). The air samples were additionally analysed for the total viable bacterial count.

Twenty grams of pooled faeces, litter and soil samples were mixed at a ratio of 1:10 with Luria/Miller-broth (LB; Roth, Karlsruhe, Germany) in stomacher bags. Boot swabs were put in stomacher bags and 200 ml LB was added. The samples were homogenized using a Stomacher 400 Circulator (Seward Limited, West Sussex, UK) at 200 rpm for 2 min. The quantitative analysis was performed by directly streaking $100 \mu\text{l}$ of the homogenized samples in triplicates on specific agar plates after serial dilution. For the quantification of *E. coli*, MacConkey agar No. 3 (Oxoid) was used (MC-). To quantify ESBL/AmpC-producing *E. coli*, 1 mg L^{-1} Cefotaxime (AppliChem, Darmstadt, Germany) was added to the MacConkey agar plates (MC+), as recommended by the EFSA (EFSA, 2011). Under optimal conditions, the quantitative detection limit of this method is $3.3 \times 10^1 \text{ cfu g}^{-1}$ and the qualitative detection limit is 1 cfu/20 g .

For the air samples, $100 \mu\text{l}$ were directly streaked on MC+ and MC- plates in triplicates. Additionally, $100 \mu\text{l}$ of the air samples were plated out on blood base agar (Blood agar Base No. 2, Oxoid) after serial dilution to determine the total viable bacterial count.

For qualitative analysis, the homogenized samples were incubated in LB medium for 24 h at 37°C . Three millilitre of air sample fluids were incubated under the same conditions after a 1:10 dilution with LB medium in Erlenmeyer flasks. Subsequently, $10 \mu\text{l}$ of each sample was streaked on MC+ and MC- agar with an inoculation loop and incubated again for 24 h at 37°C . The quantitative detection limit for the AGI-30 air samples was $8.5 \times 10^2 \text{ cfu m}^{-3}$, the qualitative detection limit was $8.0 \times 10^1 \text{ cfu m}^{-3}$. For the Coriolis μ air samples, the quantitative detection limit was $1.2 \times 10^1 \text{ cfu m}^{-3}$, the qualitative detection limit was approximately 1 cfu m^{-3} .

Species confirmation of colonies suspected to be *E. coli* was achieved using MALDI-TOF Mass Spectrometry (MALDI Microflex LT and Biotyper database, Bruker Daltonics, Bremen, Germany).

Real-time PCR and sequencing. To detect the most important ESBL and CIT-type AmpC resistance genes

(*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{CMY}), isolates from the pre-trial screenings and the wind tunnel trials were tested using real-time PCR, as previously described by Roschanski *et al.* (2014). To identify the present ESBL-/AmpC-variants, a selection of isolates was sequenced using Sanger-sequencing for each trial. The fewest isolates were analysed for the first wind tunnel trial ($n = 6$) because no AmpC-producing *E. coli* colonies were isolated from the soil-litter mixture. Most isolates ($n = 16$) were analysed for the second wind tunnel trial because isolates from two barns were characterised in this trial. For the third trial, 10 isolates were analysed. For Sanger sequencing, DNA isolation and PCR were performed as published previously (Projahn *et al.*, 2017). Purified PCR products were sent to LGC Genomics (Berlin, Germany), who provided the sequences. The obtained sequences were analysed using DNASTAR Lasergene (Madison, Wisconsin) and compared with the reference sequences of GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Phylotyping. Phylotyping of the isolates acquired from the litter used in the wind tunnel trials was performed as published by Clermont *et al.* (2013) with a modified PCR according to Projahn *et al.* (2017). Isolates that could not be assigned to one phylogroup due to unspecific band patterns were assigned a combined phylogroup.

Aerosol chamber trials

Characteristics of the aerosol chamber. The aerosol chamber trials were carried out in the aerosol chamber of the Institute for Animal Hygiene and Environmental Health (Freie Universität Berlin).

Detailed technical descriptions of the aerosol chamber, including a Figure, have been published previously (Rosen *et al.*, 2018). In brief, the aerosol chamber generates bioaerosols under different standardized climatic conditions (temperature, airflow rate and RH). The volume of the aerosol chamber is 7 m^3 . A perfusion pump transports the bacterial suspensions with a rate of 9 ml h^{-1} to an ultrasonic nebulizer. There is an axial ventilator in the ceiling of the chamber to disperse the aerosol and a separate opening in the ceiling for fresh air. The airflow rate was $100 \text{ m}^3 \text{ h}^{-1}$ and the temperature was 24°C for all experiments. The RH was set at 30%, 50% or 70% depending on the experimental setup.

The aerosol was sampled using three AGI-30 impingers at different heights (0.3, 0.8 and 1.3 m), each filled with 30 ml PBS. The airflow was approximately 12.5 L min^{-1} and the sampling time was 30 min.

Experimental design. In this experimental series, one non-resistant and two poultry-associated ESBL-/AmpC-producing strains of *E. coli* were aerosolized under

various conditions. The ESBL and AmpC-producing strains were aerosolized together in a mixed bacterial suspension. Air samples were taken during aerosolization. We investigated the effect of different RH (30%, 50% and 70%) and the influence of organic soiling (10 g L⁻¹ yeast extract and BSA, in combination) on the bacterial tenacity in the airborne state. Each *E. coli* strain was aerosolized at the three different RHs and with and without the addition of organic soiling respectively. Each experimental combination was carried out in triplicates.

The recovery rates of airborne *E. coli* were calculated by dividing the concentration of *E. coli* per m³ measured in the aerosol chamber in the experiments by the expected concentration per m³. This expected concentration was calculated for each experiment individually by multiplying the concentration of the *E. coli* suspensions (per ml) with the forward speed of the perfusion pump (9 ml h⁻¹) divided by the air exchange rate of the aerosol chamber (in m³ h⁻¹).

Preparation of the bacterial suspension and organic soiling solution. We used the well-described, non-resistant commensal laboratory strain *E. coli* K12 (DSM 423; <https://www.dsmz.de/collection/catalogue/details/culture/dsm-423>) and two resistant, poultry-associated strains that were isolated from healthy chickens in a previous project in 2011 (Hering *et al.*, 2016). The *E. coli* strain G-148-1 belongs to the multilocus sequence type (MLST) 10 and the phylogroup A. It harbours the CIT-type AmpC resistance gene *bla*_{CMY-2} and the *mcr-1* gene, which mediates colistin resistance. The second strain, first named R56 by Falgenhauer *et al.* (2016), belongs to the MLST 410 and phylogroup B1. It harbours the ESBL resistance gene *bla*_{CTX-M-15} and is additionally resistant to enrofloxacin.

Three colonies of the specific *E. coli* strain were added to 10 ml LB and incubated overnight at 37°C and 200 rpm in a shaking incubator (Multitron, Infors HT, Germany). The following day, 5 ml of this suspension was added to 100 ml of LB and incubated for 8 h. Then, 100 µl of the suspension was plated to blood base agar and incubated overnight (8 h) to achieve the exponential growth phase. The bacteria were removed from the agar plates with a plate spreader by adding 3 ml PBS and homogenized on a vortex mixer for 3 min with glass beads. To achieve the targeted concentration of 10⁹ cfu (colony forming units)/ml, a fraction of the bacterial suspension was diluted with PBS at a ratio of 1:10. A measured value of 0.5 McFarland standards in the diluted fraction verified the targeted concentration of 10⁹ cfu ml⁻¹ in the bacterial suspension. The optical density was measured at 600 nm to validate the McFarland measurement (reference range: 0.073–0.11).

The organic soiling solution containing 10 g L⁻¹ yeast extract (Merck, Darmstadt, Germany) and 10 g L⁻¹ BSA (Sigma, St Louis, USA) was prepared by adding yeast extract to distilled water at a ratio of 1:50 and autoclaving. BSA was dissolved in distilled water at the same ratio and sterilized by membrane filtration. Afterward, the solutions were mixed at a ratio of 1:1.

For the addition of organic soiling, bacterial suspensions were centrifuged at 3000 rpm for 10 min, the supernatant was discarded and the pellet was resuspended with the organic soiling solution.

Microbiological analysis of the bacterial suspensions and air samples. All bacterial suspensions and air samples were analysed quantitatively. Serial dilutions were prepared for all samples.

In the experiments with *E. coli* K12, 100 µl was streaked in triplicates on MC- agar and incubated for 24 h at 37°C. In the experiments with ESBL-/AmpC-producing *E. coli*, all samples were streaked out in triplicates on two types of MacConkey agar No. 3 plates. Both plate types were supplemented with 2 mg L⁻¹ cefotaxime. One set of plates additionally contained 4 mg L⁻¹ of enrofloxacin, allowing a phenotypical selection for the R56 strain. The other set of plates additionally contained 7 mg L⁻¹ colistin, leading to a phenotypical selection for the G-148-1 strain.

Statistical analysis

All statistical analysis was performed using R version 3.6.2 (R Foundation Vienna). Since bacterial counts (cfu) were lognormal distributed, we used the geometric mean for averaging. For statistical analysis, we used a mixed count regression. Due to overdispersion, we choose a negative binomial distribution. For the wind tunnel experiments, we used two hierarchical random effects. One random effect was used for the three soil-litter mixtures, which were measured at multiple wind velocities and within this effect, we used a random effect for the cfu plating triplicates as a repeated measures adjustment. The wind velocity and impinger type were modelled as a fixed effect interaction to assess the difference in measured cfu between AGI-30 and Coriolis µ at different wind velocities. Additionally, a model with the type of air sampler as the only fixed effect was run to compare air sampler performance overall. The probability of a qualitative detection of *E. coli* in the air samples depending on the wind velocity was analysed using logistic regression.

For the aerosol chamber experiments, a random effect for each of the experiments was utilized to account for repeated measures due to three AGI-30 impinger measurements per experiment and the combined aerosolization of two strains (*E. coli* R56 and G-148-1). We

checked for interactions using interaction plots and found an interaction between humidity and organic soiling. Additionally, the strain was included as a categorical variable in the model. *Post hoc* comparisons between all strains were adjusted for multiple comparisons using the Tukey method. For a better comparison between the three strains, the concentrations in the bacterial suspensions were normalized for this analysis. All mixed models were performed using the R package lme4 (version 1.1-21). Estimated marginal means and multiple comparison *post hoc* tests were performed using the emmeans R package (version 1.4.5). Results are reported with 95% confidence intervals. A significance threshold of 0.05 was used. Figures were created using Graphpad Prism 8 (GraphPad Software, San Diego, CA).

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References

- Acosta-Martínez, V., Van Pelt, S., Moore-Kucera, J., Baddock, M.C., and Zobeck, T.M. (2015) Microbiology of wind-eroded sediments: current knowledge and future research directions. *Aeolian Res* **18**: 99–113.
- Bakutis, B., Monstvilienė, E., and Januskeviciene, G. (2004) Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. *Acta Vet Brno* **73**: 283–289.
- Benbough, J.E. (1967) Death mechanisms in airborne *Escherichia coli*. *J Gen Microbiol* **47**: 325–333.
- Blaak, H., Hamidjaja, R.A., van Hoek, A.H.A.M., de Heer, L., de Roda Husman, A.M., and Schets, F.M. (2014) Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. *Appl Environ Microbiol* **80**: 239–246.
- Blaak, H., Van Hoek, A.H.A.M., Hamidjaja, R.A., Van Der Plaats, R.Q.J., Kerkhof-De Heer, L., De Roda Husman, A.M., and Schets, F.M. (2015) Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. *PLoS One* **10**: 1–23.
- Chinivasagam, H.N., Tran, T., Maddock, L., Gale, A., and Blackall, P.J. (2009) Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella*, *Campylobacter*, and *Escherichia coli*. *Appl Environ Microbiol* **75**: 7417–7425.
- Clauß, M. (2015) Particle size distribution of airborne microorganisms in the environment – a review. *Appl Agric For Res* **65**: 77–100.
- Clauß, M., Springorum, A.C., and Hartung, J. (2013) Jahresverlauf der Hintergrundkonzentrationen verschiedener Gruppen luftgetragener Mikroorganismen in einem urbanen, einem Agrar- und einem Forstgebiet in Norddeutschland. *Gefahrstoffe, Reinhaltung Luft Air Qual Control* **73**: 375–380.
- Clermont, O., Christenson, J.K., Denamur, E., and Gordon, D.M. (2013) The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep* **5**: 58–65.
- Cox, C.S. (1968) The aerosol survival and cause of death of *Escherichia coli* K12. *J Gen Microbiol* **54**: 169–175.
- Daehre, K., Projahn, M., Semmler, T., Roesler, U., and Friese, A. (2017) Extended-spectrum beta-lactamase-/AmpC beta-lactamase-producing Enterobacteriaceae in broiler farms: transmission dynamics at farm level. *Microb Drug Resist* **2017**: 0150.
- Day, M.J., Rodri, I., Schink, A., Wu, G., Chattaway, M.A., Donascimento, V., et al. (2016) Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, The Netherlands and the UK. *J Antimicrob Chemother* **71**: 1178–1182.
- Dierikx, C., van der Goeot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., and 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.
- Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., and Mevius, D. (2010) Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Vet Microbiol* **145**: 273–278.
- Dungan, R.S. (2010) Board-invited review: fate and transport of bioaerosols associated with livestock operations and manures. *J Anim Sci* **88**: 3693–3706.
- EFSA. (2011) 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 strains. *EFSA J* **9**: 1–95.
- Environment Agency. (2019) Review of airborne antimicrobial resistance. 1–51.
- Falgenhauer, L., Imirzalioglu, C., Ghosh, H., Gwozdziński, K., Schmiedel, J., Gentil, K., et al. (2016) Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing *Escherichia coli* ST410 in humans and animals in Germany. *Int J Antimicrob Agents* **47**: 457–465.
- Friedlander, S.K. (1977) *Smoke, Dust and Haze: Fundamentals of Aerosol Behavior*. New York: Wiley.
- Friese, A., Lu, H.M., Merle, R., and Roesler, U. (2019) Extended-spectrum beta-lactamase and AmpC beta-lactamase-producing Enterobacteriaceae in Turkey fattening farms: a cross-sectional study. *Berl Munch Tierarztl Wochenschr* **132**: 352–359.
- Friese, A., Schulz, J., Laube, H., von Salviati, C., Hartung, J., and Roesler, U. (2013) Faecal occurrence and emissions of livestock-associated methicillin-resistant *Staphylococcus aureus* (laMRSA) and ESbl/AmpC-producing *E. coli* from animal farms in Germany. *Berl Munch Tierarztl Wochenschr* **126**: 175–180.
- Funk, R. (2000) Vorstellung eines Windkanals für die Prozessforschung der Winderosion. *Mitteilungen Dtsch Bodenkundlichen Gesellschaft* **92**: 77–80.

- Funk, R., Papke, N., and Hör, B. (2019) Wind tunnel tests to estimate PM 10 and PM 2.5-emissions from complex substrates of open-cast strip mines in Germany. *Aeolian Res* **39**: 23–32.
- Funk, R., Reuter, H.I., Hoffmann, C., Engel, W., and Öttl, D. (2008) Effect of moisture on fine dust emission from tillage operations on agricultural soils. *Earth Surf Process Landforms* **33**: 1851–1863.
- Gao, L., Tan, Y., Zhang, X., Hu, J., Miao, Z., Wei, L., and Chai, T. (2015) Emissions of *Escherichia coli* carrying extended-spectrum β -lactamase resistance from pig farms to the surrounding environment. *Int J Environ Res Public Health* **12**: 4203–4213.
- Hartmann, A., Locatelli, A., Amoureux, L., Depret, G., Jolivet, C., Gueneau, E., and Neuwirth, C. (2012) Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy region). *Front Microbiol* **3**: 83.
- Heckly, R.J., Dimmick, R.L., and Windle, J.J. (1963) Free radical formation and survival of lyophilized microorganisms. *J Bacteriol* **85**: 961–966.
- Hering, J., Frömke, C., von Münchhausen, C., Hartmann, M., Schneider, B., Friese, A., et al. (2016) Cefotaxime-resistant *Escherichia coli* in broiler farms - A cross-sectional investigation in Germany. *Prev Vet Med* **125**: 154–157.
- Hoeksma, P., Aarnink, A., and Ogink, N. (2014) Effect of temperature and relative humidity on the survival of airborne bacteria. *Livestock Research Report* **864**: 1–27.
- Huijbers, P.M.C., Graat, E.A.M., Haenen, A.P.J., van Santen, M.G., van Essen-Zandbergen, A., Mevius, D.J., et al. (2014) Extended-spectrum and AmpC β -lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics. *J Antimicrob Chemother* **69**: 2669–2675.
- Jones, A.M., and Harrison, R.M. (2004) The effects of meteorological factors on atmospheric bioaerosol concentrations - a review. *Sci Total Environ* **326**: 151–180.
- Korzeniewska, E., and Harnisz, M. (2013) Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment. *J Environ Manage* **128**: 904–911.
- Laube, H., Friese, A., Guerra, B., Kreienbrock, L., Roesler, U., von Salviati, C., et al. (2013) Longitudinal monitoring of extended-spectrum-beta-lactamase/ampC-producing *Escherichia coli* at German broiler chicken fattening farms. *Appl Environ Microbiol* **79**: 4815–4820.
- Laube, H., Friese, A., von Salviati, C., Guerra, B., and Rösler, U. (2014) Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. *Vet Microbiol* **172**: 519–527.
- Leibniz-Institut für Gemüse- und Zierpflanzenbau Großbeeren. (2017) Nährstoffgehalte und N-Mineralisierung organischer Düngemittel. 1–8.
- Leverstein-van Hall, M.A., Dierikx, C.M., Stuart, J.C., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., et al. (2011) Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* **17**: 873–880.
- Lindsley, W.G., Green, B.J., Blachere, F.M., Stephen, B., Martin, B.F.L., Jensen, P.A., and Schafer, M.P. (2017) Sampling and characterization of bioaerosols. In *NIOSH Manual of Analytical Methods*, pp. 4–7. Cincinnati, OH: National Institute for Occupational Safety and Health.
- Liu, L., Wei, J., Li, Y., and Ooi, A. (2017) Evaporation and dispersion of respiratory droplets from coughing. *Indoor Air* **27**: 179–190.
- Marshall, B., Flynn, P., Kamely, D., and Levy, S.B. (1988) Survival of *Escherichia coli* with and without ColE1::Tn5 after aerosol dispersal in a laboratory and a farm environment. *Appl Environ Microbiol* **54**: 1776–1783.
- Marthi, B., Fieland, P., Walter, M., and Seidler, R.J. (1990) Survival of bacteria during aerosolization. *Appl Environ Microbiol* **56**: 3463–3467.
- Mbareche, H., Veillette, M., Bilodeau, G.J., and Duchaine, C. (2018) Bioaerosol sampler choice should consider efficiency and ability of samplers to cover microbial diversity. *Appl Environ Microbiol* **84**: 1–22.
- Ng, T.W., Ip, M., Chao, C.Y.H., Tang, J.W., Lai, K.P., Fu, S.C., et al. (2018) Differential gene expression in *Escherichia coli* during aerosolization from liquid suspension. *Appl Microbiol Biotechnol* **102**: 6257–6267.
- Pietsch, M., Irrgang, A., Roschanski, N., Brenner Michael, G., Hamprecht, A., Rieber, H., et al. (2018) Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. *BMC Genomics* **19**: 1–17.
- Poon, C.P.C. (1966) Studies on the instantaneous death of airborne *Escherichia coli*. *Am J Epidemiol* **84**: 1–9.
- Projahn, M., Daehre, K., Roesler, U., and Friese, A. (2017) Extended-spectrum-beta-lactamase and plasmid-encoded cephalosporinase-producing enterobacteria in the broiler hatchery as a potential mode of pseudovertical transmission. *Appl Environ Microbiol* **83**: e02364-16.
- Ranjan, A., Scholz, J., Semmler, T., Wieler, L.H., Ewers, C., Müller, S., et al. (2018) ESBL-plasmid carriage in *E. coli* enhances in vitro bacterial competition fitness and serum resistance in some strains of pandemic sequence types without overall fitness cost. *Gut Pathog* **10**: 1–9.
- Robé, C., Blasse, A., Merle, R., Friese, A., Roesler, U., and Guenther, S. (2019) Low dose colonization of broiler chickens with ESBL-/AmpC-producing *Escherichia coli* in a seeder-bird model independent of antimicrobial selection pressure. *Front Microbiol* **10**: 1–12.
- Roschanski, N., Fischer, J., Guerra, B., and Roesler, U. (2014) Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in enterobacteriaceae. *PLoS One* **9**: e100956.
- Rosen, K., Roesler, U., Merle, R., and Friese, A. (2018) Persistent and transient airborne MRSA colonization of piglets in a newly established animal model. *Front Microbiol* **9**: 1–12.
- Seedorf, J., Groot Koerkamp, P.W.G., Metz, J.H.M., Uenk, G.H., Phillips, V.R., Holden, M.R., et al. (1998) Concentrations and emissions of ammonia in livestock buildings in northern Europe. *J Agric Eng Res* **70**: 79–95.
- Siller, P., Daehre, K., Thiel, N., Nübel, U., and Roesler, U. (2020) Impact of short-term storage on the quantity of extended-spectrum beta-lactamase-producing

- Escherichia coli* in broiler litter under practical conditions. *Poult Sci* **99**: 2125–2135.
- Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., *et al.* (2010) Broad-spectrum β -lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS Microbiol Rev* **34**: 295–316.
- Thiel, N., Münch, S., Behrens, W., Junker, V., Faust, M., Biniash, O., *et al.* (2020) Airborne bacterial emission fluxes from manure-fertilized agricultural soil. *J Microbial Biotechnol* **13**: 1631–1647.
- Thomas, C., Idler, C., Ammon, C., Herrmann, C., and Amon, T. (2019) Inactivation of ESBL-/AmpC-producing *Escherichia coli* during mesophilic and thermophilic anaerobic digestion of chicken manure. *Waste Manag* **84**: 74–82.
- von Salviati, C., Laube, H., Guerra, B., Roesler, U., and Friese, A. (2015) Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. *Vet Microbiol* **175**: 77–84.
- Wathes, C.M., Howard, K., and Webster, A.J.F. (1986) The survival of *Escherichia coli* in an aerosol at air temperatures of 15 and 30°C and a range of humidities. *J Hyg (Lond)* **97**: 489–496.
- Wesche, A.M., Gurtler, J.B., Marks, B.P., and Ryser, E.T. (2009) Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne Pathogenst. *J Food Prot* **72**: 1121–1138.
- WHO. (2017) Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. *WHO/EMP/IAU/201712* Licence: CC BY-NC-SA 3.0 IGO.
- Yao, M., and Mainelis, G. (2006) Investigation of cut-off sizes and collection efficiencies of portable microbial samplers. *Aerosol Sci Tech* **40**: 595–606.
- Zhao, Y. (2011) *Measuring airborne microorganisms and dust from livestock houses*. PhD Thesis. Wageningen, The Netherlands: Wageningen University (2011).
- Zhao, Y., Aarnink, A.J.A., De Jong, M.C.M., and Groot Koerkamp, P.W.G. (2014) Airborne microorganisms from livestock production systems and their relation to dust. *Crit Rev Environ Sci Technol* **44**: 1071–1128.
- Zheng, B., Huang, C., Xu, H., Guo, L., Zhang, J., Wang, X., *et al.* (2017) Occurrence and genomic characterization of ESBL-producing, MCR-1-harboring *Escherichia coli* in farming soil. *Front Microbiol* **8**: 1–7.
- Zobeck, T.M., and Van Pelt, R.S. (2015) *Wind erosion*. Madison, WI: Soil Science Society of America.
- Zucker, B.A., Trojan, S., and Müller, W. (2000) Airborne Gram-negative bacterial flora in animal houses. *J Vet Med Ser B* **47**: 37–46.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Molecular characterization of the isolates from the chicken litter taken in the barns, which was subsequently used in the wind tunnel trials.

Fig. S1. LOG_{10} of the geometric mean total viable count emitted per m^2 of soil for each level of wind velocity determined with the all-glass-impinger 30 (a) and the Coriolis μ (b) for the three wind tunnel trials. The error bars indicate the upper and lower 95% confidence intervals.

Fig. S2. Sketch of the wind tunnel; modified according to Funk (2000) and Funk *et al.* (2019).