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Multidirectional dynamic model for the spread of extended-spectrum-β-lactamase-producing *Escherichia coli* in the Netherlands

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ABSTRACT

Extended-spectrum-β-lactamase-producing *Escherichia coli* (ESBL-EC) is a major public health concern. A better understanding of the dynamics of ESBL-EC transmission is required for effective prevention and control. We present here a multidirectional dynamic risk model for ESBL-EC transmission between broiler flocks, broiler farmers, and the open community, parameterized for the Netherlands. A discrete-time model was used to describe the transmission of ESBL-EC within and between populations including modeling the flock-to-human transmission via food consumption due to contamination at the slaughterhouse and/or during food preparation. The ESBL-EC prevalence reached an equilibrium prevalence of 0.65%, 24.7%, and 15.9% in the open community, farmers, and broiler flocks, respectively. The colonization of the open community could primarily be attributed to the open community itself (62%), followed by vegetable consumption (29.5%), and contact with farmers (8.5%). Model results were most sensitive to the estimated colonization and decolonization rate for humans. What-if analysis to explore the effect of interventions in the food production chain (i.e. from farm to fork) on the ESBL-EC prevalence in the open community indicated that interventions aimed at reducing the spread of ESBL-EC within broiler flocks were most effective. Interventions in the consumer phase (reduced cross-contamination in the kitchen, and reduced chicken meat consumption) resulted in a slightly lower ESBL-EC prevalence in the open community. Reducing cross-contamination at the slaughterhouse or reducing the proportion of broiler flocks with high antimicrobial use hardly had any effect on the prevalence in the open community. These results illustrate the relevance of the model for supporting the development of antimicrobial resistance risk mitigation strategies as part of public health policy making.

1. Introduction

Antimicrobial resistance (AMR) in human and animal pathogens is of global concern. Each year, AMR is responsible for an estimated 33,000 deaths and an approximate cost of 1.1 billion Euros to the European health care systems (OCDE, 2019). According to Cassini et al. (2019), the human disease burden of AMR in Europe is 170 disability-adjusted life years (DALYs) per 100,000 population, comparable to that of influenza, tuberculosis, and HIV/AIDS, which is 183 DALYs per 100,000 population (Cassini et al., 2018). Among all AMR bacteria, Extended-Spectrum-β-lactamase producing *Escherichi coli* (ESBL-EC) has gained attention because it confers resistance to antibiotics that are considered of critical importance for clinical use (WHO, 2018), such as third-generation cephalosporins, increasing the use of so-called last-resort antibiotics, e.g. carbapenems (Cantón et al., 2012; van Hout et al., 2020).

ESBL-EC are widely distributed in humans, animals, food, and the environment (Blaak et al., 2015). In the Netherlands, around 5% of the general human population is carrier of ESBL-EC (Teunis et al., 2018), but the factors associated with colonization are controversial. Leverstein-van Hall et al. (2011) suggested that transfer to humans occurs via meat consumption, but Leistner et al. (2013) did not observe

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associations between meat consumption and the carriage of ESBL-EC in humans. Dorado-García et al. (2018) did not find a close epidemiological linkage of specific ESBL-EC genes and plasmid replicon types between livestock farms and the general population, however, associations between carriage of ESBL-EC producing bacteria and people living or working on broiler farms were reported by Huijbers et al. (2014).

Different models were developed in recent years to elucidate the sources that contribute most to ESBL-EC prevalence in humans in the Netherlands. Mughini-Gras et al. (2019) used a 'top-down' source attribution model to estimate the contribution of the most important ESBL-EC reservoirs, including animal and environmental reservoirs, to human ESBL-EC carriership. We call this a 'top-down' attribution method, as the estimates are based on correlating the distribution of specific ESBL genes found in samples from humans to the distributions found in samples from potential sources. At present, a limitation of this modeling approach is that it allows for attribution of illness/colonization in only one direction, in this case from animal and environmental reservoirs to the human population and not the other way around. Evers et al. (2017) used a comparative risk assessment model looking at human ESBL-EC exposure from meat products in the Netherlands. This 'bottom up' approach models the prevalence and concentrations in meat products along the food chain. This model includes ESBL-EC concentration in products at retail, during storage in the consumers' homes (room temperature/fridge/freezer), and finally the potential cross-contamination of other products and the effect of cooking/ preparation. Comparative risk assessments are useful models to explore the effect of interventions in the food chain. These models are, however, also unidirectional, and thus do not attempt to account for the interactions between different reservoirs leading to multidirectional and dynamic spread of ESBL-EC.

According to Evers et al. (2017) an alternative extension of the existent comparative risk assessment model could be using dynamic models, such as compartmental models, that explicitly capture time and can account for multidirectional transmission between sources. This will contribute to a better understanding of ESBL-EC transmission within and between different sources, and as such provide relevant information for authorities regarding the dynamic effect of possible interventions (e.g. reduced antimicrobial use in the livestock sector or improved hygiene at slaughterhouse and consumers' homes) on the prevalence in humans.

In this paper we present a dynamic model assessing the multidirectional transmission of ESBL-EC between different populations over time. We applied the proposed model in two different human populations (open community and farmers) and broiler flocks, integrating the slaughterhouse and food preparation at the consumer level to account for transmission via the food chain. We focused on the estimation of ESBL-EC prevalence over time in the different populations and the contribution of all populations to the probability of ESBL-EC colonization for the open community. The model was used to assess the effect of ESBL-EC prevalence and the level of antimicrobial usage in broiler flocks (primary production), and the effect of measures regarding food preparation (consumer phase) on human colonization with ESBL-EC.

2. Materials and methods

2.1. Model framework

The model aims to describe the clonal spread of ESBL-EC between different sources over time. We did not include the genetic dynamics involved in the acquirement and loss of plasmids and emergence of resistance. The model was parameterized for the Netherlands and applied to broiler flocks, human populations, and food, conceived here as sources. Humans were divided into two sub-populations: people in direct contact with broiler flocks (hereafter called farmers, but this also includes e.g. veterinarians and technicians); and the open community, which are all people not in direct contact with broiler flocks. The basic structure of the model and the transmission routes considered are shown in Fig. 1.

Within the model, we assume that the broiler flock population is linked to the open community via farmers and food consumption. This assumption is motivated by the strict biosecurity measures applied in broiler farms in the Netherlands making the access of non-farmers to the flock very unlikely.

2.2. Mathematical model

The proportion of colonized individuals (n_t) in each population is assumed to increase each time interval according to the probability of colonization (p_t^{col}). Similarly, the proportion of colonized individuals is assumed to decrease each time interval according to the probability of decolonization (p^{decol}). In contrast to the probability of colonization, the probability of decolonization is assumed to be independent from the proportion of colonized individuals in the time. We implemented the model using a discrete-time approximation with a time step of one week as this provided sufficient numerical accuracy for our purposes resulting in the equations for the proportion of colonized individuals in each population:

$$n_t = (1 - n_{t-1}) * p_t^{\mathrm{col}} + n_{t-1} * \left(1 - p^{\mathrm{decol}}\right). \tag{1}$$

In the following subsections specific instances n_t, p_t^{col}, and p^{decol} will be considered for the specific populations.

2.2.1. Dynamics in the open community

The proportion of colonized individuals n_t^{h} in the open community increases as a result of exposure to ESBL-EC from four different sources: direct contact with a colonized open community individual, direct contact with a colonized farmer, consumption of chicken meat or consumption of vegetables. We denote the probabilities of colonization arising from each of these sources as r_t^{h}, r_t^{fh}, r_t^{m}, and r_t^{v} respectively. The probabilities r_t^{h} and r_t^{fh} are calculated as:

$$r_t^{\mathrm{h}} = 1 - \exp\left(-\beta^{\mathrm{h}} * n_{t-1}^{\mathrm{h}}\right) \tag{2}$$

and

$$r_t^{\mathrm{fh}} = 1 - \exp\left(-\beta^{\mathrm{h}} * n_{t-1}^{\mathrm{f}} * p^{\mathrm{hf}}\right), \tag{3}$$

where β^{h} is the transmission parameter in the open community (here defined as a dimensionless parameter as it includes a 1-week time step factor). We assume that the transmission parameter between the farmer population and the open community is the same as between open community individuals. p^{hf} is the probability that humans (open community) are in contact with a farmer in one week and n_t^{h} and n_t^{f} are the proportion of colonized individuals in the open community and farmer population at time (t), respectively.

The colonization via food is modeled by a Beta-Poisson dose-response model according to Swart et al. (In preparation):

$$r_t^{\mathrm{m}} = \left[1 - \left(1 + \frac{d_{t-1}^{\mathrm{m}}}{\gamma}\right)^{-\delta}\right] * (1 - p^{\mathrm{veg}}), \tag{4}$$

and

$$r_t^{\mathrm{v}} = \left[1 - \left(1 + \frac{d_{t-1}^{\mathrm{v}}}{\gamma}\right)^{-\delta}\right] * (1 - p^{\mathrm{veg}}), \tag{5}$$

where γ and δ are dimensionless (shape) parameters, p^{veg} is the probability that an individual in the population is vegetarian and does not consume chicken meat, d_t^{m}, *and* d_t^{v} are the average dose (expressed as colony forming units (cfu)) ESBL-EC on the amount of chicken meat and vegetables consumed by humans at time t (t being a one week period), respectively. The calculation of d_t^{m}, *and* d_t^{v} will be addressed at the end of the Section 2.2.5. We assumed that only meat consumers will be

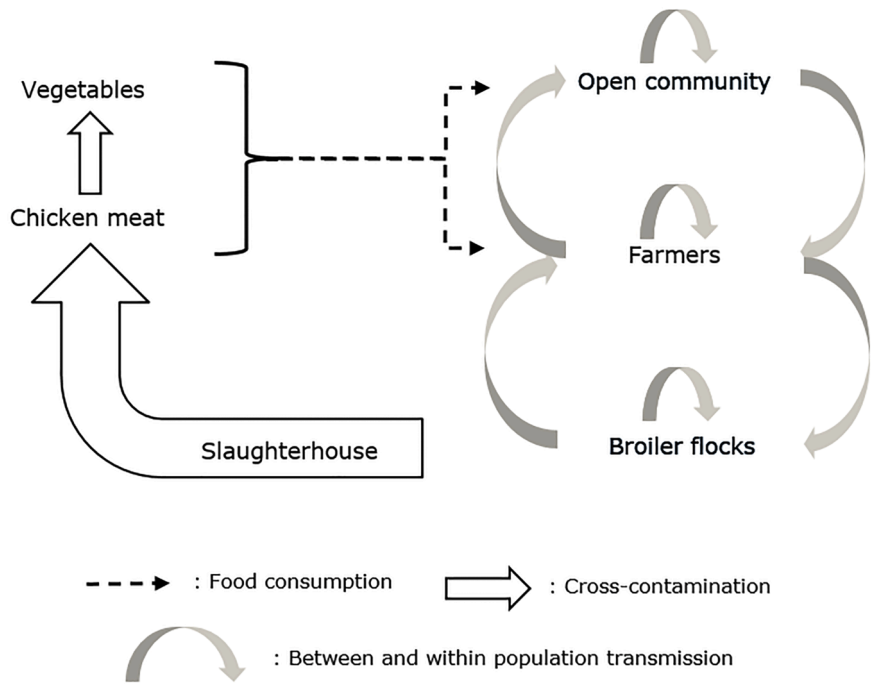

Fig. 1. Schematic representation of AMR transmission between humans and poultry flocks (adapted from Huijbers et al. 2015a). The arrows depict the transmission routes and the associated dynamical aspect of the model: curly arrows describe the routes considered in the model for clonal spread of ESBL-EC between different populations; dashed arrows describe transmission via food consumption, and solid white arrows describe cross-contamination processes.

exposed to ESBL-EC contaminated vegetables since contamination was modelled as the result from cross-contamination during chicken preparation.

The total probability of colonization of an individual in the open community (p_t^{colh}) is given by the probability of the union of events

$$p_t^{\mathrm{colh}} = 1 - \left[\left(1 - r_t^{\mathrm{h}}\right) * \left(1 - r_t^{\mathrm{fh}}\right) * \left(1 - r_t^{\mathrm{m}}\right) * \left(1 - r_t^{\mathrm{v}}\right)\right]. \tag{6}$$

It is assumed that the probability of decolonization in one week's time (p^{decolh}) is independent of the proportion of colonized individuals and given by

$$p^{\mathrm{decolh}} = 1 - \exp\left(-\theta^{\mathrm{h}}\right), \tag{7}$$

where θ^{h} is the decolonization rate integrated over one time unit.

Finally, substituting n_t by n_t^{h}, p_t^{col} by p_t^{colh}, and p^{decol} by p^{decolh} in Eq. (1), the model equation for the proportion of colonized humans in the open community, based on taking into account both the newly acquired and lost colonization within one timestep, reads:

$$n_t^{\mathrm{h}} = \left(1 - n_{t-1}^{\mathrm{h}}\right) * p_t^{\mathrm{colh}} + n_{t-1}^{\mathrm{h}} * \left(1 - p^{\mathrm{decolh}}\right), \tag{8}$$

where n_0^{h} is the initial prevalence in the open community at time=0, which was set at 5%.

2.2.2. Dynamics in the farmer population

In the farmer population, individuals are colonized and decolonized with probabilities p^{colf} and p^{decolf}, respectively. The proportion of colonized individuals n_t^{f} increases given the exposure to ESBL-EC by five different sources: direct contact with a colonized open community individual, contact with a colonized farmer, contact with the broiler flock, consumption of chicken meat or consumption of vegetables. The probabilities of colonization given each source are r_t^{hf}, r_t^{f}, r_t^{cf}, r_t^{m}, *and* r_t^{v}, respectively. The probabilities r_t^{hf}, r_t^{f}, and r_t^{cf} are calculated as:

$$r_t^{\mathrm{hf}} = 1 - \exp\left(-\beta^{\mathrm{h}} * n_{t-1}^{\mathrm{h}}\right), \tag{9}$$

$$r_t^{\mathrm{f}} = 1 - \exp\left(-\beta^{\mathrm{h}} * n_{t-1}^{\mathrm{f}}\right), \tag{10}$$

and

$$r_t^{\mathrm{cf}} = \left(1 - n_{t-1}^{\mathrm{f}}\right) * \left(n_{t-1}^{\mathrm{c}}\right) * \mathrm{p}^{\mathrm{cf}}, \tag{11}$$

where the transmission parameter between the open community and the farmer populations is assumed be the same as between open community individuals (β^{h}). p^{cf} is a constant representing the probability of colonization given the contact (of one week duration) of a susceptible farmer with a positive flock with a within-flock prevalence n_t^{c} (explained below in the Section 2.2.3). The estimate of p^{cf} accounts for the hygienic measures taken by the farmer to avoid being exposed to fecal-oral colonization, e. g. washing hands after dealing with the flock, changing clothes, and preventing the contact of hands with mouth during the work. r_t^{m} *and* r_t^{v} are assumed to be the same for the farmers and the open community.

The total probability of colonization of an individual in the farmer population (p_t^{colf}) is given by the probability of the union of events

$$p_t^{\mathrm{colf}} = 1 - \left[\left(1 - r_t^{\mathrm{hf}}\right) * \left(1 - r_t^{\mathrm{f}}\right) * \left(1 - r_t^{\mathrm{cf}}\right) * \left(1 - r_t^{\mathrm{m}}\right) * \left(1 - r_t^{\mathrm{v}}\right)\right]. \tag{12}$$

It is assumed that the probability of decolonization in one week's time for farmers (p^{decolf}) is the same as that used for the open community given by Eq. (7).

Substituting n_t by n_t^{f}, p_t^{col} by p_t^{colf}, and p^{decol} by p^{decolf} in Eq. (1), gives us the final equation for the proportion of colonized farmers

$$n_t^{\mathrm{f}} = \left(1 - n_{t-1}^{\mathrm{f}}\right) * p_t^{\mathrm{colf}} + n_{t-1}^{\mathrm{f}} * \left(1 - p^{\mathrm{decolf}}\right), \tag{13}$$

where n_0^{f} is the initial prevalence in farmers at time=0, which was set at 5%.

2.2.3. Dynamics in the broiler flock population

Considering that broilers are clustered in flocks, we modeled 500 independent broiler flocks (i), allowing the effect of some parameters to affect flocks separately (e.g., effect of antimicrobial usage on ESBL-EC,

which will be approached ahead). The dynamics in the broiler flock population mimic a six-week broiler-flock fattening period, plus one extra week between production cycles (week zero), when the farm is empty.

Given the fact that broiler flock populations have a periodicity, the model uses two separate timelines. The first one represents the time in weeks for humans (t) and the second the time in weeks for the flocks, called t^{flock}. At the start of the simulation, each flock i enters the model in a random week of its production cycle, t_i^{flock}. The starting value of t_i^{flock} is generated randomly from zero to six resulting in flocks with different ages when the simulation starts. The t_i^{flock} value increases by one every week until it equals 6. In the next time step of the model, the value of t_i^{flock} is set back to 0 (Eq. (14)), mimicking the one-week sanitation period before a new flock is introduced.

$$t_{i,t}^{\text{flock}} = \begin{cases} t_{i,t-1}^{\text{flock}} + 1, & if\ t_{i,t-1}^{\text{flock}} < 6 \\ 0, & if\ t_{i,t-1}^{\text{flock}} = 6 \end{cases}. \tag{14}$$

Broilers are colonized and decolonized with probabilities p_t^{colc} and p^{decolc}, respectively. The proportion of colonized broilers within flock i ($n_{i,\ t}^{\text{c}}$) increases given the exposure to ESBL-EC to two different sources: direct contact with a colonized broiler or contact with a colonized farmer. The probabilities of colonization given each source are r^{c} and r^{fc}, respectively, and calculated as

$$r_{i,\ t}^{\text{c}} = 1 - \exp\left\{ -\beta^{\text{c}} * \left[\text{AMU}_i * \left(1 + \lambda^{\beta^{\text{c}}}\right) + (1 - \text{AMU}_i)\right] * n_{i,\ t-1}^{\text{c}} \right\}, \tag{15}$$

and

$$r_{i,\ t}^{\text{fc}} = n_{t-1}^{\text{f}} * \left(1 - n_{i,\ t-1}^{\text{c}}\right) * \text{p}^{\text{fc}} \tag{16}$$

where β^{c} is the within-flock transmission parameter (here defined as a dimensionless parameter as it includes a 1-week time step factor); and p^{fc} incorporates the biosecurity measures adopted by farmers when dealing with chickens, reducing the fecal-oral route. The final probability of colonization of a broiler in the broiler flock population ($p_{i,t}^{\text{colc}}$) is given by the given by the probability of the union of events

$$p_{i,t}^{\text{colc}} = 1 - \left[\left(1 - r_{i,\ t}^{\text{c}}\right) * \left(1 - r_{i,\ t}^{\text{fc}}\right)\right]. \tag{17}$$

All broilers are decolonized with the same probability p^{decolc}

$$p^{\text{decolc}} = 1 - \exp\left\{ -\theta^{\text{c}} * \left[\text{AMU}_i * \left(1 - \lambda^{\theta^{\text{c}}}\right) + (1 - \text{AMU}_i)\right] \right\} \tag{18}$$

where θ^{c} is the is the decolonization rate for chickens in one week.

Eqs. (15) and (18) accounting for the fact that the use of antimicrobials affects the colonization (Luiken et al., 2019) of ESBL-EC in broilers. We use a factor called $\lambda^{\beta^{\text{c}}}$ and $\lambda^{\theta^{\text{c}}}$ that increases the transmission parameter (β^{c}) and reduces the decolonization rate (θ^{c}), respectively (Luiken et al., 2019) for flocks with a high antimicrobial use. The status of each flock for antimicrobial use (high or normal) (AMU_i) is set using a Bernoulli distribution $\text{AMU}_i \sim \text{Bernoulli}(p^{\text{AMU}})$, where p^{AMU} is the proportion of farms with high antimicrobial use.

Substituting n_t by $n_{i,\ t}^{\text{c}}$, p_t^{col} by $p_{i,t}^{\text{colc}}$, and p^{decol} by p^{decolc} in Eq. (1), gives us the final equation for the proportion of colonized chickens in broiler flock i at time t ($n_{i,\ t}^{\text{c}}$)

$$n_{i,\ t}^{\text{c}} = \left(1 - n_{i,\ t-1}^{\text{c}}\right) * p_{i,t}^{\text{colc}} + n_{i,\ t-1}^{\text{c}} * \left(1 - p^{\text{decolc}}\right), \tag{19}$$

where $n_{i,\ 0}^{\text{c}}$ is the initial prevalence in broiler flock i when entering the farm as 1-day old chickens, which was set at 1%.

To generate the prevalence of ESBL-EC in broiler flocks entering the slaughterhouse (p_t^{c}), the average value of $n_{i,\ t}^{\text{c}}$ of all broiler flocks that are at week six ($t_{i,\ t}^{\text{flock}} = 6$) is calculated. This prevalence feeds the slaughter model (Section 2.2.4). Table 1 lists the input parameters used for the prevalence calculations in humans and broilers, as well their descriptions, values and sources.

Table 1
Input parameters used to model the clonal spread of ESBL-EC between open community, farmers, and broiler flocks.

Parameter	Description (unit)	Value	Source
β^{h}	Transmission parameter for humans (colonized person/week)	0.037	Haverkate et al. (2017)
θ^{h}	Rate of decolonization for humans (decolonization/week)	0.063	Haverkate et al. (2017)
p^{hf}	Proportion of humans in the open community who have contact with farmers	0.0036	Mughini-Gras et al. (2019)
γ	Parameter used in the dose-response model (dimensionless)	713	Swart et al. (In preparation)
δ	Parameter used in the dose-response model (dimensionless)	0.267	Swart et al. (In preparation)
p^{veg}	Proportion of vegetarians in the Dutch population	0.045	Geurts et al. (2017)
β^{c}	Transmission parameter for broilers (colonized chicken/week)	0.7	Calibrated to fit the outcome with observed data*
θ^{c}	Rate of decolonization for chickens (decolonization/week)	0.6	Calibrated to fit the outcome with observed data*
p^{cf}	Probability of colonization of farmer given contact with a flock per week	0.19	Huijbers et al. (2014)
p^{fc}	Probability of colonization of chicken given contact with a farmer per week	0.01	Authors' best guess
p^{AMU}	Proportion of broiler flocks with high antimicrobial usage	0.01	Authors' best guess
$\lambda^{\beta^{\text{c}}}$	Increase of β^{c} in case of high antimicrobial usage (i.e., 46% increase)	0.46	Luiken et al. (2019)
$\lambda^{\theta^{\text{c}}}$	Reduction of θ^{c} in case of high antimicrobial usage (i.e., 46% decrease)	0.46	Luiken et al. (2019)

* Observed ESBL-EC prevalence in sampled broilers at slaughter during the last years in the Netherlands varied from 18% to 10% (MARAN, 2021).

2.2.4. Dynamics at the slaughterhouse

The prevalence of broilers entering the slaughterhouse (p_t^{c}) is used as input for the slaughterhouse module based on the model described by Nauta et al. (2005), allowing to estimate the expected contamination (bacterial load (cfu)) on a carcass $E(N_{\text{ext}})$. Details on the slaughterhouse module are given in Supplementary material 1. We used the processing steps described by Pacholewicz et al. (2015): scalding, defeathering, evisceration, and chilling. At the end of the slaughter process the bacterial concentration ($\text{conc}_{S,\ t}$ (cfu/g)) ESBL-EC on carcasses is obtained by dividing the expected bacterial load (cfu) on the carcasses by the average carcass weight (μ_w):

$$\text{conc}_{S,\ t} = E\left(N_{\text{ext}S,\ t}\right)/\mu_w \tag{20}$$

where μ_w is 1.9 kg according to Pacholewicz et al. (2016). The parameters and variables used in this module are summarized and explained in Supplementary material 1.

2.2.5. Dynamics at the consumer level

For the consumer level, we assume that all chicken meat is fresh meat. In other words, cutting, cooling, and cooking are the only relevant processes in the consumer phase. We assume that ESBL-EC contamination on the chicken meat is only superficial, and that ESBL-EC is inactivated by heating at the consumer phase. The model takes, however, into account that ESBL-EC colonization via food consumption can occur via cross-contamination of vegetables that are prepared together with

raw chicken meat.

The consumer phase mimics the storage and food preparation at home based on the model described by Evers et al. (2017). The input for the consumer phase is the ESBL-EC concentration in chicken meat ($\log10(\text{conc}_{S,\ t})$) obtained as the output of the slaughterhouse module. The consumer phase module encompasses bacterial growth during storage, cross-contamination between meat and vegetables, and bacterial inactivation; details are given in Supplementary material 2. At the end of the consumer phase, the ESBL-EC dose (cfu) on a weekly portion of meat (d_t^{m}) and vegetables (d_t^{v}) is assessed and used in the dose-response model of Eqs. (4) and (5).

2.3. Model calculations

For each source in the model, the average proportion of colonization over time is calculated. Inter-individual variability for chickens and humans is not accounted for in the calculations. The sources of variability in the model are the time for the flocks when the simulation starts ($t_{i,\ 0}^{\text{flock}}$) and the status of each flock for antimicrobial use (AMU_i). To account for this variability, 5000 iterations were run with the model. The values of all other input parameters were given as point estimates. The impact of parameter uncertainty on model results was assessed by uncertainty analysis. All calculations were made using R (R Core Team, 2019) and the scripts are available on: https://git.wur.nl/wbvr_epi/public_esbl

2.4. Output parameters

The outcomes explored in this model are the ESBL-EC prevalence in the open community after running the model for 200 weeks (n_{200}^{h}). The relative contribution of each source to the colonization of individuals in the open community, farmers, and flocks was calculated according to Supplementary material 3. The expected ESBL-EC contamination on food after slaughter and at the consumer phase at week 200 are also reported.

2.5. Uncertainty analysis

Out of 13 input parameters used for the transmission dynamics in the open community and farmers, 11 were changed to assess the effect of parameter uncertainty on the estimated prevalence of ESBL-EC in the open community at week 200. The parameters p^{veg}, and p^{AMU} were assessed in the what-if analysis (Section 2.6). For γ, δ, β^{c}, θ^{c}, and p^{fc} no information was available, and a range of possible (reasonable) values was tested. The other parameters (β^{h}, θ^{h}, p^{cf}, p^{hf}, $\lambda^{\beta^{\text{c}}}$, and $\lambda^{\theta^{\text{c}}}$) were explored using the lower and upper bounds of their 95% confidence interval range as given in scientific publications (Table 2).

2.6. What-if analysis

Six different scenarios were explored to assess the effect of interventions along the production chain (i.e., from farm to fork) on the ESBL-EC prevalence in the open community at week 200. Three scenarios included possible changes at the pre-harvest/slaughterhouse level, mimicking a reduced proportion of farms using high levels of antibiotics, increased biosecurity measures reducing within-flock spread, and reduced cross-contamination at the slaughterhouse during dressing. The other three scenarios mimicked changes in the behavior of the open community, reduction of the proportion of people consuming chicken meat (more vegetarians), reduction of the size of a chicken meat portion, and increased hygiene during food preparation at the kitchen leading to less cross-contamination at consumer level. The scenarios were set with parameter values that were different than those used in the baseline model (Table 3).

Table 2
Input parameters changed to assess the effect of parameter uncertainty on the prevalence of ESBL-EC in the open community at week 200.

Parameter changed	Baseline	Min - Max	Source	Description
β^{h}	0.037	0.017 – 0.083	Haverkate et al. (2017)	Transmission parameter for humans
θ^{h}	0.063	0.032 – 0.125	Haverkate et al. (2017)	Rate of decolonization for humans
β^{c}	0.7	0.56 – 0.84	Authors' best guess	Transmission parameter for broilers
θ^{c}	0.6	0.048 – 0.72	Authors' best guess	Rate of decolonization for broilers
p^{cf}	0.19	0.12 – 0.19	Huijbers et al. (2014)	Probability of colonization of farmer given contact with a flock
p^{fc}	0.01	0.001 – 0.02	Authors' best guess	Probability of colonization of chicken given contact with a farmer
p^{hf}	0.003645	0.003 – 0.0042	Mughini-Gras et al. (2019)	Proportion of humans in the open community who have contact with farmers
$\lambda^{\beta^{\text{c}}}$	0.46	0.05 – 0.99	Luiken et al. (2019)	Increase of β^{c} in case of high antimicrobial usage
$\lambda^{\theta^{\text{c}}}$	0.46	0.05 – 0.99	Luiken et al. (2019)	Reduction of θ^{c} in case of high antimicrobial usage
γ	713	570 – 855	Authors' best guess	Parameter used in the dose-response model (dimensionless)
δ	0.267	0.21 – 0.32	Authors' best guess	Parameter used in the dose-response model (dimensionless)

Min and max are the upper and lower bounds of the 95% confidence interval, respectively.

Table 3
Scenarios assessed in the what-if analysis to estimate the effect of interventions on the prevalence of ESBL-EC in the open community.

	Values used for input parameters	
Scenario	Baseline	Scenarios
Low chicken meat consumption (more vegetarians)	$1 - p^{\text{veg}} = 0.955$	$1 - p^{\text{veg}} = 0.71$
Low cross-contamination at the kitchen*	$\text{frac_cross} = 0.47$	$\text{frac_cross} = 0.35$
Reduced portion size (g/week)*	$\text{consum}^{\text{ch}} = 34.5$	$\text{consum}^{\text{ch}} = 25.9$
Low contamination at slaughterhouse**	$\mu_m = \{1.3,\ 4.3,\ 4.3\}$; $p_{\text{fec}} = \{0.03\}$	$\mu_m = \{0.6,\ 3.6,\ 3.6\}$; $p_{\text{fec}} = \{0.01\}$
Low spread within the flock	$\beta^{\text{c}} = 0.7$ $\theta^{\text{c}} = 0.6$	$\beta^{\text{c}} = 0.56$ $\theta^{\text{c}} = 0.72$
Low proportion of farms with high antimicrobial use	$\text{p}^{\text{AMU}} = 0.01$	$\text{p}^{\text{AMU}} = 0.0075$

* Details about the parameters are provided in Supplementary material 2.
** Input parameters used for the scenario "low contamination at slaughterhouse" (scalding, defeathering and evisceration), are described in more detail in Supplementary material 1 tables S1 and S2.

3. Results

3.1. ESBL-EC transmission dynamics between the open community, farmers and broiler flocks

The prevalence in the open community started at 5% and decreased to 0.65% at week 200. On the other hand, the prevalence in the farmer population increased during the first 50 weeks, from 5% to 24.7%,

keeping this value until week 200. Over the 500 simulated flocks, the average within-flock prevalence at slaughter was 1% at the beginning of the simulation and increased to 15.9% by week 50, remaining around this value until week 200 (Fig. 2).

Of the relative contributions of each source to the colonization of the open community, that of the open community itself was largest, followed by vegetable consumption, and contact with farmers (Table 4). The colonization in farmers was attributed most to contact with broiler flocks and other farmers, with a very low attribution from open community individuals, and vegetable consumption. For the flocks, almost all colonization is attributed to within-flock colonization, and a small part is attributed to contact with farmers (Table 4).

3.2. Dynamic at slaughterhouse and consumer level

The mean ESBL-EC load on carcasses was 0.7 $\log_{10}$ cfu ESBL-EC/g at week 1, increasing to 1.1 $\log_{10}$ cfu ESBL-EC/g by week 50, leveling off at the same value until week 200. At the consumer level, the effect of cooking reduced the dose on chicken meat to zero, making the exposure via this source zero. Given cross contamination, the dose on vegetables at consumption was 0.12 cfu ESBL-EC/portion at week 1, increasing to 0.33 cfu ESBL-EC/portion at week 200 (Supplementary material 4).

3.3. Uncertainty analysis

The ESBL-EC prevalence in the open community at week 200 given the changes in 11 input parameters was compared to the baseline and depicted in a tornado plot (Fig. 3). A considerable increase was observed when the colonization and decolonization parameters for the open community (β^c and θ^c) were increased and decreased, respectively (Fig. 3). Only the five parameters with the largest changes are shown in the tornado plot for depicting purposes.

3.4. What-if analysis

The ESBL-EC prevalence in the open community at week 200 in each what-if scenario was compared to the baseline and depicted in a tornado plot (Fig. 4). The most effective intervention was to reduce the within-flock spread at broiler farms, reducing the prevalence in the open community from 0.65% to 0.32%. Reducing the cross-contamination in the kitchen, the meat portion size, and the proportion of meat consumers (non-vegetarians) had similar effect and reduced the ESBL-EC prevalence in the open community to approximately 0.53% in all scenarios. The low cross-contamination in the slaughterhouse and the low proportion of flocks using high amounts of antimicrobial had hardly any effect on the ESBL-EC prevalence in the open community (reduction from 0.65% to 0.64%) (Fig. 4).

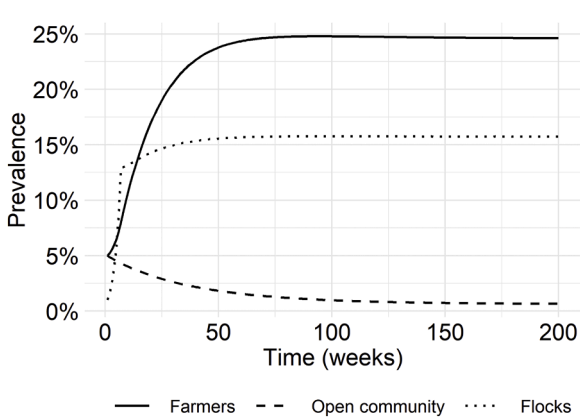

Fig. 2. ESBL-EC prevalence in farmers, broiler flocks before slaughter, and the open community population over a 200-week simulation time.

Table 4
Relative contribution of sources to colonization in the open community, farmers, and poultry flocks at week 200.

	Source of ESBL-EC (attributing to the different populations)				
Population	OC	Farmer	Flock	Meat	Vegetables
OC*	62%	8.5%	–	0	29.5%
Farmer	1.2%	45.9%	52.3%	0	0.6%
Flock	–	3.6%	96.4%	–	–

* OC: open community.

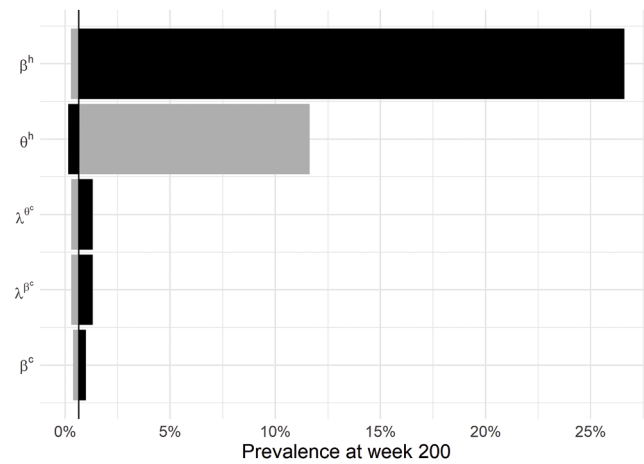

Fig. 3. Tornado plot depicting the estimated ESBL-EC prevalence at week 200 in the open community given changes in uncertain input parameters. The baseline value for the ESBL-EC prevalence is 0.65% (vertical line). Gray bars give the results for the minimum value of the input parameter; black bars give the results for the maximum value of the input parameter. Only the five parameters with the largest changes on the ESBL-EC prevalence in the open community at week 200 are depicted in the tornado plot.

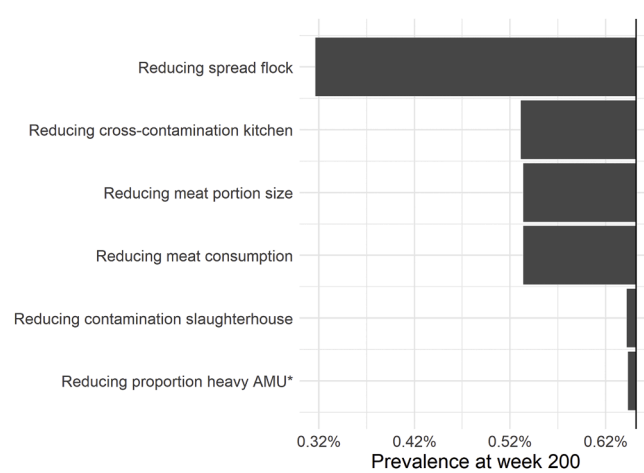

Fig. 4. Tornado plot showing the calculated ESBL-EC prevalence at week 200 in the open community for six different what-if scenarios. The vertical line at 0.65% represents the baseline value for the ESBL-EC prevalence. *AMU-=antimicrobial use.

4. Discussion

We developed a multidirectional dynamic risk model to describe the spread of ESBL-EC between and within different populations over time. The model was applied to humans and broilers, using data from the Netherlands. So far, approaches aimed at ranking sources for antimicrobial resistance acquirement in humans were mainly based on

descriptive molecular data (Dorado-García et al., 2018; Huijbers et al., 2015a, 2014; Leistner et al., 2013). The inclusion of different sources was approached by Mughini-Gras et al. (2019), who considered different populations as sources for ESBL-EC in humans, and Evers et al. (2017), who conducted a comparative exposure assessment including different meat sources via food consumption. Here, we included different populations and connected those populations in a dynamic risk assessment model. The model allows interventions at a certain point in time and to assess the effects on the ESBL-EC prevalence in humans, answering relevant questions regarding the spread of antimicrobial resistance.

Among the included sources, farmers, food and broiler flocks are less important sources for the community-acquired ESBL-EC carriage than the open community itself. This is in accordance with the findings of Mughini-Gras et al. (2019), who reported that around 60% of the open community colonization is attributable to human-to-human transmission. The ESBL-EC colonization probability given the consumption of chicken meat was zero, because the chicken meat products are assumed to be externally contaminated only. Consequently, the exposure via meat consumption is eliminated by bacterial inactivation during chicken meat cooking, and colonization occurs only via cross-contamination of vegetables. This phenomenon was already discussed by Evers et al. (2017).

For farmers, the probability of colonization by food consumption and via contact with the open-community individuals were the same as the probability estimated for the open community, but both sources were not the most relevant for farmers. In fact, flock-farmer and the farmer-farmer contact were the first and second most relevant sources for ESBL-EC colonization of farmers. Colonization in broiler flocks is heavily driven by within-flock spread with a small contribution of farmers as a source. These results are in accordance with the results obtained by Dorado-García et al. (2018). The authors discuss that there is ESBL-EC gene similarity between human farming communities and their animals, suggesting an epidemiological link between broiler farms and farmers.

At week 200, only 0.65% of the open community individuals were colonized by ESBL-EC. This proportion is lower than the overall prevalence of ESBL-EC carriage observed in the Netherlands, which is around 5% (ESBLAT, 2018; Huijbers et al., 2013). The model presented here considered only broiler flocks, farmers, and food as sources to the open community. It could be that further extension of this model to include more sources of ESBL-EC, would result in a higher ESBL-EC colonization proportion in the open community. For the farmers, the proportion of colonized individuals obtained here (24.7%) is similar to that reported by Dierikx et al. (2013) and Huijbers et al. (2014), varying from 14 to 33%. Also, more than 98% of the ESBL-EC colonization in farmers is (relatively) attributed to farmers-farmers and farmers-broilers contact. This is in accordance with previous findings from Huijbers et al. (2015b), who suggests that contact with live broilers is a risk factor for ESBL-EC carriage.

The average within-flock prevalence of colonized chickens was 15.9% at week 200. The monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands (MARAN) does not report within-flock ESBL-EC prevalence, but between 2019 and 2020, the ESBL-EC prevalence in sampled broilers at slaughter varied from 18% to 10% (MARAN, 2021). The same report shows that the prevalence of ESBL-EC in broilers declines in the Netherlands every year. The higher prevalence observed in our simulations is in agreement with Dame-Korevaar et al. (2019b) who observed that eventually all broilers within the flock became colonized within 72 h after inoculation with ESBL-EC. This resulted in a high ESBL-EC prevalence. The study of Dame-Korevaar et al. (2019b) was, however, performed in small groups of broilers under experimental conditions and did not follow the broilers for the full fattening period of six weeks. In a longer observation period, Cardenas-Rey et al. (2021) reported that more than 50% of the broilers got colonized by ESBL-EC until the 5th week of the production period. Furthermore, in our simulations, all broiler flocks were assumed to be ESBL-EC positive, starting with 1% within-flock prevalence at the day of arrival at the farm. As a consequence, all flocks were ESBL-EC colonized at the moment of slaughter given the parameters used for within-flock transmission in the model. In reality, a new production round can start with broiler chicks free from ESBL-EC and remain free until slaughter. This was not accounted for in the current model calculations and will probably have resulted in an overestimate of the average within-flock prevalence. At the other hand, flocks might also start with a high prevalence of ESBL-EC (Dame-Korevaar et al., 2017; Huijbers et al., 2016).

Exploring the effect of parameter uncertainty (uncertainty analysis) on the open community prevalence in equilibrium at week 200, can be seen as an internal validation of the model. We conclude that the results are behaving logically given the variation on the input parameters i.e., increasing parameters implicating higher exposure results in higher prevalence, and reducing the same parameters results in lower prevalence. The parameters for human colonization (β^{h}) and decolonization (θ^{h}) were much more relevant than parameters regarding colonization of livestock. This is not surprising given the relatively high contribution of human to human transmission to the ESBL-EC prevalence in the open community. The huge impact of these parameters on model results also indicate that the absolute model outcome for the ESBL-EC prevalence in the open community is highly uncertain. The relative source attribution results are however, more robust and the model can therefore be used to explore the impact of interventions. Changes in parameters related to the effect of antimicrobial use ($\lambda^{\beta^{c}}$ and $\lambda^{\theta^{c}}$) and the colonization (β^{c}) in the broilers had less effect on the ESBL-EC prevalence in the open community. Nevertheless, more information is needed about the within-flock colonization rate (β^{c}), which appeared as the fifth most relevant parameter to the outcome. In our model we calibrated this parameter to achieve a within-flock ESBL-EC prevalence around 18% and 10%, which was observed in sampled broilers at slaughter in the Netherlands during the last years (MARAN, 2021). Finally, the other parameters included in the uncertainty analysis (γ, δ, p^{hf}, p^{cf}, p^{fc}, and θ^{c}) had limited effect on the outcome, which is not surprising since they are not directly related to the open community ESBL-EC transmission dynamics.

Based on the what-if analysis, we conclude that interventions aimed at reducing the within-flock ESBL-EC spread are most effective in reducing the ESBL-EC prevalence in the open community (Fig. 4). Several interventions have been suggested that might reduce the prevalence of ESBL-EC in broilers, such as housing measures (e.g. subdividing flocks) (Dame-Korevaar et al., 2020b), or increased biosecurity, such as disinfecting the floor between production rounds (Mo et al., 2016) or wearing gloves by farm personnel (Jones et al., 2013). In addition, competitive exclusion products, which promote establishment of natural intestinal bacteria preventing colonization with certain bacteria as ESBL-EC, could be used to prevent colonization of young broilers (Dame-Korevaar et al., 2020a, 2020b). Interventions at the consumer level, such as decreasing meat consumption (reducing number of non-vegetarians or meat portion size) or preventing cross-contamination at the kitchen also contributed to a reduced ESBL-EC prevalence in the open community, but to a lesser extent. The effect of interventions reducing cross-contamination at the slaughterhouse are expected to have relatively little effect on the ESBL-EC prevalence in the open community. It should be kept in mind that we only evaluated interventions in the food production chain (farm to fork). We did not explore the effectiveness of interventions aimed at reducing human-to-human transmission, whereas our model calculations implicated this to be the main source of ESBL-EC in the open community (Table 4).

Decreasing the proportion of flocks with high levels of antimicrobial use had the smallest impact on the ESBL-EC prevalence in the open community (Fig. 4). Although it seems that a small number of farms with high antimicrobial usage has only minor effect on the outcome, it does not mean that the antimicrobial usage in livestock is not relevant for the

selection and spread of ESBL-EC. Antimicrobial usage has an impact on other parameters such as the within-flock spread, but also on further spread via the environment. It is hypothesized that antimicrobial use (including in livestock production) plays a role in the selection of antimicrobial resistant bacteria in the environment, exposing humans by several activities such as swimming in open water, hiking, camping, or eating fish, fruits, and vegetables (Blaak et al., 2014; Fagerström et al., 2019; Mughini-Gras et al., 2019; Müller et al., 2016). To better estimate the effect of high antimicrobial usage at farm level, the model should be extended with additional reservoirs of ESBL-EC being part of the One-Health approach, including other livestock animals, plant producers, and environmental sources such as surface water and wildlife.

We could not incorporate the full complexity of ESBL-EC transmission in the model, e.g. acquiring and losing resistance genes at the bacterial level was not accounted for. The model simulates clonal spread of ESBL-EC once it has been introduced into the system and we assumed it to be already present in both humans (open community and farmers) and broilers. The choice of the initial prevalence in humans was based on field observations, but did not affect model results. The modelled transmission dynamics finally resulted in an equilibrium with stable prevalence levels in both humans and broilers (Fig. 2). Already now, with the connections between the different sources modelled as simple as possible, we faced substantial uncertainty in model input parameters for each stage of the model: direct transmission within and between populations, and indirect transmission from livestock to humans via food consumption (slaughterhouse and consumer phase in the model). The model was developed to answer questions on source attribution and the effectiveness of interventions to reduce ESBL-EC prevalence. Multidirectionality and dynamicity were considered essential for this purpose. The model allowed us to explore what-if scenarios and how interventions/changes along the food production chain impact the human ESBL-EC prevalence, helping public health policymakers in risk management aimed at antimicrobial resistance risk mitigation.

5. Conclusion

This multidirectional and dynamic risk model provides insight into the contribution of different sources to ESBL-EC prevalence in the general human population, similar to source attribution models. It can also be used to explore the effect of interventions in the livestock sector or food production chain on the prevalence in the human population, similar to comparative exposure assessments. Based on model results, we conclude that intraspecies transmission (i.e. within sources) contributed most to ESBL-EC prevalence in both humans and broilers. Interventions aiming at reduced within-flock prevalence at the broiler farm were most effective in reducing the human ESBL-EC prevalence attributed to livestock production.

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CRediT authorship contribution statement

Eduardo de Freitas Costa: Writing – original draft, Methodology, Formal analysis, Investigation, Methodology, Conceptualization. **Thomas J. Hagenaars:** Supervision, Writing – review & editing, Methodology, Investigation, Conceptualization. **Anita Dame-Korevaar:** Writing – review & editing, Methodology, Methodology, Investigation, Conceptualization. **Michael S.M. Brouwer:** Writing – review & editing, Methodology, Conceptualization. **Clazien J. de Vos:** Funding acquisition, Project administration, Supervision, Writing – review & editing, Investigation, Methodology, Conceptualization.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2022.100230.

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