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ORIGINAL ARTICLE

Modeling of interventions for reducing external *Enterobacteriaceae* contamination of broiler carcasses during processing

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Abstract

This article presents a mathematical model for the *Enterobacteriaceae* count on the surface of broiler chicken during slaughter and how it may be affected by different processing technologies. The model is based on a model originally developed for *Campylobacter* and has been adapted for *Enterobacteriaceae* using a Bayesian updating approach and hitherto unpublished data gathered from German abattoirs. The slaughter process in the model consists of five stages: input, scalding, defeathering, evisceration, washing, and chilling.

The impact of various processing technologies along the broiler processing line on the *Enterobacteriaceae* count on the carcasses' surface has been determined from literature data. The model is implemented in the software R and equipped with a graphical user interface which allows interactively to choose among different processing technologies for each stage along the processing line. Based on the choice of processing technologies the model estimates the *Enterobacteriaceae* count on the surface of each broiler chicken at each stage of processing. This result is then compared to a so-called baseline model which simulates a processing line with a fixed set of processing technologies.

The model calculations showed how even very effective removal of bacteria on the exterior of the carcass in a previous step will be undone by the cross-contamination with leaked feces, if feces contain high concentrations of bacteria.

KEYWORDS
broiler processing, cross-contamination, *Enterobacteriaceae*, mathematical modeling

1 | INTRODUCTION

The impact of processing on broiler carcass contamination has various important facets. One concerns the economic impact of reducing spoilage bacteria important for enhancing shelf-life of the products. At the same time, the reduction of pathogenic bacteria is important from a food safety perspective. Also, nonpathogenic organisms may be relevant for food safety due to their ability to carry and transfer antimicrobial resistance (AMR) genes (D'Costa et al., 2006; Hummel et al., 1977; Jiang et al., 2017). Meat or meat products can act as a vehicle for resistant bacteria, which may lead to exposure of large parts of the human population.

A sizable part of the meat consumption is of poultry origin. In the European Union for example about one-third of the meat consumed per capita originates from poultry (OECD/FAO, 2019). Studies on the prevalence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* using selective microbiological detection methods in broilers and broiler meat demonstrated their wide distribution with

more than 80% positive samples in livestock and more than 50% in food samples (Casella et al., 2017; Davis et al., 2018; Hering et al., 2016; Kaesbohrer et al., 2019).

Meat processing is an important stage at which microorganisms can spread across the handled carcasses (Hardie et al., 2019; Zwirzitz et al., 2020). Previous modeling approaches of the bacterial transmission during broiler processing focused mainly on pathogenic microorganisms like *Campylobacter* (Hartnett, 2001; Hayama et al., 2011; Nauta et al., 2007) Some of studies did a model-based evaluation of the impact of processing interventions for *Salmonella* in chicken (Bucher et al., 2012; Gonzalez et al., 2019). In one study (Parette, 2018) the reduction of *Enterobacteriaceae*, *Salmonella* spp., and *Campylobacter* spp. in poultry processing have been studied based on a Monte Carlo approach.

At the same time similar approaches for studying the spread of nonpathogenic bacteria known to be carriers of resistant traits have not been undertaken. Sporadic work has been done using limited statistical modeling to track resistant ESBL/AmpC *E. coli* in broiler flocks (Huijbers et al., 2016) or during processing (Pacholewicz et al., 2015). Our work presented here aims at this knowledge gap in order to provide a full mechanistic model that can be used to study the influence of processing technologies on the spread of resistance carrying bacteria along the poultry slaughter processing line.

It was the focus of the German joint research project EsRAM ("Entwicklung stufenübergreifender Reduktionsmaßnahmen für Antibiotikaresistente Erreger beim Mastgeflügel") to study interventions that may potentially lead to a reduction of ESBL- and ampicillin class C (AmpC)-producing *E. coli* along the broiler production chain. During the EsRAM project a literature review was conducted (Projahn et al., 2018) which looked at bacterial reduction on carcasses for different processing technologies. During EsRAM also new data has been gathered on *Enterobacteriaceae* on broiler carcasses from German abattoirs and from experimental rearing and processing. We aim to combine this new data with the results from the literature review of Projahn et al. (2018) and the existing mathematical model (Nauta et al., 2007; Nauta, van der Fels-Klerx, et al., 2005) on *Campylobacter* colonization of carcasses along the broiler processing line in order to close the above mentioned knowledge gap.

2 | MATERIALS AND METHODS

For the assessment of different intervention measures, a published mathematical model for the external bacterial concentration of broiler carcasses along the processing line was chosen (Nauta, Jacobs-Reitsma et al., 2005; Nauta, van der Fels-Klerx et al., 2005) and transferred into a software application. The model was originally implemented in the software @Risk (Palisade Corporation) and was reimplemented for this work in R ver. 3.5.1 (R Core Team, 2018). The source code of the model is provided in the Supporting Information (2.7z) of this article. The implementation of the model included a graphical user interface (GUI) using the R package shiny (Chang et al., 2018). This allows the user to choose easily from a list of interventions and get an estimate of the reduction of bacterial contamination due to the chosen intervention. Furthermore, the GUI allows changing the parameters of the distributions from which values for the initial bacterial load of feces in the colon (given as cfu/g *E. coli*) and the exterior of the carcasses (measured in CFU/carcass) are drawn. Data on the enumeration of the *Enterobacteriaceae* in feces and on carcasses, collected within the project, were used as starting values.

2.1 | Mathematical model

2.1.1 | Mathematical structure

The mathematical model is only briefly described here, for more details we refer to the original publications of the model (Nauta, Jacobs-Reitsma et al., 2005; Nauta, van der Fels-Klerx et al., 2005).

The model assumes a chicken slaughter processing line with six processing stages:

- input,
- scalding,
- defeathering,
- evisceration,
- washing and
- chilling.

The model mathematics perform a kind of bookkeeping about how many bacteria are on the chicken carcass and in its environment and how many bacteria move between both at each stage along the processing line (see Figure 1). There are three places in the model where bacteria can be at the exterior of the broiler carcass, in leaked feces and in the environment of the carcass (which means the surroundings of the carcass at the current processing stage, e.g., the scalding water is the environment of the carcass in the scalder).

Furthermore, the model considers three types of processes that describe what happens to bacteria at each of the three places:

- bacteria on the exterior of the carcass can become inactivated, or they can move from the surface of the chicken to the environment.
- bacteria from leaked feces might move to the surface of the chicken or to the environment.
- bacteria in the environment of the chicken can become inactivated, or they can move to the chicken exterior.

The model describes which proportion of bacteria moves from one place to the other at each individual processing

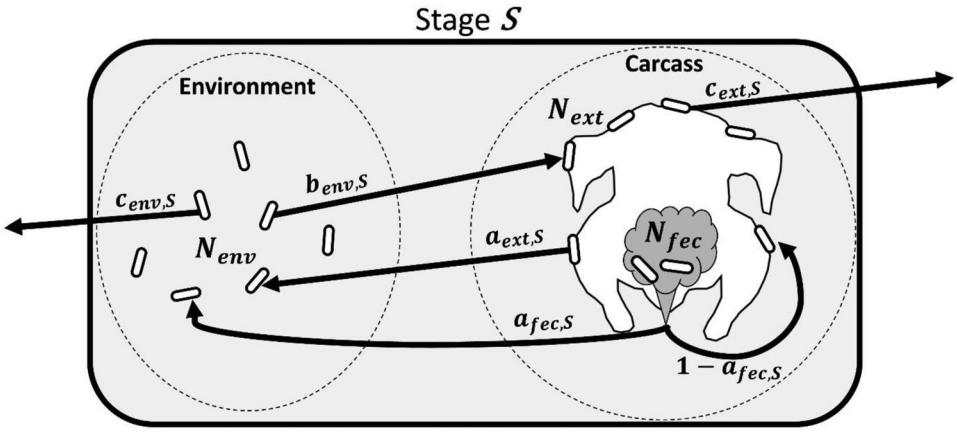

FIGURE 1 The processes that the model considers at each stage S of the processing line. The rod-shaped entities represent bacteria in the environment of the carcass, on the exterior of the carcass and in the feces in the carcass' gut. The current number of bacteria in the environment, on the carcass' exterior and in the gut's feces are called N_{env}, N_{ext} and N_{fec}, respectively. The arrows show how bacteria move, with $a_{ext,S}$, $c_{ext,S}$, $b_{env,S}$, $c_{env,S}$, $a_{fec,S}$ being the proportions of bacteria which move between environment and carcass during processing at the stage.

stage using the following two recursive formulas:

$$N_{ext,\,S}(i) = \left(1 - a_{ext,S}\right)\left(1 - c_{ext,S}\right) N_{ext,S-1}(i) + b_{env,S} N_{env,S}(i-1) + \left(1 - a_{fec,S}\right) N_{fec,S}(i) \quad (1)$$

$$N_{env,\,S}(i) = a_{ext,S}\, N_{ext,S-1}(i) + (1 - b_{env,S})\left(1 - c_{env,S}\right) N_{env,S}(i-1) + a_{fec,S} N_{fec,S}(i) \quad (2)$$

Here, $N_{ext,\,S}(i)$ stands for the number of bacteria on the external of the i-th carcass while it is at processing stage S.

$N_{env,\,S}(i)$ stands for the number of bacteria in the environment of stage S while the i-th carcass is at that stage. The subscript "$S-1$" or "$i-1$" describe the previous processing stage and the previous carcass, respectively. $N_{fec,S}(i)$ stands for the number of bacteria originating from feces, that leaked from the intestine of the i-th carcass at station S. This number is calculated in the model using Equation (3).

$$N_{fec,S}(i) = w_{fec,S}(i) \cdot C_{fec}(i) \quad (3)$$

$C_{fec}(i)$ is the number of cfu per gram feces that leaked from the intestine of the i-th carcass while $w_{fec,S}(i)$ is the mass of the feces in gram which leaked from the i-th carcass at station S . Not all carcasses leak feces. The way this is incorporated in the model is that for one model run a probability p_{fec} is used to determine whether a carcass leaks feces. For that, in each model run we draw for each chicken a value p_{leak} from the uniform distribution $U(0,1)$. If $p_{leak} \leq p_{fec}$ then we set $w_{fec,S}(i) = m_s$ otherwise we set $w_{fec,S}(i) = 0$. Here m_s is the mean mass of leaked feces at stage S.

The model parameters $a_{ext,S}$, $c_{ext,S}$, $b_{env,S}$, $c_{env,S}$, $a_{fec,S}$ are proportions, that is, they have values between 0 and 1. They indicate which proportion of the bacteria present at this stage moves from one place to the other. While the letter of a parameter indicates where the bacteria are going to, their subscript indicates from where the bacteria are coming. All parameters with letter a describe which proportion of bacteria goes to the environment (e.g., $a_{ext,S}$ indicates the proportion of bacteria which goes from the exterior to the environment). All parameters with letter b describe the proportion of bacteria going to the exterior of the carcass and parameters with letter c describe the proportion of bacteria becoming inactivated.

Equations (1) and (2) describe mathematically what happens at each stage except for the "input" stage. At "input" the model is initialized. This means that the initial colonization with *Enterobacteriaceae* of the feces in the colon (given in cfu per gram feces) and on the exterior of the carcasses (given in cfu per carcass) are drawn for each animal from predefined distributions. Here we assumed the distributions to be log-normally distributed. The corresponding decadic logarithms of these values are then normally distributed log cfu values per gram feces or per carcass. Thus, in the model calculations we draw the log cfu values for feces and the carcass' exterior from normal distributions.

As mentioned above the GUI of the model app allows changing the distribution parameters (mean and standard deviation) describing the initial external carcass colonization as well as the internal feces colonization using slider buttons. Based on the selected values, the model calculates the new external colonization at the next processing stage in a recursive way using the formulas in Equations (1) and (2).

2.1.2 | Initial conditions

The model calculations simulate 100 flocks with 500 animals each (running the model with more realistic flock sizes like 50,000 did not affect the model outcome and only increased the execution time) and use as initial conditions the initial external and fecal colonization of the animals. The

distributions for the initial external colonization as well as for the colonization of the feces were determined using empirical data from the partners of EsRAM project.

External colonization

For the initialization of the statistical model data of one examination trial of the EsRAM consortium were included. The data originated from sampling carcasses from 16 broiler flocks at a commercial broiler abattoir. Sampling took place at five processing steps in the slaughter line: before scalding, after scalding, after plucking, before chilling, after chilling. At each processing step four carcasses were taken out of the line and breast skin samples were taken aseptically. So, per flock four samples per processing step were achieved. In order to initialize the model with realistic values for the external colonization of carcasses we used only the samples taken before scalding (which corresponds to the stage "input" in the model). Thus, 16 mean carcass colonization values were calculated (one mean for each flock and each mean was the average of the colonization values for the four animals sampled from each flock). The external colonization was determined by performing microbiological analysis for *Enterobacteriaceae* count within 24 h in the laboratory after sampling. Results in cfu/g breast skin were transformed to logarithmic scale ($\log_{10}$).

This empirical data on the colonization of the breast skin samples were extrapolated to a whole surface colonization for the model calculations since the model calculates log cfu per carcass instead of log cfu per g (see also the Supporting Information [4.7z]).

Following Nauta, Jacobs-Reitsma et al. (2005) for each simulated flock an individual within-flock-distribution for the initial external contamination was derived. This was done in a two-step process: starting with the first of the 100 flocks in a first step a value was drawn from the between-flock-distribution. This drawn value was then used as mean of a within-flock-distribution for that first simulated flock. For the next modeling step, this value was used as the mean to define a normal distribution with a given standard deviation (see below). The second step consisted then in drawing from this newly defined within-flock-distribution 500 values, each one indicating the initial log cfu counts on the exterior of each of the 500 animals.

Assuming that the between-flock-distribution is a normal distribution, its mean μ_{bf} was set to 7.93 $\log_{10}$ cfu/carcass, which is the arithmetic mean of all 16 mean log cfu counts (one mean for each of the 16 sampled flocks). Correspondingly, the standard deviation σ_{bf} of the between-flock-distribution was set to 0.21 $\log_{10}$ cfu/carcass, which is the standard deviation of all 16 mean log cfu counts. The standard deviation of the within-flock-distribution σ_{wf} was set to 0.38 $\log_{10}$ cfu/carcass, which is the mean of the 16 log cfu standard deviations (one standard deviation for each of the 16 sampled flocks).

Internal colonization

In order to estimate the initial values for the internal colonization we used the results from another EsRAM study (Robe et al., 2021). Samples from caecum and colon contents taken from these animals for enumeration of *Enterobacteriaceae* showed in the colon a mean of 6.56 $\log_{10}$ cfu/g feces and a standard deviation of 0.84 $\log_{10}$ cfu/g feces, respectively. Thus, numeric log values of $C_{fec}(i)$ were drawn from a normal distribution $N(6.56,\ 0.84)$.

2.2 | Baseline process model

The baseline process model was defined by a given set of parameter values which were assumed to describe the slaughtering process in a broiler abattoir with a given set of processing steps. In field work performed in the EsRAM project data collected from one of three visited abattoirs were used to fix the baseline parameters. This particular plant was chosen since it featured a maximum number of processing technologies of interest, namely technologies considered in Projahn et al. (2018).

The baseline abattoir was equipped with a triple-tank immersion scalder. Broiler chickens were subjected to a low-temperature scalding at about 51–55°C for three minutes. Afterwards carcasses were moved to a closed defeathering machine with a subsequent washing unit installed. An inside-out washer followed the evisceration line. Finally, carcasses were subjected to an air chilling process for about 3 h (average carcass core temperature after chilling of 2.3°C).

2.2.1 | Bayesian updating of model parameters

The model described in Equations (1) and (2) was originally developed to study how the number of *Campylobacter* on chicken carcasses changes during slaughter. In order to adapt the model to our case of *Enterobacteriaceae* we followed a Bayesian updating approach described by Kurowicka et al. (2010) which is based on importance sampling.

The Bayesian updating approach worked in principle as follows. A prior estimate of the $\log_{10}$ cfu of *Enterobacteriaceae* on the carcasses after each stage in the processing line is calculated based on the model Equations (1) and (2) using a complete set of model parameters for *Campylobacter* spp. Then these model parameters are updated using data of *Enterobacteriaceae* counts on carcasses obtained from the above-mentioned baseline abattoir in order to adapt the model parameters to *Enterobacteriaceae*.

The initial model parameters for *Campylobacter* spp. were derived from an expert elicitation (Van der Fels-Klerx et al., 2005). The complete set of model parameters at a given stage S is considered to be a random variable $\theta_S = [a_{ext,S},\ b_{env,S},\ a_{fec,S},\ c_{env,S},\ w_{fec,S}(i),\ c_{ext,S},\ p_{fec}]$ described by a multivariate distribution, which Kurowicka et al. (2010) denotes as f_θ. It is assumed that each stage S has a different dynamic with respect to the moving of bacteria between environment and carcasses. Hence we denote here the distributions of model parameters at a stage S as $f_{\theta,S}$. We obtained samples from $f_{\theta,S}$ (M. Nauta, personal communication, February 12, 2018) which we used to calculate our

prior estimate of the $\log_{10}$ cfu of *Enterobacteriaceae* on the carcasses after each stage. For a depiction of the distributions of the parameters, see Nauta, Jacobs-Reitsma, et al. (2005, p. 127). For each stage S the samples contained 501 complete sets of model parameters θ_S drawn from $f_{\theta,S}$. Thus, one can write the samples as $\theta_S = t_n,\ n = 1, \ldots, 501$.

Kurowicka et al. (2010) updated $f_{\theta,S}$ to $g_{\theta,S}$ where the latter is an updated distribution describing the model parameters based on new data of *Campylobacter* counts on carcasses. In contrast, we updated $f_{\theta,S}$ to $h_{\theta,S}$ where the latter is an updated distribution describing the model parameters based on data of *Enterobacteriaceae* counts on carcasses.

Starting at the first stage we take the first complete set of model parameters, t_1, and run the model with 500 chickens with initial internal and external *Enterobacteriaceae* colonization derived from our slaughterhouse data before the first stage. Then the external colonization of all chicken after passing that stage is calculated based on the model Equations (1) and (2) using $\theta_S = t_1$. The $\log_{10}$ cfu external colonization of all chicken after being processed at the first stage is averaged according to $m\,(n = 1) = \frac{1}{500}\sum_{i=1}^{500} log_{10}(N_{ext,S=Stage1}(i))$. Then we do the same for the remaining 500 complete sets of parameters (i.e., from t_2 to t_{501} calculating $m(n = 2)$ to $m(n = 501)$). Thus, we obtained a sampling distribution of the mean external colonization $m(n)$ after processing at the first stage which represents our prior estimate for the external colonization based on $f_{\theta,S}$.

In the next step, we estimated a quantity proportional to the likelihood of observing each $m(n)$ given the data. In this case it is the data on the external colonization of carcasses with *Enterobacteriaceae* gathered in processing plants during the EsRAM project. This likelihood was estimated by using the R function dnorm() (pseudocode: $g(n) = \text{dnorm}(m(n), \mu_{S=Stage1}, \sigma^2_{S=Stage1})$) in order to numerically determine the probability density at each value $m(n)$ for a normal distribution $N(\mu_{S=Stage1},\ \sigma^2_{S=Stage1})$. The parameters $\mu_{S=Stage1}$ and $\sigma^2_{S=Stage1}$ were the mean and variance of the $\log_{10}$ cfu values for the external colonization of carcasses with *Enterobacteriaceae* as found in the slaughterhouse data after the first stage. As a result, we got 501 values $g(n)$ as a numerical estimate proportional to the likelihood of observing each $m(n)$ given the data. By normalizing the values $g(n)$ through $w(n) = \frac{g(n)}{\sum_{n=1}^{501} g(n)}$ we end up with normalized weights $w(n)$ which are then used to resample from our sample of $f_{\theta,S=Stage1}$, that is, $\{t_1, t_2, \ldots, t_{501}\}$. Resampling means that the complete set $\theta_S = t_{n=1}$ will be drawn with the probability $w(n = 1)$, the complete set $\theta_S = t_{n=2}$ with probability $w(n = 2)$ and so on. After resampling 501 times from $\{t_1, t_2, \ldots, t_{501}\}$ we got a new updated sample with complete sets of model parameters $\{t_1^{up}, t_2^{up}, \ldots, t_{501}^{up}\}$ and we assume this to be a sample from the distribution $h_{\theta,S=Stage1}$.

Finally, we sampled each model parameter over all $t_1^{up}, t_2^{up}, \ldots, t_{501}^{up}$, for example, $\bar{a}^{up}_{ext,S=Stage1} = \frac{\sum_{n=1}^{501} a^{up}_{ext,S=Stage1}(n)}{501}$ and used these updated mean model parameters in the final processing line model.

The same updating procedure was performed at all subsequent stages. The updated model parameters were then used in the model calculations in the shiny app. See Table 1 for the parameter values before and after updating.

We provided the source code in R for our updating process in the supplementary material (in the file Supplement3.7z). This R code is an adaptation of the original code written in Matlab which was used in Kurowicka et al. (2010) and shared with us (D. Kurowicka, personal communication, November 2, 2018).

2.3 | Intervention process model

The interventions at the level of processing technologies considered in the model can take effect in two different ways. These two ways reflect two different approaches followed by the various authors of the studies reviewed in Projahn et al. (2018) when investigating processing technologies.

In one of these two ways, authors compared the processing technology used in our baseline processing plant with an alternative technology. First, these authors measured cfu counts found on carcasses at a given stage after using the baseline processing technology. Then the authors compared these cfu counts with the cfu counts after using an alternative technology at the same stage. The mean cfu counts on carcasses after using the technology from the baseline processing plant and after using the alternative technology resulted in a mean reduction due to the alternative technology compared to the baseline processing technology. As a simple example let us assume it is a mean 1 $\log_{10}$ reduction. Therefore, this reduction represents a reduction that the alternative technology provides compared to the baseline technology. Correspondingly this kind of reduction is incorporated in our model by reducing the result of the model calculation from the baseline process (in our example: the cfu counts on each carcass as calculated at the end of the considered stage of the baseline process model is reduced by 1 $\log_{10}$, that is, multiplied by the factor 0.1). In our model, the relative reduction of the intervention is applied after modeling the baseline process according to Equations (1) and (2) and the resulting external contamination. In this sense, the effect of the intervention (i.e., the alternative processing technology) is additionally considered to the effect calculated through the baseline process model. In the app interventions of this kind, when chosen from the drop-down menu are marked with an "(A)" at the end which stands for "additionally considered," since the effect of the intervention was considered additionally to the results of the baseline process model calculations.

In the second of the two ways, authors only look at an alternative processing technology other than that used in our baseline processing plant for a given stage. Here, these authors compare the mean cfu counts on carcasses before and after the use of the alternative processing technology in order

TABLE 1 Mean model parameters before and after Bayesian updating approach

Model parameters before updating						
Stage	$a_{ext,S}$	$b_{env,S}$	$a_{fec,S}$	$c_{env,S}$	$c_{ext,S}$	p_{fec}
Scalding	0.76450539	8.14E-06	0.99999844	0.04903812	0.70983493	0.48399142
Plucking	0.86521098	2.80E-02	0.9999868	0.10365409	0.05105988	0.69552954
Evisceration	0.46305888	1.11E-05	0.99378606	0.08478044	0.0415988	0.6573509
Washing	0.34214247	5.99E-03	1	0.06314914	0.21213199	0
Chilling	0.09009878	8.04E-03	1	0.01705675	0.19484161	0
Model parameters after updating						
Stage	$a_{ext,S}$	$b_{env,S}$	$a_{fec,S}$	$c_{env,S}$	$c_{ext,S}$	p_{fec}
Scalding	0.7236513	8.54E-06	0.99999856	0.05238084	0.70174661	0.52306267
Plucking	0.9976000	1.68E-04	0.99999186	0.11330000	0.06922	0.6933
Evisceration	0.38310878	1.13E-05	0.99449981	0.08366268	0.04226497	0.63666267
Washing	0.24499485	9.96E-03	1	0.05760156	0.19164983	0
Chilling	0.07559658	8.72E-03	1	0.01628155	0.07404469	0

to determine the relative reduction due to use of the alternative processing technology. Let us again assume as a simple example that some authors found a mean 1 $\log_{10}$ reduction after the use of the alternative technology compared to the cfu count before carcasses enter the stage with the alternative technology. Therefore, this reduction represents the total reduction due to the alternative technology applied at this processing step. Correspondingly, this kind of reduction is incorporated in the model in the following way: We did not perform the model calculations based on the Equations (1) and (2) at this stage but rather replace them by simply reducing the cfu counts of all carcasses as they were after leaving the previous stage (in our example we reduce the cfu counts on each modeled carcass after the previous stage by 1 $\log_{10}$). These interventions (i.e., the alternative technologies) are marked with a "(R)" at the end, which stand for "replaced" since we replaced the usual baseline model calculations from Equations (1) and (2) by a simple reduction reported in the corresponding study.

In other words, the reductions due to interventions of type "(R)" and "(A)" are distinct as far as the modeling calculations are concerned (and are therefore implemented differently as described in the previous two paragraphs). On the one hand a reduction due to an intervention of type "(A)" represents an incremental reduction in addition or relative to the baseline process as described in Equations (1) and (2). On the other hand, a reduction due to an intervention of type "(R)" represents the total reduction due to the intervention without any reference to the baseline process. It contains the complete information about what happens to the cfu count due to the intervention in that stage of the processing and calculations based on Equations (1) and (2) are obsolete.

Interventions which are currently not available for commercial use, but which were studied in a laboratory or experimental setting are flagged with the comment "(experimental)" behind the intervention name in the drop-down menus. Determining log reductions of interventions from the literature.

In this section and in the Supporting Information 1, we report how we derived log reductions from data generated in the EsRAM project and from the literature reviewed in (Projahn et al., 2018). The focus in (Projahn et al., 2018) was laid on *E. coli* as a representative of the *Enterobacteriaceae*.

Some authors cited in (Projahn et al., 2018) used standard deviations (SD) and others used standard errors (SE) in order to describe their results. To record which statistic was used we explicitly mention this in brackets using "(mean ± SD)" or "(mean ± SE)." We used error propagation calculations to assess the uncertainty of the bacterial reduction estimates we derived from the literature. In order to quantify the variability of the estimated reductions we used always standard errors. To quantify the central tendency we used (arithmetic) means as estimates of the reductions.

The model simulates the changing number of bacteria on broiler carcasses during processing. Here only processing methods allowed in the European Union were considered. Therefore, interventions, which use anything beyond plain potable water (e.g., chlorinated water) for washing the carcasses, have been excluded.

We analyzed the literature reviewed in Projahn et al. (2018) for the following stages: preprocessing in the slaughterhouse, scalding, defeathering, evisceration, washing, and chilling. Furthermore, we analyzed data from the EsRAM project for interventions at the farm level. In the following we demonstrate by means of an example how we derived the bacterial reduction from data generated on some interventions studied in the EsRAM project and from data described in the literature reviewed in Projahn et al. (2018). First, we will show how we derived the log reduction from EsRAM data and afterwards from literature data. At the end we explain how the determined reductions were integrated in the model calculations.

The EsRAM data we used for our demonstration originated from one trial conducted during the EsRAM project. The trial dealt with an intervention at the farm level, namely the reduced stocking density (Robe et al., 2021). In that experiment a control group of broiler chicken (Ross 308) was reared under conventional farming conditions. This included no pen enrichment, commercial grower ration provided by the poultry industry partners of the EsRAM project, feed and water ad libitum, and stocking density of 39 kg per sqm. The birds were challenged with predefined resistant ESBL-/AmpC–producing *E. coli* strains. An intervention group was reared in the same way with the exception of a reduced stocking density of 25 kg per sqm (Robe et al., 2021). Both groups were reared in a controlled environment and subsequently brought to an experimental slaughter facility. There the external colonization (breast skin) with *Enterobacteriaceae* in general and ESBL-/AmpC-producing *E. coli* strains was measured quantitatively using swab sampling at each station of the slaughter process (Projahn et al., 2021). At their arrival at the experimental slaughter facility, the animals of the control group had a mean and standard deviation for the external colonization with *Enterobacteriaceae* of 4.15 ± 0.79 $\log_{10}$ cfu/20 sqcm. The animals of the reduced stocking density group had at their arrival a mean external colonization of 2.69 ± 0.81 $\log_{10}$ cfu/20 sqcm. Note that the unit "cfu/20 sqcm" comes from the swab sampling technique where a sterile screen with a quadratic opening of 20 sqcm was placed on the broiler's breast skin and swabbed.

To determine a best estimate for the $\log_{10}$ reduction Δc of the external contamination due to the reduction of the stocking density we proceeded in the following way. We subtracted the logs of the means of the external contamination for the control group and the intervention group. The resulting 1.46-$\log_{10}$ reduction means then that the external contamination has been reduced by a factor of $10^{-1.46} = 0.034$. Since the log reduction is a relative measure of reduction this 1.46-$\log_{10}$ reduction is independent of the units used for the absolute counts (measured in cfu/sqcm by Projahn et al. (2021) or cfu/carcass by our processing model). The log reduction applies to both units in the same way, namely it describes a reduction of the bacterial counts by a factor of 0.034. Hence, we always derived the log reduction for each intervention and used it to take the effect of the intervention into account in our model.

In order to estimate the variability of the reduction values we used Gaussian error propagation for estimating the standard error of the differences of the means following the procedure described in Hamilton (2003). Gaussian error propagation in this case gave for the standard error a value of 0.2. Thus, lower stocking density leads to a 1.46 ± 0.2-$\log_{10}$ reduction (mean $\pm$ se).

The best estimate for reduction Δc and the standard error were integrated into the model calculations in the following way. In order to introduce some variability into the reduction we assumed it to be normally distributed with mean Δc and a standard deviation which corresponded to the calculated standard error. As described above the interventions can be of type "(R)" or type "(A)." Since in our current example we derived the log reduction by comparing the baseline process "control" (animals reared with a stocking density of 39 kg per sqm) with the alternative approach (animals reared with a stocking density of 39 kg sqm) we have here an example of an intervention of type (A). For each modeled carcass a reduction value, say "reduct," was drawn from a normal distribution with a mean of 1.46 and a standard deviation of 0.2. After the carcasses external colonization had been determined by the model it was reduced by a factor of $10^{-\text{reduct}}$ to reflect the effect of the intervention measure at primary production.

For some interventions the authors of the corresponding publications did not provide enough information to calculate the standard error. In this case we assumed a value (see the file "Supplement1.docx"). This uncertainty estimates were used to add variability to the reduction of the applied interventions. The literature data we used for our demonstration is based on data from high pressure spray wash using potable water published (Giombelli, 2013; Giombelli et al., 2015). In this case the spray wash equipment was operated with a pressure of 1000 kPa for carcasses with and without visible gastrointestinal contamination (VGC). The data in Giombelli (2013) are summarized in Table 2.

In order to estimate the mean log reduction, we subtracted the mean log cfu values in the last two columns in Table 2 from each other. Then, using error propagation we calculated the standard error (where we took the reported 50 animals as sample size for each of the groups) and arrived at Table 3 which contains the log reduction values.

Giombelli (2013) performed Tukey tests for the 1000 kPa data. The results showed only in the "washing" intervention of the "With VGC" condition a significant difference between the mean log cfu values before and after washing.

We chose the 0.56 ± 0.14-$\log_{10}$ reduction for the 1000 kPa spray wash. If the intervention "1000 kPa (R)" is chosen, this 0.56 ± 0.14-$\log_{10}$ reduction was used to determine the normal distribution from which the reduction values for each carcass was drawn. And it was the reduction value drawn for each carcass which was used as a substitute intervention for the baseline washing process, which is based on a low pressure outside washer.

3 | RESULTS AND DISCUSSION

The results presented here consist in an open-source reimplementation in R of a model for the change of bacterial counts on carcasses during processing. We adapted the model parameters for the case of *Enterobacteriaceae* using a Bayesian updating approach and empirical data from the EsRAM project. The effect of various technological or husbandry-based interventions on the bacterial contamination was estimated based on results reported in the literature and integrated in the model. The results from our analysis of the literature on bacterial reduction due to different interventions are summarized in Table 4. A GUI was implemented using the R package shiny in order to allow easy changes on the initial bacterial load on the carcasses and the applied interventions.

TABLE 2 Data from Giombelli (2013, Table III.5) considering the *Escherichia coli* contamination before and after spray washing (with 1000 kPa pressure) and/or trimming for the case that the carcasses showed visible gastrointestinal contamination (VGC) or not

VGC status	Intervention	Mean ± SD *E. coli* (log cfu/g) before (mean ± SD)	Mean ± SD *E. coli* (log cfu/g) after (mean ± SD)
Without VGC	Washing	4.68 ± 0.49	4.25 ± 0.62
With VGC	Washing	4.85 ± 0.67	4.29 ± 0.68
	Trimming	4.85 ± 1.11	4.53 ± 1.09

TABLE 3 Log_{10} reduction calculated using error propagation using data from Giombelli (2013) considering the *Escherichia coli* contamination before and after spray washing (with 1000 kPa pressure) or trimming for the case that the carcasses showed visible gastrointestinal contamination (VGC) or not

VGC Status	Intervention	Log_{10} reduction of *E. coli* (mean ± SE)
Without VGC	Washing	0.43 ± 0.11
With VGC	Washing	0.56 ± 0.14
	Trimming	0.32 ± 0.22

3.1 | The role of fecal (or internal) colonization

Choosing different combinations of interventions lead to different courses of the log cfu curves along the processing line. Usually, the curve for the processing model declines steadily. But there are combinations that lead to a kind of dipping behavior of the curve in the sense that it declines from one station to the next and then rises again as it proceeds to the next processing stage. This dipping behavior can be seen if one chooses at the farm level the reduced stocking density and at the scalding stage the 65.8°C for 2.5 min treatment. This behavior is caused by cross-contamination with bacteria from leaked feces with relatively high fecal colonization. If the fecal colonization is set to its minimum value of 3 log_{10} cfu/g, cross-contamination is reduced and contamination level declines monotonically.

This finding from the model calculations provides theoretical evidence that the level of the internal colonization plays an important role in the external colonization along the processing line. It shows that in case of contamination the level of internal colonization might thwart highly effective reduction interventions that work only on the exterior of the carcass at a prior processing step. In that case, it leads to raising colonization numbers on the exterior of the carcass as shown in Figure 2 (A). One can see that usually the log cfu counts drop continuously along the slaughter process. But in Figure 2 (A) the curves which started at the lowest mean external cfu counts (at 4 log_{10} cfu/carcass and 2 log_{10} cfu/carcass) show a different behavior. The curve starting at 4 log_{10} cfu/carcass shows dipping in that it drops from "input" to "scald" and then raises from "scald" to "defeather." The curve starting as 2 log_{10} cfu/carcass raises from "input" through "scald" to "defeather." The dipping behavior shown in Figure 2(A) is due to a disproportionally high colonization of feces compared to the external colonization. Due to cross-contamination at this step, the fecal load pushes the external colonization disproportionally up. If the external colonization falls below a certain level (e.g., because we set log_{10} cfu values at "input" low by hand as in Figure 2(A) or because there might be highly effective interventions that removes many bacteria on the exterior of the carcass) the bacteria from leaking feces pushes the colonization up again, that is, we see the dipping behavior. This means that even very effective removal of bacteria on the exterior of the carcass will be undone by the cross-contamination with leaked feces as displayed by the model mechanics implemented here.

One can see that this behavior of the model is due to the leakage of colonized feces. If we set the fecal colonization to zero, we see a monotonous drop of the log cfu curves irrespective of the initial mean value of the external colonization as shown in Figure 2(C). The fecal colonization was set to zero by setting the mean and standard deviation of the normal distribution from which $C_{fec}(i)$ values were drawn to zero. Note that setting the fecal colonization to zero in the model is equivalent to setting the amount of leaked feces to zero (see Equation (3). This means that we could have achieved the same effect if we would have set $w_{fec,S}(i)$ to zero. Both ways would avoid that any bacteria from the feces can contaminate the exterior of carcasses in the model.

An intermediate situation is shown in Figure 2(B). Here the fecal load is chosen in such a way that it drops proportionally to the external colonization. Here the log values from $C_{fec}(i)$ have been drawn from distributions with means that were always 1.5 log_{10} lower than the means from the distributions from which the log cfu of the external colonization are drawn. For example, for the mean log external colonization 8 cfu/carcass the mean log cfu internal colonization was set to 6.5 cfu/g feces, for the mean log colonization 6 cfu/carcass the mean log fecal colonization was set to 4.5 cfu/g feces, etc. The standard deviations for the fecal (i.e., internal) colonization distributions were also lowered as the means of the fecal colonization dropped. The standard deviations were always 0.13 times the values of the means from the fecal distributions. For example, for the mean 6.5 cfu/g feces the corresponding standard deviation was set to 0.85 cfu/g feces,

TABLE 4 Summary of the interventions and corresponding reductions for all stages which have been incorporated into the interactive model app. The mean ± SE $\log_{10}$ reduction values are marked with "(A)" and "(R)" in order to indicate whether the corresponding reduction is of the "additionally considered" type (i.e., considered additionally to the model calculations of Equations (1) and (2)) or the "replaced" type (i.e., replacing the model calculations Equations (1) and (2) as described in Materials and Methods section. The reductions resulted from comparing the bacterial counts to baselines defined in the individual studies

Stage	Intervention	Mean Log_{10} Reduction	Reference
At farm level	Competitive exclusion	0.72 ± 0.20 (A)	(Projahn et al., 2021)
	Reduced stocking density	1.46 ± 0.22 (A)	(Projahn et al., 2021)
Pre-processing	Brush breast/vent	0.30 ± 0.10 (A)	(Pacholewicz et al., 2016)
	Cloacal plugging	0.39 ± 0.08 (A)	(Musgrove et al., 1997)
Scalding	Immersion scalding 59.5°C for 2.5 min	1.57 ± 0.09 (R)	(Notermans et al., 1975)
	Immersion scalding 62.5°C for 2.5 min	3.00 ± 0.09 (R)	(Notermans et al., 1975)
	Immersion scalding 65.8°C for 2.5 min	4.00 ± 0.09 (R)	(Notermans et al., 1975)
	Counterflow triple tank	2.60 ± 0.10 (R)	(Berrang et al., 2003)
Defeathering	Hot water rescald	0.50 ± 0.33 (A)	(Berrang et al., 2000)
	Hot water spray	0.70 ± 0.33 (A)	(Berrang et al., 2000)
Evisceration	Skin removal	1.40 ± 0.10 (A)	(Berrang et al., 2002)
Washing	High pressure spray wash (1000 kPa)	0.56 ± 0.14 (R)	(Giombelli, 2013)
	Steam treatment 100°C for 5 sec	1.44 ± 0.30 (R)	(James et al., 2007)
	Steam treatment 100°C for 10 sec	1.60 ± 0.07 (R)	(James et al., 2007)
	Steam treatment 100°C for 12 sec	2.26 ± 0.33 (R)	(James et al., 2007)
	Steam treatment 100°C for 20 sec	2.83 ± 0.30 (R)	(James et al., 2007)
	Steam treatment 138°C for 1.1 sec	1.27 ± 0.03 (R)	(Kozempel et al., 2003)
	Hot water 80°C for 10 sec	0.94 ± 0.18 (R)	(James et al., 2007)
	Hot water 80°C for 20 sec	1.68 ± 0.12 (R)	(James et al., 2007)
Chilling	Salting	1.77 ± 0.24 (A)	(Shin et al., 2012)
	Crust freezing	0.80 ± 0.52 (R)	(Chaves et al., 2011; James et al., 2007)
	Immersion chilling	1.00 ± 0.25 (R)	(Berrang et al., 2008; Chaves et al., 2011; Dickens et al., 2000; James et al., 2007; Souza et al., 2012)

for the mean 4.5 cfu/g the standard deviation was set to 0.59 cfu/g feces.

As Nauta et al. (2009) reports, this dipping effect has also been found in the original version of the (Dutch) model for *Campylobacter* and in another *Campylobacter*-in-chicken-model developed in the United Kingdom (Hartnett, 2001). However, Nauta et al. (2009) observe that the dipping happens for the Dutch *Campylobacter* model at scalding, while in the UK model it happens at plucking. This difference is, according to Nauta et al. (2009), attributed to different model assumptions, which primarily are based on different expert opinions. Whether this difference is just a difference of opinion or a true difference between countries is unclear.

The dipping effect itself shows according to Nauta et al. (2009) the large impact of fecal contamination of carcasses. As shown above we reproduced this effect.

We additionally note that the dipping effect is not only about the large impact of fecal contamination per se. What is really important is the ratio of internal and external colonization. If the ratio of internal and external colonization remains constant there will be no dipping effect however high the fecal contamination is as exemplified in Figure 2(B).

3.2 | Limitations and further assumptions

The model assumes that each chicken is alone at each processing stage when processed and thus does not consider that multiple animals are together at one processing stage at the same time, what is what might happen for example in defeathering devices or in immersion scalding or immersion chilling. Thus, cross-contamination effects whenever multiple animals are being processed together in the same environment at the same time, say in a scalding tank, are not considered in the model. However, since Equations (1) and (2) consider that bacteria can cross-contaminate carcasses via the environment there is an indirect contact between animals incorporated in the model, but only in a strictly sequential way. For the example of scalding that means that in the model one chicken is being scalded alone in a scalding tank. Then it leaves the tank and a second chicken comes into the tank. The model now allows the first chicken to cross contaminate the second via the scalding water but not vice versa. In a more realistic setting both chicken would be scalded at the same time in the tank side by side allowing possible cross contamination by each other.

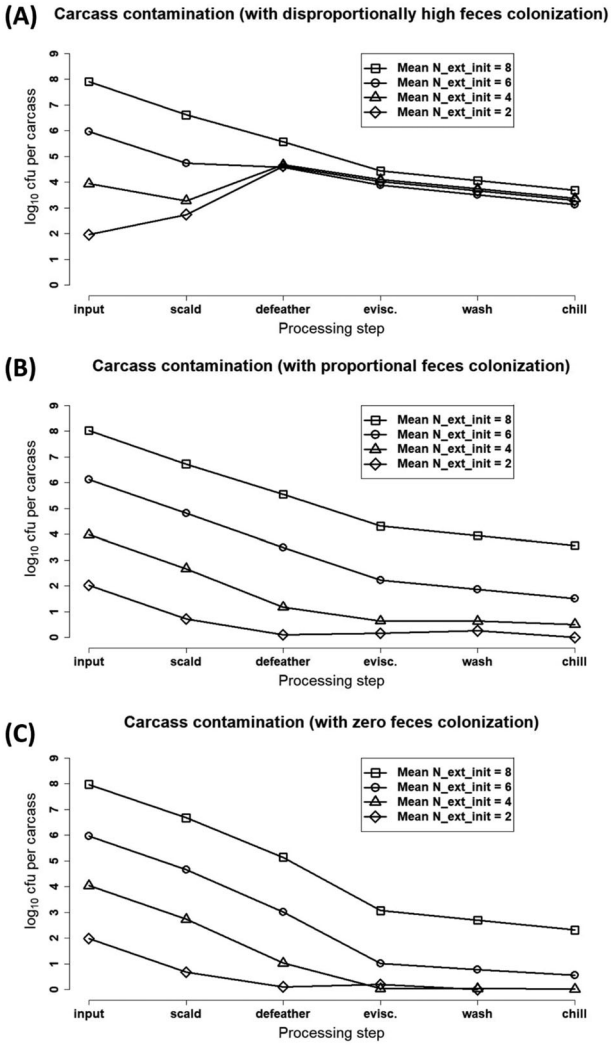

FIGURE 2 Subfigure(A) shows how the external bacterial colonization of the carcasses changes over the course of slaughtering if the log values from (***i***) are drawn from a normal distribution with mean of 6.56 and a standard deviation of 0.84. One sees that as the external colonization drops below a certain level in the initial phase it subsequently raises again instead of dropping (dipping behaviour). Thus, we have a disproportional high fecal colonization compared to the external colonization. Subfigure (B) shows the same model setup except that the log values from (***i***) are drawn from distributions with proportional fecal colonization levels. That is, the means of the distributions from which the log cfu of (***i***) are drawn are always 1.5 log10 lower than the value of the means from the distributions from which the log cfu of the external colonization are drawn and the standard deviations from the fecal distributions are always 0.13 times the values of the means from the fecal distributions. Subfigure (C) shows the model results for the same model setup except that we here assume that no bacteria arise from leaked feces. In this case the external colonization drops down to values near zero

Furthermore, the model assumes constant process effectiveness, meaning that each carcass is processed exactly with the same effectiveness. This ignores possible effects, like a less efficient reduction in case of initially very high colonization. In the same way an extremely low initial colonization will be diminished by the predefined reduction factor even though the very low initial colonization does not allow for such a high reduction at the given stage. This can lead to negative $\log_{10}$ values which indicate that the number of bacteria on the carcass has fallen below 1. Such numbers of bacteria have no biological sense, therefore in the model the external colonization of each carcass calculated using Equations (1) and (2) was rounded down to the next integer. That means that if the number of bacteria on the carcass in the model calculations falls below the value of 1 the model rounds it down to zero.

Given the minimal possible colonization numbers users can choose and given the values of the model parameters $a_{ext,S}$, $c_{ext,S}$, $b_{env,S}$, $c_{env,S}$, $a_{fec,S}$ for the baseline model, it never happens that the baseline model reaches negative $\log_{10}$ values for external colonization. However, given that by choosing interventions the colonization numbers down through the corresponding reduction, negative $\log_{10}$ values are possible which are then rounded to zero.

There are three limitations of the current model with respect to the way that the effect of different processing technologies (= interventions) are incorporated in the model. The first limitation is that incorporating interventions can lead to situations where the model parameters are ignored. If in a production stage an intervention of type (R) is chosen, the bacterial load on the carcasses at that production stage is not determined by the model parameters. If on a stage an intervention of type (A) is chosen, then the model parameters from the baseline model are used to calculate an intermediate result to which the reduction determined from the literature is added. Thus choosing an intervention at a given stage means skipping partly or completely the usual ways of the model to describe the situation at a given processing stage based on the model parameters $a_{ext,S}$, $c_{ext,S}$, $b_{env,S}$, $c_{env,S}$, $a_{fec,S}$. And this means that the processes of cross-contamination of carcass and environment are no longer simulated but replaced by an estimated reduction.

The second limitation is that the model can only take one intervention per stage into account. Thus, combined effects of multiple interventions at one stage cannot be modeled.

The third limitation is that choosing interventions at multiple processing stages can lead to a large overestimation of the modeled bacterial reduction along the processing line. We caution about the selection of interventions and discourage the use of interventions in multiple production stages as this means to in effect to use less of the model and just add up estimated reductions from the literature in an unrealistic manner. We rather suggest to choose intervention only at one or two stages in one model run.

Another limitation in this context is that the model might under- or overestimate the bacterial reduction depending on the settings chosen. The reason is that there is probably an effect of varying efficacy in bacterial reduction. That is, that efficacy in bacterial reduction for a given intervention might depend on the $\log_{10}$ cfu loads on the carcasses. Since we determined the log-reductions from individual studies with their individual $\log_{10}$ cfu loads on the carcasses, load-dependent effects might be missed. Take for example the 0.56- $\log_{10}$ reduction from Table 3. This reduction was

determined for animals at the washing stage using spray washing with 1000 kPa with animals having a mean $\log_{10}$ cfu load between 4.68 and 4.85. If the user would have chosen, the settings in the model app in such a way that the mean $\log_{10}$ cfu load would be well below 4.85 then choosing 1000 kPa wash as intervention with an assumed 0.56-$\log_{10}$ reduction would probably lead to an overestimation of the reduction. This overestimation would come from an assumed diminishing efficacy of the reduction effect with lower numbers of cfu load. The opposite might happen if the model settings are chosen such that the mean $\log_{10}$ cfu load on carcasses at the washing stage would be well above 4.85. In that case, the assumed 0.56-$\log_{10}$ reduction would probably lead to an underestimation of the reduction effect. This underestimation would come from the assumed effect that for around 4.85 $\log_{10}$ cfu the 1000 kPa wash leads to the 0.56-$\log_{10}$ reduction but for higher $\log_{10}$ cfu counts the reduction efficacy might be higher. Thus, the model might over- or underestimate the effect of reduction of an intervention at a stage depending on the difference between the modeled cfu count on the carcass in the considered scenario and the cfu count on the carcass in the study from which the reduction effect was estimated. A large difference in cfu counts on carcasses between model and study can for example arise through choosing the intervention with the highest efficacy in bacterial reduction at each stage. This would lead to low cfu counts on the carcasses, which in turn would likely lead to an overestimation of the bacterial reduction by the model.

Even though we used data from a real abattoir the model does not claim to represent this particular abattoir. No systematic comparisons have been made between the variations of empirical values from the abattoir and the model outcomes to fine tune the model in a way as to represent closely this particular abattoir. For example, the mean weight of animals of the various flocks slaughtered varied between 1.9 kg and 3.2 kg. These data were aggregated for the model calculations. It is likely that the flocks originated from different primary production sites, which might vary in many ways, from husbandry conditions to breeds. And it is also likely that the machinery of the abattoir reduces bacteria on carcasses from such different sources differently, for example, it might be that it reduces bacteria differently on lighter animals than on heavier ones. Furthermore, the machinery of the abattoir is adapted to the expected higher or lower slaughter weight introducing even more variability into the system. The model does not capture all these idiosyncrasies. In this sense, the model does not describe any specific abattoir.

We calculated the log reductions for various interventions asserting that these were independent of the units used to describe the absolute bacterial counts. There is a tacit assumption in this assertion, namely that the found log reduction is independent of the sampling procedure. For example, the EsRAM data on the reduction in external contamination due to reduction of the stocking density were collected by swab sampling using the units $\log_{10}$ cfu/20 sqcm of broiler breast skin. The derived log reduction was then used in the model as if the determined log reduction found on the breast is the same everywhere on the carcass. Since there were no replicates in the EsRAM animal trial for determining the effect of farm level interventions (Robe et al., 2021) results of these studies constitute only limited evidence and replication for studying further the found reduction effects of the interventions is desirable.

Our choices of the log reduction might be systematically overconfident estimates since publication bias (Fanelli, 2013; Young et al., 2008) leads to positive results being overrepresented in the published literature. Furthermore, we took the log reductions for the model from the literature reviewed in Projahn et al. (2018) and used them even when these reductions were not statistical significant. Correspondingly, one should consider the results of the model calculations for a set of chosen interventions as upper limits for the bacterial reduction attainable.

The model considers external and internal colonization. If one generally assumes that the external colonization only reflects contact with feces from the same bird and is therefore in line with internal colonization, then in the extreme case of no internal colonization by resistant bacteria a similar absence of external contamination with resistant *Enterobacteriaceae* is to be expected. However, this assumes that there are no external sources of resistant bacteria before the start of the considered processing stage leading to external contamination of the carcass. Most probably, external contamination of a carcass reflects previous introduction of the bacteria into the fattening farms or during transport to the abattoir. Empirical results from the EsRAM project showed that the internal colonization with resistant bacteria might be reduced by the use of competitive exclusion (CE) (Methner et al., 2019). Our model results showed that internal colonization might present a relevant factor for the external colonization or contamination of carcasses during processing. The model shows that if feces contain high concentration of bacteria, they might counteract through cross-contamination even highly effective reduction interventions during processing (cf. section 3.1).

4 | OUTLOOK

The model in its current form is the result of the available resources of its time during the development in the context of the EsRAM Project. We will continue to develop the model further as new data becomes available and as the model structure will be varied.

4.1 | New data

As a working hypothesis we assumed that we do not need to differentiate between external contamination with resistant and with susceptible bacteria. Thus, we assume that acquiring resistance against antibiotics has no influence on tenacity, growth, or resilience against the processes during slaughtering. The model in its current version just keeps track of *Enterobacteriaceae* in general. This is due to the fact that

there is a lack of sufficient data concerning the quantitative measurement of external contamination of resistant *Enterobacteriaceae* along the broiler processing line. Gathering of data on bacterial loads of resistant *Enterobacteriaceae* on carcasses and fecal content would be useful to gauge the validity of the working hypothesis. As more studies become available for individual interventions one could employ evidence synthesis approaches like meta-analysis to get better estimates for reductions. These estimates in turn can then be used for Bayesian updating of the model parameters.

Furthermore, the model can be developed further in order to take into account the idiosyncrasies of individual abattoirs. For example, an extended comparison of the model outputs and new data on empirical cfu counts for a real-life abattoir in its various modes of operation could help improving the model so that it can predict better the microbial status of the carcasses throughout the slaughter process.

4.2 | Model structure

Using the Bayesian updating approach described above could be used to incorporate the reducing interventions more implicitly in the model. As a result reductions of interventions described in the literature would be used to update the model parameters instead of applying reduction factors themselves on the outcome of the model calculations in the explicit way it is done currently.

The model structure shown in Figure 1 could be adapted in such way as to consider multiple animals to be present in a stage. This would allow to generalize the model from the current strictly individualized and sequential processing structure to a more group oriented one. Instead of considering only one animal in a stage the model would, were appropriate, consider several animals in a stage. This would be useful to model situation as in immersion scalding or immersion chilling where a number of animals at the same stage might contaminate each other as they are processes together in the same water.

Thus, the model in its current version is considered to be a starting point for further model-based investigations of interventions for the reduction of external contamination in the slaughter process of broilers.

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REFERENCES

Berrang, M. E., Buhr, R. J., Cason, J. A., & Dickens, J. A. (2002). Microbiological consequences of skin removal prior to evisceration of broiler carcasses. *Poultry Science*, *81*(1), 134–138.

Berrang, M. E., Dickens, J. A., & Musgrove, M. T. (2000). Effects of hot water application after defeathering on the levels of *Campylobacter*, coliform bacteria, and *Escherichia coli* on broiler carcasses. *Poultry Science*, *79*(11), 1689–1693.

Berrang, M. E., Meinersmann, R. J., Buhr, R. J., Reimer, N. A., Philips, R. W., & Harrison, M. A. (2003). Presence of *Campylobacter* in the respiratory tract of broiler carcasses before and after commercial scalding. *Poultry Science*, *82*(12), 1995–1999.

Berrang, M. E., Meinersmann, R. J., Smith, D. P., & Zhuang, H. (2008). The effect of chilling in cold air or ice water on the microbiological quality of broiler carcasses and the population of *Campylobacter*. *Poultry Science*, *87*(5), 992–998.

Bucher, O., Fazil, A., Rajic, A., Farrar, A., Wills, R., & McEwen, S. A. (2012). Evaluating interventions against Salmonella in broiler chickens: Applying synthesis research in support of quantitative exposure assessment. *Epidemiology and Infection*, *140*(5), 925–945.

Casella, T., Nogueira, M. C. L., Saras, E., Haenni, M., & Madec, J. Y. (2017). High prevalence of ESBLs in retail chicken meat despite reduced use of antimicrobials in chicken production, France. *International Journal of Food Microbiology*, *257*, 271–275.

Chang, W., Cheng, J., Allaire, J., Xie, Y., & McPherson, J. (2018). shiny: Web application framework for R: R package version 1.2.0.

Chaves, B. D., Han, I. Y., Dawson, P. L., & Northcutt, J. K. (2011). Survival of artificially inoculated *Escherichia coli* and *Salmonella* Typhimurium on the surface of raw poultry products subjected to crust freezing. *Poultry Science*, *90*(12), 2874–2878.

D'Costa, V. M., McGrann, K. M., Hughes, D. W., & Wright, G. D. (2006). Sampling the antibiotic resistome. *Science*, *311*(5759), 374–377.

Davis, G. S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, B. A., Liu, C. M., & Price, L. B. (2018). Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC Microbiology*, *18*, https://doi.org/10.1186/s12866-018-1322-5

Dickens, J. A., Berrang, M. E., & Cox, N. A. (2000). Efficacy of an herbal extract on the microbiological quality of broiler carcasses during a simulated chill. *Poultry Science*, *79*(8), 1200–1203.

Fanelli, D. (2013). Positive results receive more citations, but only in some disciplines. *Scientometrics*, *94*(2), 701–709.

Giombelli, A. (2013). Métodos de análise e mapeamento de microorganismos patogênicos e indicadores de qualidade e higiene na criação e no abate de frangos. (PhD), Universidade Federal de Minas Gerais. http://www.bibliotecadigital.ufmg.br/dspace/bitstream/handle/1843/BUOS-98SG7X/audecir_giombelli_tese_de_doutorado_final.pdf

Giombelli, A., Hammerschmitt, D., Cerutti, M. F., Chiarini, E., Landgraf, M., Franco, B. D., & Destro, M. T. (2015). High pressure spray with water shows similar efficiency to trimming in controlling microorganisms on poultry carcasses. *Poultry Science*, *94*(10), 2589–2595.

Gonzalez, R. J., Sampedro, F., Feirtag, J. M., Sanchez-Plata, M. X., & Hedberg, C. W. (2019). Prioritization of chicken meat processing interventions on the basis of reducing the *Salmonella* residual relative risk. *Journal of Food Protection*, *82*(9), 1575–1582.

Hamilton, M. (2003). *The biofilm laboratory: Step-by-step protocols for experimental design, analysis, and data interpretation* (p. 16–17). Cytergy.

Hardie, K. M., Guerin, M. T., Ellis, A., & Leclair, D. (2019). Associations of processing level variables with *Salmonella* prevalence and concentration on broiler chicken carcasses and parts in Canada. *Preventive Veterinary Medicine*, *168*, 39–51.

Hartnett, E. (2001). Human infection with *Campylobacter* spp. from chicken consumption, A quantitative risk assessment. PhD thesis at the University of Strathclyde, Glasgow. https://stax.strath.ac.uk/downloads/9019s269z?locale=en

Hayama, Y., Yamamoto, T., Kasuga, F., & Tsutsui, T. (2011). Simulation model for *Campylobacter* cross-contamination during poultry processing at slaughterhouses. *Zoonoses and Public Health*, *58*(6), 399–406.

Hering, J., Fromke, C., von Munchhausen, C., Hartmann, M., Schneider, B., Friese, A., Rösler, U., Kreienbrock, L., & Hille, K. (2016). Cefotaxime-resistant *Escherichia coli* in broiler farms-A cross-sectional investigation in Germany. *Preventive Veterinary Medicine*, *125*, 154–157.

Huijbers, P. M. C., Graat, E. A. M., van Hoek, A. H. A. M., Veenman, C., de Jong, M. C. M., & van Duijkeren, E. (2016). Transmission dynamics of extended-spectrum beta-lactamase and AmpC beta-lactamase-producing *Escherichia coli* in a broiler flock without antibiotic use. *Preventive Veterinary Medicine*, *131*, 12–19.

Hummel, R. P., Miskell, P. W., & Altemeier, W. A. (1977). Antibiotic-resistance transfer from nonpathogenic to pathogenic bacteria. *Surgery*, *82*(3), 382–385.

James, C., James, S. J., Hannay, N., Purnell, G., Barbedo-Pinto, C., Yaman, H., Araujo, M., Gonzalez, M. L., Calvo, J., Howell, M., & Corry, J. E. (2007). Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *International Journal of Food Microbiology*, *114*(2), 195–203.

Jiang, X. L., Ellabaan, M. M. H., Charusanti, P., Munck, C., Blin, K., Tong, Y. J., Weber, T., Sommer, M. O. A., & Lee, S. Y. (2017). Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. *Nature Communications*, *8*, https://doi.org/10.1038/ncomms15784

Kaesbohrer, A., Bakran-Lebl, K., Irrgang, A., Fischer, J., Kampf, P., Schiffinanne, A., Werckenthin, C., Busch, M., Kreienbrock, L., & Hille, K. (2019). Diversity in prevalence and characteristics of ESBL/pAmpC producing *E. coli* in food in Germany. *Veterinary Microbiology*, *233*, 52–60.

Kozempel, M., Goldberg, N., Dickens, J. A., Ingram, K. D., & Craig, J. C. Jr. (2003). Scale-up and field test of the vacuum/steam/vacuum surface intervention process for poultry. *Journal of Food Process Engineering*, *26*(5), 447–468.

Kurowicka, D., Nauta, M., Jozwiak, K., & Cooke, R. (2010). Updating parameters of the chicken processing line model. *Risk Analysis*, *30*(6), 934–944.

Methner, U., Friese, A., & Rosler, U. (2019). Competitive exclusion: A tool to combat extended-spectrum beta-lactamase-producing *Escherichia coli* strains in chickens. *Research in Veterinary Science*, *123*, 124–128.

Musgrove, M. T., Cason, J. A., Fletcher, D. L., Stern, N. J., Cox, N. A., & Bailey, J. S. (1997). Effect of cloacal plugging on microbial recovery from partially processed broilers. *Poultry Science*, *76*(3), 530–533.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., & Havelaar, A. (2009). A comparison of risk assessments on *Campylobacter* in broiler meat. *International Journal of Food Microbiology*, *129*(2), 107–123.

Nauta, M., Jacobs-Reitsma, W. F., Evers, E. G., van Pelt, W., & Havelaar, A. (2005). Risk assessment of *Campylobacter* in the Netherlands via broiler meat and other routes. https://www.rivm.nl/bibliotheek/rapporten/250911006.html

Nauta, M., Jacobs-Reitsma, W. F., & Havelaar, A. H. (2007). A risk assessment model for *Campylobacter in* broiler meat. *Risk Analysis*, *27*(4), 845–861.

Nauta, M., van der Fels-Klerx, I., & Havelaar, A. (2005). A poultry-processing model for quantitative microbiological risk assessment. *Risk Analysis*, *25*(1), 85–98.

Notermans, S., & Kampelmacher, E. H. (1975). Heat destruction of some bacterial strains attached to broiler skin. *British Poultry Science*, *16*(4), 351–361.

OECD/FAO. (2019). OECD-FAO Agricultural outlook (Edition 2019). https://doi.org/10.1787/eed409b4-en). https://www.oecd-ilibrary.org/content/data/eed409b4-en

Pacholewicz, E., Liakopoulos, A., Swart, A., Gortemaker, B., Dierikx, C., Havelaar, A., & Schmitt, H. (2015). Reduction of extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* through processing in two broiler chicken slaughterhouses. *International Journal of Food Microbiology*, *215*, 57–63.

Pacholewicz, E., Lipman, L. J., Swart, A., Havelaar, A. H., & Heemskerk, W. J. (2016). Pre-scald brushing for removal of solids and associated broiler carcass bacterial contamination. *Poultry Science*, *95*(12), 2979–2985.

Parette, L. A. (2018). *Quantitative microbial risk assessment for parts, ground, and MSC poultry product including intervention analysis and exploration of Enterobacteriaceae as an indicator organism in poultry processing*. University of Arkansas. https://scholarworks.uark.edu/cgi/viewcontent.cgi?article=4579&context=etd

Projahn, M., Pacholewicz, E., Becker, E., Correia Carreira, G., Bandick, N., & Kaesbohrer, A. (2018). Reviewing interventions against Enterobacteriaceae in broiler processing: using old techniques for meeting the new challenges of ESBL E. coli? BioMed Research International. https://doi.org/10.1155/2018/7309346

Projahn, M., Sachsenroeder, J., Correia-Carreira, G., Becker, E., Martin, A., Thomas, C., Hobe, C., Reich, F., Robé, C., Roesler, U., Kaesbohrer, A., & Bandick, N. (2021). Impact of on-farm interventions against CTX-Resistant *Escherichia coli* on the contamination of carcasses before and during an experimental slaughter. *Antibiotics (Basel)*, *10*(3), https://doi.org/10.3390/antibiotics10030228

R Core Team. (2018). R: A language and environment for statistical computing: R Foundation for statistical computing.

Robe, C., Daehre, K., Merle, R., Friese, A., Guenther, S., & Roesler, U. (2021). Impact of different management measures on the colonization of broiler chickens with ESBL- and pAmpC- producing *Escherichia coli* in an experimental seeder-bird model. *PLoS One*, *16*(1), e0245224.

Shin, D., Kakani, G., Molina, V. A., Regenstein, J. M., Choe, H. S., & Sanchez-Plata, M. X. (2012). Effect of kosher salt application on microbial profiles of poultry carcasses. *Poultry Science*, *91*(12), 3247–3252.

Souza, L. C. Jr., Pereira, J. G., Spina, T. L., Izidoro, T. B., Oliveira, A. C., & Pinto, J. P. (2012). Microbiological evaluation of chicken carcasses in an immersion chilling system with water renewal at 8 and 16 hours. *Journal of Food Protection*, *75*(5), 973–975.

Van der Fels-Klerx, H. J., Cooke, R. M., Nauta, M. N., Goossens, L. H., & Havelaar, A. H. (2005). A structured expert judgment study for a model of campylobacter transmission during broiler-chicken processing. *Risk Analysis*, *25*(1), 109–124.

Young, N. S., Ioannidis, J. P. A., & Al-Ubaydi, O. (2008). Why current publication practices may distort science. *Plos Medicine*, *5*(10), 1418–1422.

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F. F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M., & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *NPJ Biofilms and Microbiomes*, *6*(1). https://doi.org/10.1038/s41522-020-0136-z

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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