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# Estimating the likelihood of ESBL-producing *E. coli* carriage in slaughter-aged pigs following bacterial introduction onto a farm: a multiscale risk assessment

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**Short title:** A risk assessment to predict the likelihood of ESBL-producing *E. coli* carriage on commercial pig farms

# Abstract

The transmission of antimicrobial resistance (AMR) between animals, their environment, food and humans is a complex issue. Previous pharmacokinetic-pharmacodynamic (PKPD) models indicate that extended-spectrum β-lactamase (ESBL) resistant bacterial populations may be self-sustaining through horizontal and vertical gene transfer, even in the absence of antimicrobial pressure. However, models focusing purely on the biochemical aspects fail to incorporate the complicated host population dynamics which occur within a farm environment. Models of disease transmission within commercial farm environments can provide further insight to the on-farm transmission dynamics of AMR between animals and their environment, as well as predict the effect of various on-farm interventions. Here, we present a risk assessment which predicts the likelihood that slaughter-aged pigs would carry resistant bacteria after a single introduction of ESBL *E. coli* on commercial pig farms. We incorporate outputs from a PKPD model which explores the complex host/gastrointestinal bacteria interplay after antimicrobial treatment; with an on-farm model of bacterial transmission. The risk assessment is designed to be adaptable for the simultaneous transmission of multiple bacteria and resistant strains. We predicted that after introduction onto a pig farm, ESBL *E. coli* bacteria are likely to persist on the farm for more than a year, leading to a high batch prevalence (39.4% slaughter pigs, $5^{th}$ and $95^{th}$ percentiles: 0.0-57.5) and high faecal shedding. A comparison of different farm management types suggested that all-in-all-out housing was a protective measure for both prevalence in slaughter-aged pigs and faecal shedding rates. We applied two main interventions at the farm level, an enhanced cleaning and disinfectant (C&D) protocol and isolation of pigs in sick pens for the duration of their antibiotic treatment. Both interventions were able to reduce the number of pigs shedding more than 2 $\log_{10}$ ESBL *E. coli* from 18.7% ($5^{th}$ and $95^{th}$ percentiles: 5.9-30.4) in the baseline scenario, to 7.2% ($5^{th}$ and $95^{th}$ percentiles: 0.0-21.5) when an enhanced C&D protocol was applied, 0.1% ($5^{th}$ and $95^{th}$ percentiles: 0.0-0.3) when sick pens were used and 0.1% ($5^{th}$ and $95^{th}$ percentiles: 0.0-0.3) when a combination of enhanced C&D plus sick pens was used. Both scenarios also reduced the prevalence in batches of pigs going to slaughter. This effect was largest when sick pens were used, where 75% of batches had 0% positive pigs. The results suggest that a single introductory event is sufficient to cause a substantial risk of carriage in slaughter-aged pigs. Further quantitative microbial risk assessments (QMRA) are needed to consider the onwards risk posed to later parts of the food chain.



**Keywords:** antimicrobial resistance, pharmacokinetic-pharmacodynamic model, transmission model, intervention, slaughter, farm management

# 1 Introduction

The World Economic Forum has identified antimicrobial resistance (AMR) as a "global risk", stating that "while viruses may capture more headlines, arguably the greatest risk of hubris to human health comes in the form of antibiotic-resistant bacteria" (World Economic Forum, 2013). With no major new class of antibiotics having been discovered since 1987 (World Health Organization, 2015), there is the very real concern that bacterial diseases that are currently not life threatening may once again prove fatal.

ESBLs are predominantly plasmid-encoded enzymes found in *Enterobacteriaceae* which confer resistance to a variety of beta-lactam antibiotics (EFSA, 2011). The first human clinical isolates expressing ESBLs were identified in Germany in 1983 (Knothe *et al.*, 1983; Kliebe *et al.*, 1985) with carriage in animals and meat products reported since 2000 (EFSA, 2011). ESBL-producing *Enterobacteriaceae* may cause a range of clinical infections in people from urinary tract infections (UTI) to more serious bloodstream infections (Pitout and Laupland, 2008). They have also been involved in serious large scale antimicrobial resistant bacterial disease outbreaks in hospital settings (NethMap, 2019). However, it is not only the direct transmission of pathogenic bacteria that is a concern, but also the transmission of non-pathogenic AMR bacteria, such as commensal *E. coli,* which could potentially colonise the human gastrointestinal tract (GIT) and then confer resistance to pathogenic bacteria ingested subsequently.

There is ongoing debate as to the contribution that the food chain plays with regards to the transmission and spread of AMR bacteria in humans. This is likely to vary widely by bacterial species, transmission routes, and other factors. For example, the majority of human extended-spectrum beta (β)-lactamase producing *E. coli* (hereafter, ESBL *E. coli*) carriage, is acquired from other humans (Mughini-Gras *et al.*, 2019). Recent studies indicate that the attribution from the open population (defined as clinically healthy individuals who had not travelled to high-risk regions nor engaged in farming activities) may be as high as 60.1% (Mughini-Gras *et al.*, 2019). However, it is accepted that humans can acquire zoonotic pathogens such as *Salmonella* and shiga toxin-producing *Escherichia coli* (STEC) from consumption of meat (EFSA, 2019) and the World Health Organisation argues that food is one of the possible transmission routes of AMR from animals to humans (World Health Organization, 2015). In the same recent modelling study, food consumption (meat, seafood and vegetables) accounted for 18.9% of human ESBL carriage cases (Mughini-Gras *et al.*, 2019). In the United Kingdom, 4.7% of pork products tested at retail were positive for ESBL/AmpC *E. coli,* whereas 2.5% of *Campylobacter* spp. isolates from retail chicken meat displayed multi-drug resistance profiles (Willis *et al.*, 2018). It is therefore prudent to consider how to limit the acquisition of antimicrobial resistant bacteria via the food chain. It is recognised that the livestock industry may play a large role in dissemination of AMR through both direct occupational and non-occupational contact with farm animals (Mughini-Gras *et al.*, 2019), and indirect methods such as the dissemination of antibiotics into the environment (World Health Organization, 2015; Casey L. Cazer *et al.*, 2017; Filippitzi *et al.*, 2019). The need to control resistant bacteria in the livestock industry, both in order to reduce onwards transmission to humans, and to maintain treatment options for animals, has been well documented in the literature.

Many generic models have previously been developed to investigate the transmission of AMR at the bacterial level from donor bacteria to recipient bacteria. These models indicate that resistant bacterial populations can persist in the absence of antimicrobial pressure, through horizontal and vertical gene transfer (Freter *et al.*, 1983; Bergstrom *et al.*, 2000; Volkova *et al.*, 2012). However, models focusing purely on the biochemical, within-host aspects fail to incorporate the complicated host population dynamics which occur within a farm environment. For example, heterogeneous animal mixing and ingestion habits, the movement of animals within and between farms, cleaning and disinfection protocols, and treatment of animals for a multitude of farm pathogens, may all impact on the persistence of a population of endemic bacteria. Moreover, biochemical bacterial growth studies often use broths or growth mediums which replicate ideal growth conditions and can have quite different results to growth within faeces in a farm environment. A previous mathematical model comparing the impact of antimicrobial usage with the emergence of resistant bacteria in finisher pig farms found that both the transmission coefficient between pigs and the spontaneous clearance rate of drug-resistant bacteria influenced whether the resistant bacteria reached a steady endemic state on the farm or whether the bacteria could be cleared from the farm (Abatih *et al.*, 2009). The combination of pharmacokinetic-pharmacodynamic (PKPD) models with on-farm models of bacterial transmission and realistic farm management practices, would offer a unique opportunity to understand the disease dynamics in a more realistic environment.

The evidence regarding the presence, prevalence and microbial load of AMR bacteria in slaughter-age animals is limited. It is therefore imperative to gain further insight into on-farm AMR transmission dynamics with a view to assessing the level of resistance in slaughter-age animals and the impact of potential control measures. This risk assessment aims to predict the likelihood of resistant bacterial carriage in slaughter-aged pigs following a single introduction of ESBL *E. coli* onto commercial pig farms. The prevalence and concentration in pigs is further explored through scenarios designed to understand the effect of different, practical, on-farm interventions. Uniquely, we show how outputs from PKPD models can be combined with farm transmission models to add further confidence to a farm-based risk assessment. It is hoped that this will provide useful information for further studies, such as a full farm-to-consumption QMRA and cost-benefit models.

# 2 Methods

This risk assessment combined output from a PKPD model with a farm transmission model. The final output was the prevalence and concentration of resistant bacteria in the faeces of pigs sent to the abattoir. While the results are demonstrated using ESBL-producing commensal *E. coli* (hereafter, ESBL *E. coli*) on commercial UK pig farms treated (or not) with amoxicillin (AMOX), it is designed to be easily adapted for other bacteria and resistant strains, including multiple resistant strains, and other antibiotics. The model tracked the ingestion, excretion and carriage of both sensitive and resistant *E. coli* independently. An overview is provided here with full details available in appendix 1 and 2.

## 2.1 PKPD model

The development of the within-host semi-mechanistic PKPD model (hereafter simplified as PKPD model) was based on previous works in cattle from Cazer *et al.* (2017) and Volkova *et al.* (2017), and adapted to pigs. The aim was to predict the impact of an antimicrobial treatment (as an input of the model) on the selection of ESBL *E. coli* within the GIT and excretion towards faeces (output of the model), at the individual and population level (taking into account the inter-individual variability). The model was divided into a PK part describing the fate of AMOX within the GIT and a PD component to describe the level variations of sensitive *E. coli* and ESBL *E. coli* within the colon (and towards faeces) (Figure 1).

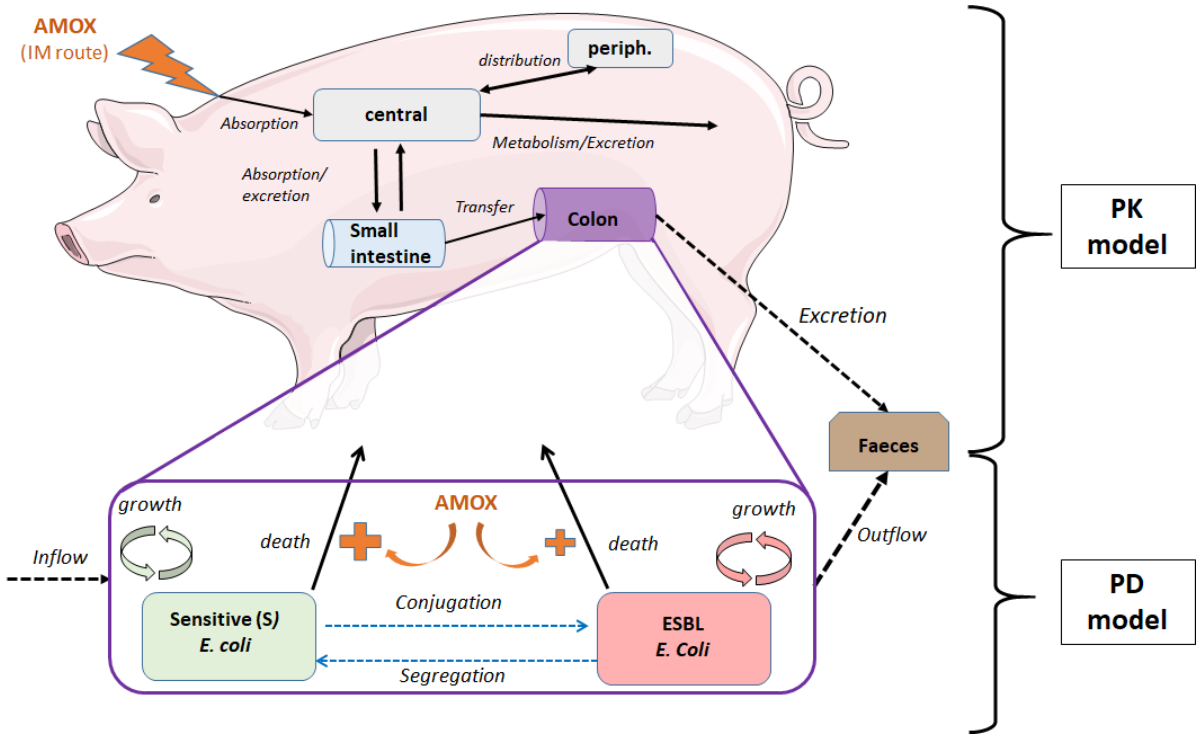


*Figure 1: Overview of pharmacokinetic-pharmacodynamic (PKPD) model for amoxicillin and E. coli within a pig gastrointestinal tract (GIT). The PK submodel describes the PK of amoxicillin (AMOX) after intramuscular administration within the central system and the digestive tract. The PD submodel describes the dynamics of sensitive and resistant E. coli within the GIT and the impact of AMOX. (See Appendix 1 for more details).*

A hybrid PK model was developed with empirical compartments for the central system (central and peripheral compartments) based on previous population PK studies of amoxicillin in pigs (Rey *et al.*, 2014; Agersø and Friis, 1998) and more mechanistic compartments for the GIT (with physiological volumes and transit rates) (see appendix A1.1 for equations and parameter distributions).

The PD model involves one sensitive and one resistant sub-population of *E. coli* (i.e. ESBL) within the colon, the latter harbouring resistance genes within a plasmid. Note that the sensitive *E. coli* was modelled here as a ubiquitous, commensal bacteria and not indicative of an independent *E. coli* infection in the pigs. Each bacterial population grew following a logistic model until they reached the (shared) maximal capacity of bacterial load within intestines, meaning that they compete for the same ecological niche (nutrients, space, etc.) (Blanquart, 2019; Davies *et al.*, 2019). Bacteria were also affected by a natural death constant. Moreover, there was an inflow of bacteria from the environment (via feeding/coprophagia) and an outflow by faecal excretion. The AMOX concentrations within the colon impacted the bacteria with an increase in the death of bacteria. Finally, a transmission of plasmid from resistant to sensitive bacteria by conjugation process was also considered as well as a potential plasmid loss by segregation (see appendix A1.2 for equations and parameter distributions).

The considered scenario was a treatment with AMOX given by intramuscular injections once a day for five consecutive days at 15 mg/kg, in post-weaning pigs of 17 kg. The treatment started at t=50 hours (and ended at t=146 hours) with an integrative step of 3 hours. Different amounts of inflow of resistant bacteria (InflowR, see Appendix A1.2) were considered from t=0 hours until end of simulations: 2, 4, 6 or 8 $\log_{10}$ cfu/g ESBL *E. coli* per day. The daily quantities of sensitive and resistant bacteria excreted towards faeces were outputs from the model used to connect with the farm transmission model (see section 2.3).

Monte Carlo simulations were performed, based on the distribution of each parameter, to generate a population of 5000 pigs. All simulations were done in Rstudio (RStudio, 2018) with the simulX function of the mlxR package from Lixoft (Lavielle, 2020).

## 2.2 Farm transmission model

The farm transmission model was an individual based, stochastic, susceptible-infected-susceptible (SIS) model of pigs within commercial pig farms, where "infected" indicated that the pigs were colonised with resistant bacteria. It was based on a previous model of *Salmonella* transmission on commercial pig farms in the European Union (Hill *et al.*, 2016). The model consisted of two main components: a pig management and a bacterial transmission component (Figure 2). The model was run with 500 iterations, each iteration representing production from one farm over a 365-day period. The time step of the model was one day.

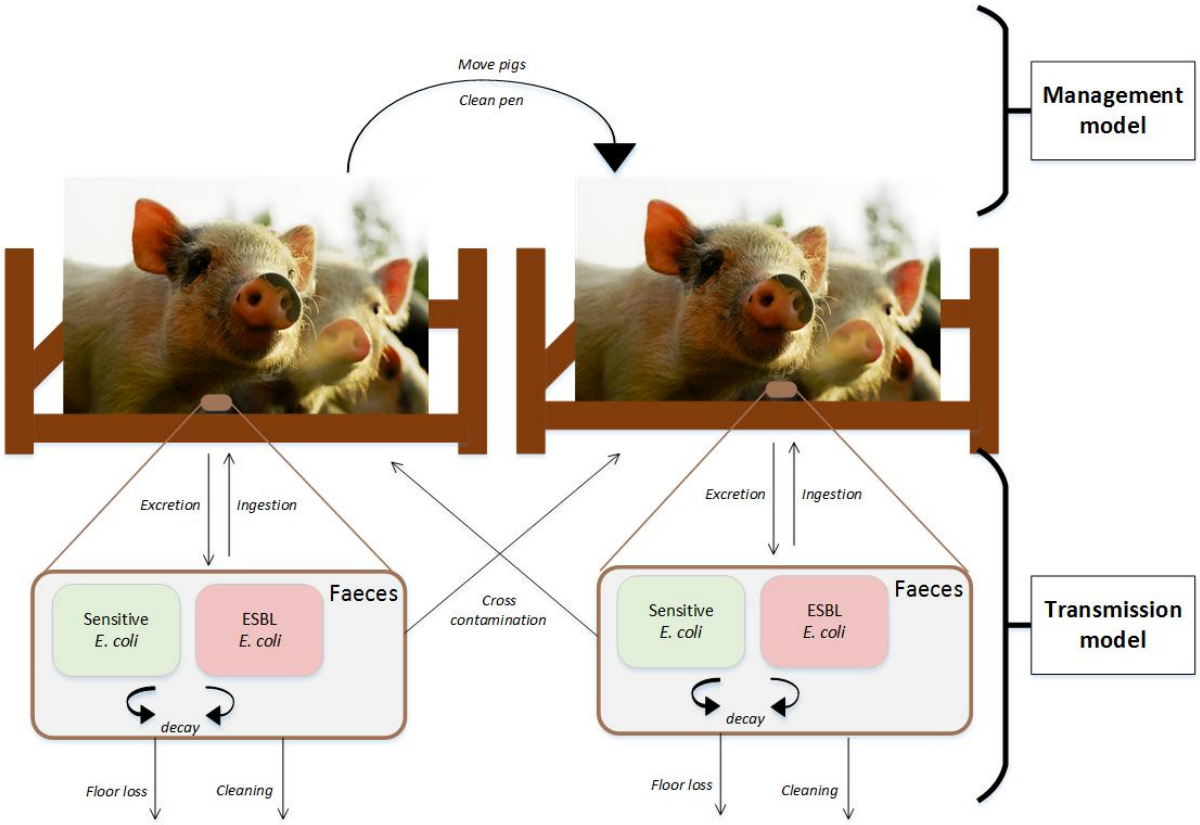


*Figure 2: Overview of farm model which simulated the colonisation and transmission of resistant bacteria in pigs within a commercial farm environment. The model has both management and transmission components. The management component tracked individual pigs as they moved through the farrowing, weaner, grower and finisher stages. The transmission component simulated the build-up (and loss) of bacteria within the pen environment, ingestion of bacteria and subsequent colonisation and faecal excretion. Faecal excretion rates were calculated from the output from the PKPD model (Figure 1).*

The farms were designed as breeder-finisher systems, where pigs stay on the same farm from birth until they go to the abattoir. The model incorporated different practices of commercial pig farms. Farms may run an all-in-all-out (AIAO) production system (where batches of pigs were kept together in one room for each of the weaning, growing and finishing stages, without direct contact with other batches all the way through rearing), or a continuous production system which allows mixing of different batches of pigs at each stage of development. Farms could also use solid or slatted floor in the pig pens. Different production systems were randomly selected for each iteration but the probability of selection was weighted towards that of the UK commercial pig industry, as in Hill et al. (2016). Therefore, the majority of farms had a continuous system on solid floor (43.2%), followed by an all-in-all-out (AIAO) system on solid floor (39.2%), continuous system on slatted floor (10.9%) and AIAO on slatted floor (6.6%). The baseline model sums the predicted number of positive pigs over all batches/farms to estimate the prevalence of colonised pigs going to slaughter. Comparisons of the relative risk in different farm types was also explored.

The management component tracked individual pigs as they moved through the farrowing, weaner, grower and finisher stages. Transport to slaughter occurred weekly when one batch of pigs (consisting of four pens of 40 finishers) leaves the farm. The first five batches of pigs sent to slaughter were excluded from analysis to allow sufficient introduction of bacteria to occur.

The transmission component simulated the build-up (and loss) of bacteria within the pen environment, ingestion of bacteria and subsequent colonisation and faecal excretion. Faecal excretion rates were calculated from the output from the PKPD model (see below). Bacterial levels in the environment could change due to the natural decay of the pathogen over time, loss through flooring (if slatted flooring is used) or cleaning and cross-contamination to or from neighbouring pens (see appendix A2 for equations and parameter distributions).

The model was initiated by simulating a single introductory event on day 0, in which five piglets started excreting 8 $\log_{10}$ cfu/g ESBL *E. coli* (Hansen *et al.*, 2013). The model randomly selected the five piglets on each iteration. These could reside in any of the piglet pens and were not necessarily within the same pen. Piglets were chosen as the group for this introduction event as these were furthest in age from the finisher pigs, and so were the group least likely to still show carriage by slaughter age (thereby simulating a "worst case" scenario). The consequence of choosing five piglets was explored in the sensitivity analysis. Moreover, the baseline model randomly assigned five colonised piglets to any piglet pen. We ran an additional piglet scenario to assess whether the carriage of ESBL *E. coli* in slaughter-aged pigs was dependent upon the initial location of the colonised piglets on day one. Specifically, we ran a scenario to see whether slaughter-aged pigs could still carry ESBL *E. coli* if the five colonised piglets were initially within the same pen (therefore simulating infection from one sow), or one colonised piglet was placed in five different pens (therefore simulating a low-level excretion from five infected sows).

## 2.3 Integrating the PKPD output with the farm transmission model

The PKPD model provided an estimate of the bacterial excretion rates from 5,000 pigs who ingested either 2, 4, 6 or 8 $\log_{10}$ cfu/g ESBL *E. coli* per day, both for pigs which were, and were not, treated with AMOX. For pigs treated with AMOX, faecal excretion rates of resistant bacteria were shown to peak one day after antimicrobial treatment ceased (day six), and then had a log linear decrease over time. To calculate the maximum faecal excretion rate for each combination of ingestion and antibiotic status, we fitted different models (linear, log linear, polynomial) to determine the best fit for the percentiles of pigs on day six. The polynomial model proved the best fit. To calculate the daily rate decrease after the peak, we fitted a linear equation to the 50$^{th}$ percentile of pigs in the PKPD model from the peak to the end of the simulation (again, allowing for ingestion and antibiotic status).

It was assumed that there would be a variety of endemic diseases circulating on the pig farm, such as nervous system and respiratory diseases. On each day in the farm transmission model, healthy pigs had a probability of becoming "sick" with such a disease and being treated with antibiotics. Due to the predominance of these diseases in weaner age piglets and older (Merck, 2016), it was assumed that piglets wouldn't be classed as sick. Therefore, piglets (up until 28-days-old) were never treated with antibiotics. The youngest age at which pigs could be treated with antibiotics was in the weaner stage.

At each time step in the farm transmission model, the amount of ESBL *E. coli* ingested by each pig was calculated. The relevant outputs from the PKPD model were selected depending on the ingestion value and whether the pig had or hadn't been treated with antibiotics. On the first day that a pig ingested ESBL *E. coli*, we assigned a percentile to each pig by sampling from the uniform distribution set around the $10^{th}$ and $90^{th}$ percentiles for the dose ingested. Note that due to uncertainty from the PKPD model, it was decided not to use more extreme percentiles ($5^{th}$ and $95^{th}$) as it was not clear if these would be biologically plausible. This value was entered into the polynomial equation to calculate the maximum faecal excretion rate of ESBL *E. coli* for that pig. On subsequent days, the model determined whether the amount ingested had changed sufficiently from the previous day (i.e. whether the new ingestion amount was at least 2 $\log_{10}$ cfu/g higher or lower than the previous day, such that the pig should enter a new ingestion category in the PKPD output). If this was the case, then we resampled from the uniform distribution which was entered into the new polynomial equation. If the ingestion category did not change, then we assumed that the faecal excretion rate remained constant for six days. On subsequent days, (assuming no change in ingestion category), the pig would decrease by the rate determined by the linear equation from the $50^{th}$ percentile of pigs.

## 2.4 Scenarios for farm-based interventions

During the baseline model simulations, we assumed that pigs could be treated with antibiotics whilst still residing within the pig pen that they were in prior to becoming classed as "sick". As an initial exploration into whether isolation of sick pigs helped to reduce resistant bacteria transmission, we simulated an additional intervention where sick pigs were moved out of the communal pen and into a separated "sick pen" (the hypothesis being that pigs treated with antibiotics would have the highest rate of excretion, which may reduce by the time they leave the sick pen). These pens were modelled to occur in complete isolation, with no cross-contamination between pens. They were cleaned weekly. There was one sick pen per age group and all sick pigs of the same age resided in the same sick pen for the duration of time that they are treated with antibiotics. When pigs completed their antibiotic treatment they were returned to their original batch and considered to be healthy again, such that they could potentially become sick again and re-start antibacterial treatment.

A further intervention was modelled to understand the effect of an enhanced cleaning and disinfection (C&D) protocol. Cleaning has been found to be less than 100% effective at eliminating *E. coli* within lairage pens, with up to 2.8 $\log_{10}$ CFU/$cm^2$ remaining after cleaning (FSA, 2006). Parameters related to cleaning in the baseline model were taken from estimates used in the *Salmonella* model (Hill et al. 2015). The C&D scenario simulated a situation where enhanced cleaning was used, which removed all contamination after a cleaning event.

# 3 Results

Simulations using the PKPD model highlighted the impact of the AMOX treatment on the quantities of sensitive and resistant bacteria excreted towards faeces (Figure 3). Indeed, during the treatment period (from t=50 to t=146h), there was a huge increase of ESBL *E. coli* therefore resistant bacteria stayed at a dominant level at least 14 days after the end of treatment. The daily inflow of resistant bacteria was the major parameter influencing the level of excretion of resistant bacteria, for both treated and untreated pigs, and this was confirmed by the results of the sensitivity analysis (see appendix A3.1).

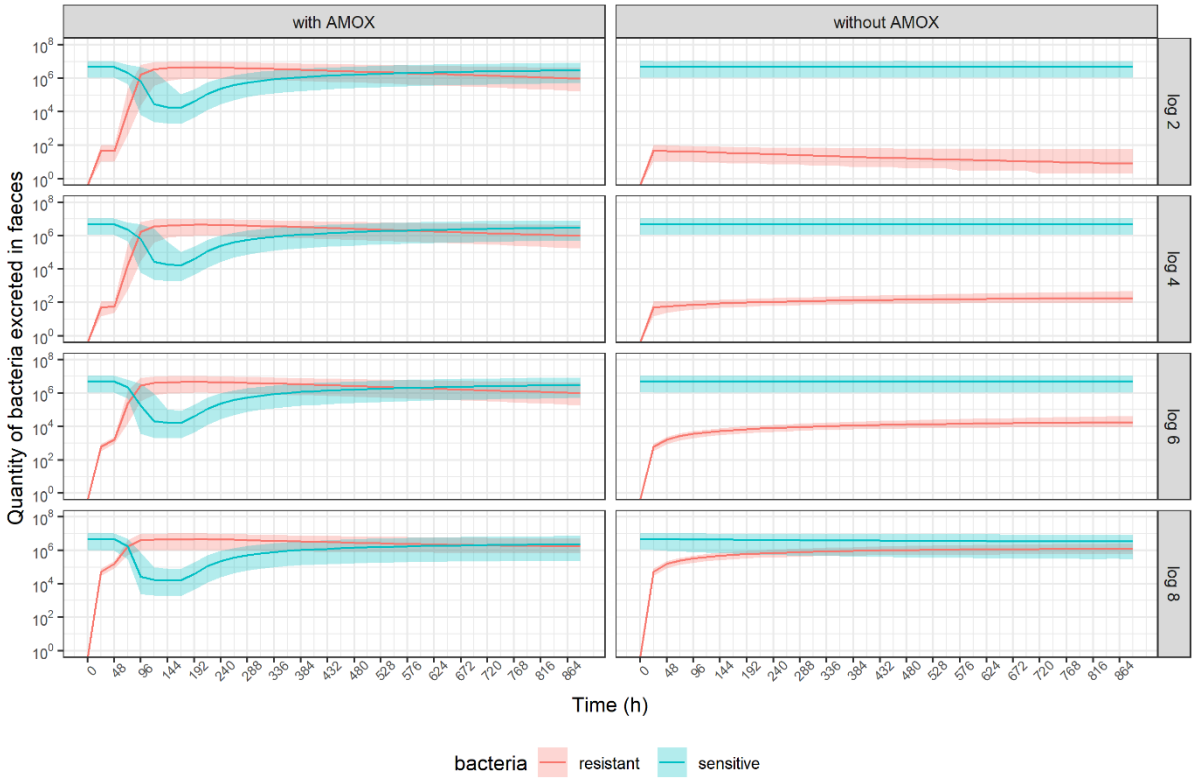


*Figure 3: Outputs of the PKPD model for the quantity of sensitive E. coli and resistant (ESBL) E. coli for a simulated population of 5000 pigs. Scenarios were simulated for different daily inflows of ESBL E. coli (levels indicated on right y axis), with and without Amoxicillin treatment (indicated on top x axis). The median estimates are represented by the coloured lines and the shaded areas indicate the 10th and 90th percentiles.*

Results from the overall risk assessment suggested that after an introduction of ESBL *E. coli* into the piglet pens, bacteria would seed into the finisher pig pens and produce positive slaughter-aged pigs (Figure 4). These levels would then remain high until the end of the simulation at 52 weeks. The average prevalence in batches of slaughter-aged pigs (defined as a group of 40 finisher pigs from four pens going to slaughter at the same time) in the baseline scenario was 39.4% (5$^{th}$ and 95$^{th}$ percentile: 0.0%-57.5%). However, there was variation between pens and iterations, with occasional finisher buildings remaining negative in some iterations. The average pig prevalence within positive batches was 46.3% (5$^{th}$ and 95$^{th}$ percentile: 4.4-58.1).

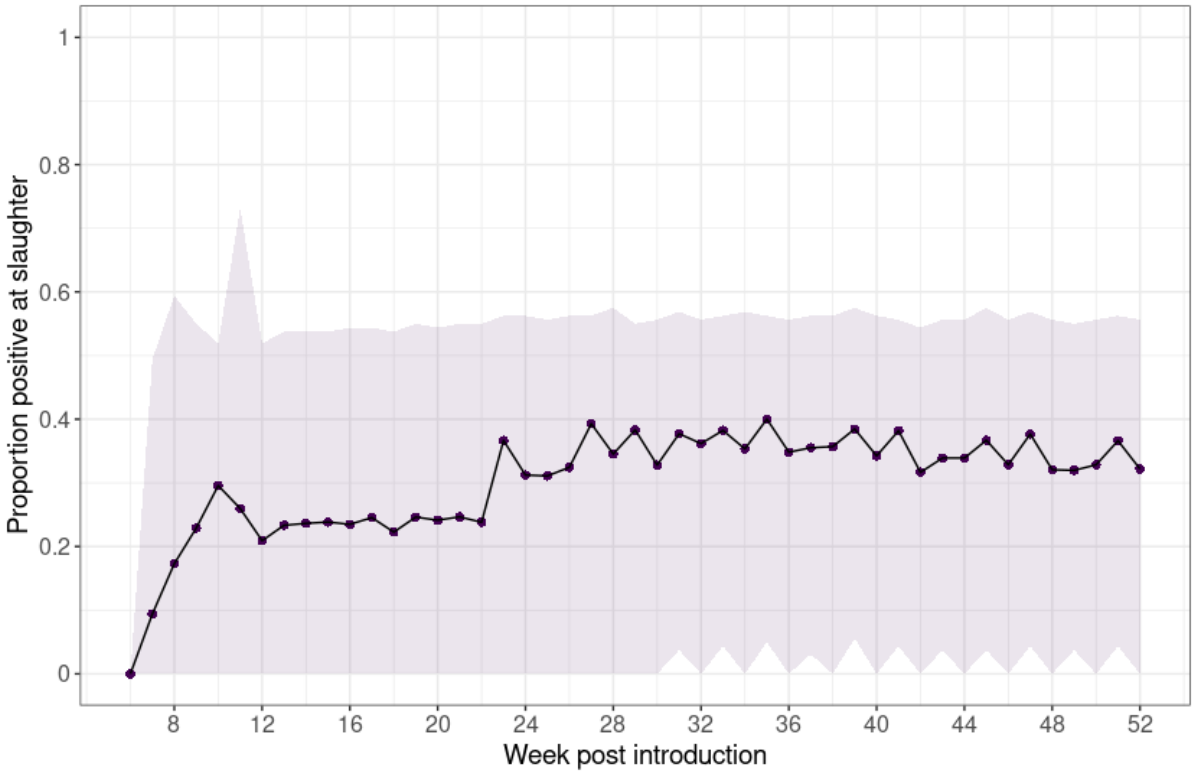


*Figure 4: Prevalence of ESBL E. coli carriage in batches of slaughter–aged pigs within a commercial pig farm environment. Five colonised piglets were simulated to enter the farm on day 1. Slaughter-aged pigs were removed weekly from the farm. Results from the first five weeks have been removed from the analysis in order to allow full transmission within the farm (results shown for weeks 6 onwards). Shaded regions refer to the 5th and 95$^{th}$ percentiles from 500 iterations.*

A comparison of different farm types suggested that AIAO housing was a protective measure for both prevalence in slaughter-aged pigs and faecal shedding rates. The average batch prevalence in pigs raised in AIAO systems on slatted floor was 26.9% (5$^{th}$ and 95$^{th}$ percentiles: 0.0-51.6), whereas those on solid floor had a reduced average batch prevalence of 5.0% (5$^{th}$ and 95$^{th}$ percentiles: 0.0-56.3) (Figure 5A). The prevalence was higher in continuous systems, where pigs reared on slatted floor had an average prevalence of 34.4% (5$^{th}$ and 95$^{th}$ percentiles: 0.0-52.5) and those raised on solid floor had a prevalence of 48.1% (5$^{th}$ and 95$^{th}$ percentiles: 0.0-59.4). Similarly, pigs raised in AIAO systems had lower faecal shedding rates, with 13.0% pigs (5$^{th}$ and 95$^{th}$ percentiles: 6.9-15.2) on slatted floor and 10.3% pigs (5$^{th}$ and 95$^{th}$ percentiles: 5.1-14.4) on solid floor shedding over 2 $\log_{10}$ cfu/g (Figure 5B). In comparison, 22.0% pigs (5$^{th}$ and 95$^{th}$ percentiles: 20.1-23.8) on slatted floor and 26.4% pigs (5$^{th}$ and 95$^{th}$ percentiles: 18.5-30.9) on solid floor, raised in a continuous system, shed over 2 $\log_{10}$ cfu/g.

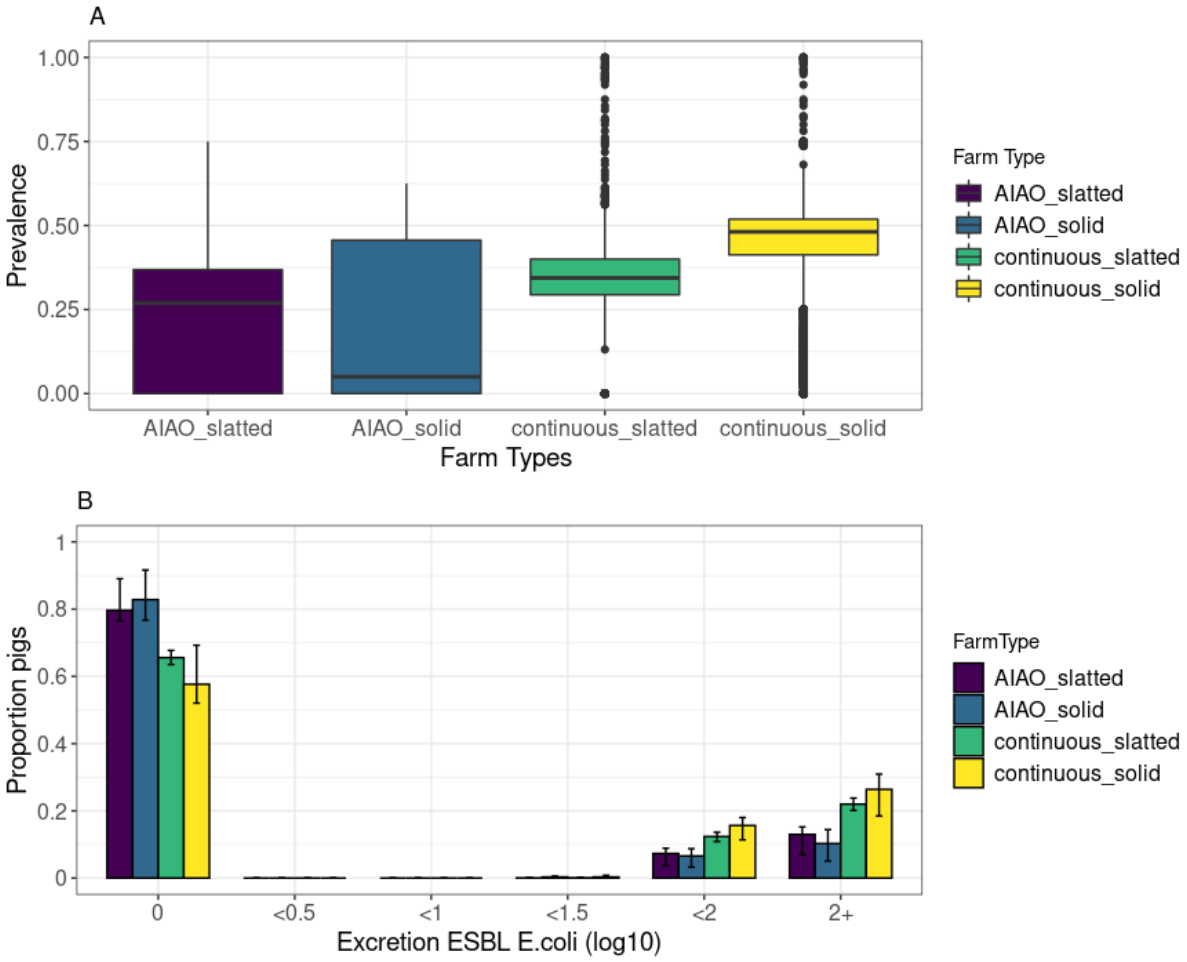


*Figure 5: Batch prevalence (A) and faecal shedding rates (B) for ESBL producing E. coli in slaughter – aged pigs reared in the following commercial farm management types: All-In-All-Out (AIAO) system on slatted floor (AIAO_slatted), AIAO system on solid floor (AIAO_solid), continuous system on slatted floor (continuous_slatted) and continuous system on solid floor (continuous_solid). In figure B, error bars show 5th and 95th confidence limits.*

The scenario results suggested that both enhanced C&D and the use of sick pens acted to reduce the prevalence in batches going to slaughter (**Erreur ! Source du renvoi introuvable.**A). The average batch prevalence under the C&D scenario was reduced to 6.3% ($5^{th}$ and $95^{th}$ percentiles: 0.0-53.8). The use of sick pens or sick pens plus C&D both reduced this further, to 0.0% ($5^{th}$ and $95^{th}$ percentiles: 0.0-44.4%) and 0.0% ($5^{th}$ and $95^{th}$ percentiles: 0.0-47.5%) respectively. Moreover, all interventions acted to reduce the shedding rates in positive pigs (**Erreur ! Source du renvoi introuvable.**B). In the baseline scenario, 69.8% ($5^{th}$ and $95^{th}$ percentile: 52.5–90.0) of pigs were not excreting ESBL *E. coli* at slaughter. Of those pigs that were positive, the majority (18.7%, $5^{th}$ and $95^{th}$ percentiles: 5.9-30.4) shed over 2 $\log_{10}$ cfu/g. In the C&D, sick pens, and sick pens plus C&D scenarios, 7.2% pigs ($5^{th}$ and $95^{th}$ percentiles: 0.0-21.5), 0.1% pigs ($5^{th}$ and $95^{th}$ percentiles: 0.0-0.3) and 0.1% pigs ($5^{th}$ and $95^{th}$ percentiles: 0.0-0.3) respectively, shed over 2 $\log_{10}$ cfu/g.

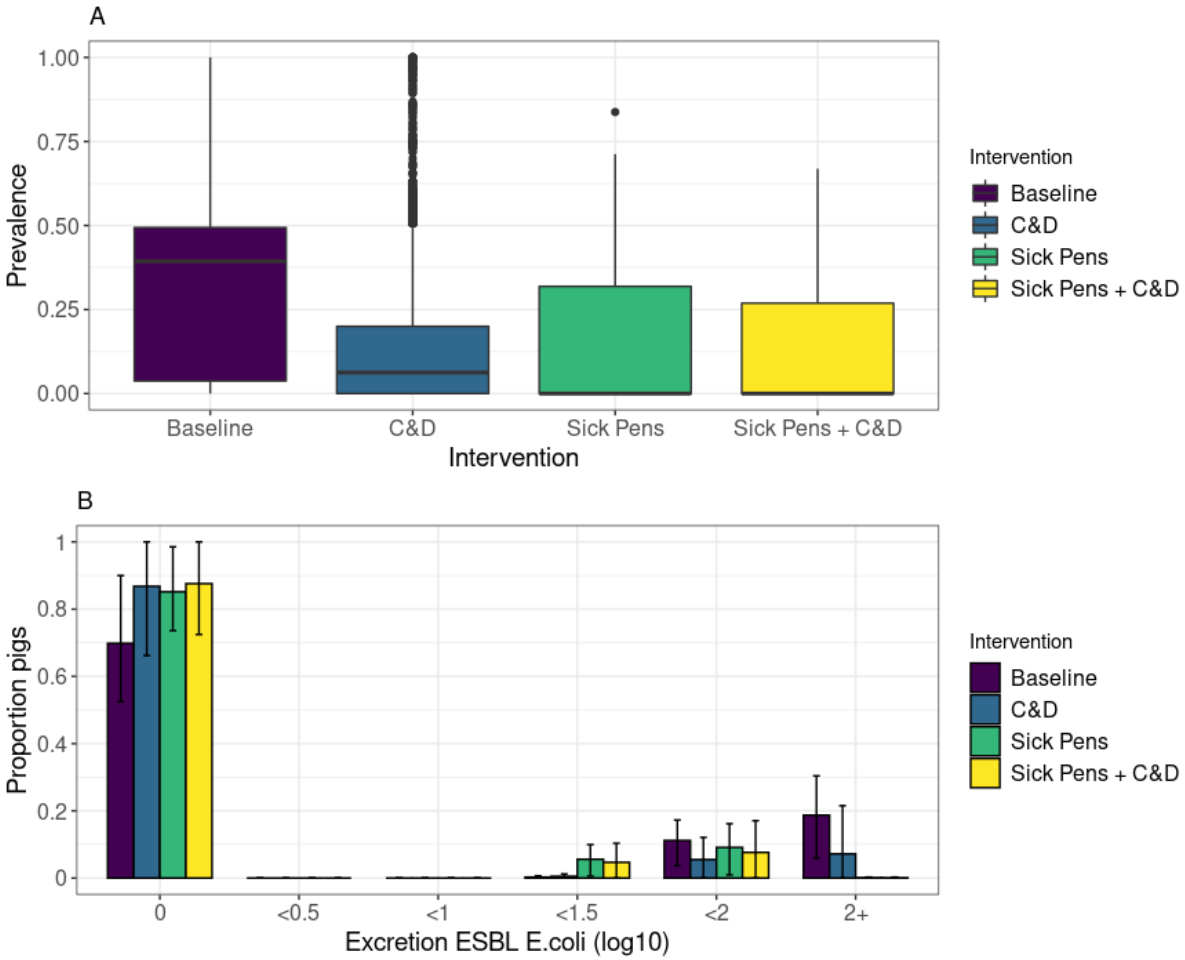


*Figure 6: Batch prevalence (A) and faecal shedding rates (B) for ESBL producing E. coli in slaughter – aged pigs within a commercial farm environment. The baseline model is compared against the following interventions: enhanced cleaning and disinfection (C&D), the use of sick pens to isolate pigs on antimicrobial treatment (Sick Pens), and a combination of sick pens plus enhanced C&D (Sick Pens + C&D). In figure B, error bars show 5th and 95th confidence limits.*

Results from the sensitivity analysis suggested that the average prevalence at slaughter-age was most sensitive to the old faecal loss through the floor and the decay of bacteria within the pen environment. It was less sensitive to the proportion of pigs treated with antibiotics per day and the cross contamination rates between pens (Figure A3.2). The piglet scenario analysis showed that slaughter-aged pigs could carry resistant bacteria after a single introduction of ESBL *E. coli* on commercial pig farms even if five colonised piglets were placed within the same pen, or if one colonised piglet was placed in five different pens (see Figure A3.3).

# 4 Discussion

Here, we have proposed a method for incorporating a farm model which simulates the transmission of bacteria on a commercial pig farm, with a PKPD model which simulates the impact of antimicrobial treatment on the selection of ESBL *E. coli* within the GIT. The results suggest that after an initial introduction of the resistant bacteria onto the farm, prevalence rates will reach a steady plateau. This suggests that if ESBL *E. coli* are introduced in sufficient quantity onto a commercial pig farm, then on average, they are able to persist on the farm for at least 365 days and are likely to be present in slaughter-aged pigs. The risk assessment predicted an average prevalence of ESBL *E. coli* of 39.4% ($5^{th}$ and $95^{th}$ percentiles: 0.0-57.5) in slaughter-aged pigs. The latest UK veterinary antibiotic resistance and sales surveillance report (VARSS report, 2017) found that 15% of caecal samples from healthy pigs at slaughter were positive for ESBL *E. coli* (VMD, 2018), suggesting a reasonable level of similarity (albeit with broad predicted percentiles). The results outlined may be useful for subsequent farm-to-consumption QMRAs in order to consider the impact on human exposure, or for further cost-benefit analyses to understand the financial costs of farm-based interventions.

Uniquely, this risk assessment shows how the addition of output from a PKPD model can add further confidence to a farm-based risk assessment. This is especially important in predicting microbial load in positive animals, which is fundamental for understanding the risk of onwards transmission. Due to the large bacterial carrying capacity within the host GIT, results from the PKPD model suggest that pigs excrete a high concentration of resistant bacteria on a daily basis. A previous model of AMR transmission in pig finisher farms suggested that the proportion of pigs carrying resistant bacteria was largely influenced by transmission rates between pigs and spontaneous clearance rates of bacteria from the host (Abatih *et al.*, 2009). Competitive exclusion using probiotic flora has been found to be an effective method for reducing ESBL *E. coli* transmission between broiler chickens (Ceccarelli *et al.*, 2017). Further work should focus on reducing the carriage of resistant bacteria within pigs.

An additional strength of the current risk assessment is in its ability to assess the comparative impact of different control measures. In many infectious disease processes, the minority of high-shedding animals are responsible for the majority of transmission, the so-called “super-shedding” animals (Chase-Topping *et al.*, 2008). The use of sick pens had the largest effect on this, reducing the percentage of pigs shedding over 2 $\log_{10}$ cfu/g from 18.7% in the baseline scenario to 0.1%. Enhanced C&D also created a beneficial effect, although not to the same extent as the use of sick pens. The ability for farmers to apply strict biosecurity measures or isolation of sick pens is likely to vary between commercial establishments. The current risk assessment did not consider whether applying an enhanced C&D protocol would have an additional indirect benefit for AMR transmission, by helping to reduce levels of endemic diseases and therefore antimicrobial usage. Additionally, it did not consider whether heavy disinfectant use would offer a selective advantage to resistant bacteria by preferentially removing sensitive bacteria. This has previously been suggested for various antiseptics and heavy metals (Martin and Maris, 1995; Potenski *et al.*, 2003; Braoudaki and Hilton, 2004; Soumet *et al.*, 2012) however, the exact role that the use of disinfectants has on driving AMR resistance is controversial (Cheng *et al.*, 2019). Further work is needed to investigate this factor before it can reliably be incorporated into QMRA models. The use of sick pens here was designed as an initial exploration into whether segregating sick pigs would reduce resistant bacteria transmission amongst pigs (with the hypothesis that pigs being treated with antibiotics would have the highest

rate of excretion and that this high rate would have reduced by the time that pigs left the sick pen). Further work would be useful to understand the variations by which farmers may use sick pens. For example, we have assumed a total segregation of sick pigs with no pen-to-pen transmission and that all pigs would return to their original pen after ceasing their antibiotic treatment. Realistic variations upon this may be that sick pens are incompletely isolated therefore allowing some transmission to non-sick pens; different numbers of sick pens and pigs per sick pen; more or less frequent cleaning of the sick pens; and allowing pigs to remain in sick pens without returning to their original pen.

The PKPD model was based on a previous model for cattle (Cazer *et al.*, 2017; Volkova, Cazer and Gröhn, 2017) and adapted to pigs thanks to an extensive literature search to get physiological, pharmacokinetics and bacterial data. Other authors have published similar PKPD model for swine and ampicillin (Ahmad *et al.*, 2016) but they used the plasma concentrations as a surrogate for the impact of antimicrobial drug on the intestinal *E. coli*. This is however an over-simplification of reality as the PK of drugs within the GIT is not only due to a passive equilibrium of the drug between plasma and the intestinal lumen, but rather a combination of several simultaneous mechanisms of excretion, reabsorption and transit that depends on the location within the GIT. Our model included these processes to be more physiological and was developed in order to be easily adapted to other antimicrobials and bacterial species. One limit concerns the lack of published longitudinal data on faecal concentrations of amoxicillin and faecal *E. coli* in pigs treated with AMOX that could be used to validate our PKPD model. However, our simulations are in accordance with published experiments in pigs treated with ampicillin (which is close to AMOX) and showing a strong increase of the ESBL *E. coli* levels in faeces after an IM treatment (Bibbal *et al.*, 2007).

Despite the importance of the β-lactamase enzyme on the resistance phenomena by protecting the resistant bacteria, the degradation of AMOX by these enzymes within intestines did not seem to be an influential factor concerning the excreted quantity of AMOX according to the sensitivity analysis. This is in agreement with older studies looking at the inactivation of amoxicillin by biological and non-biological processes in human faeces (de Vries-Hospers *et al.*, 1993; Jansen *et al.*, 1992). However, recent studies in mice have highlighted a positive influence of these enzymes on the protection against ampicillin for intestinal bacteria not expressing β-lactamase enzyme, suggesting the degradation of the β-lactam drug as the main factor explaining these findings (Gjonbalaj *et al.*, 2020). Overall, the importance of the gut microbiota on the metabolism of antimicrobial drug is an ongoing subject (Zimmermann *et al.*, 2019) and new data may help to refine the PKPD model.

The farm transmission model considered bacterial persistence within faeces in the pen environment as a potential source of infection, but did not explicitly model any biofilm formation within the pens. This has been shown to enable resistant *Salmonella Typhimurium* to persist in the environment (Tassinari *et al.*, 2019). Interestingly, both the natural decay rate of ESBL *E. coli* and the rate at which old faeces are lost from the pen floor had a large effect on the overall prevalence according to the sensitivity analysis. In transmission studies of ESBL *E. coli* in broiler flocks, prevalence within birds dropped to zero in the absence of antimicrobial usage but the pathogen was found to persist within the pen environment (Dame-Korevaar *et al.*, 2017). If the resistant bacteria being modelled were found to successfully create biofilms within farm environments, this would affect parameters such as the cleaning coefficients which have been incorporated into this current risk assessment.

Conversely, it may be possible that biofilm formation negatively affects the availability of bacteria to animals within the environment, as evidenced by the zero prevalence in broiler flocks despite persistence within the environment (Dame-Korevaar *et al.*, 2017).

Due to the closed nature of the pig farms modelled here, we had assumed that infected replacement sows could enter the farm and shed to piglets (rather than bacteria entering via entry of older animals). The previous *Salmonella* model allowed the pathogen to enter the farm via infected feed, the environment (such as wildlife reservoirs), or infected sows (Hill *et al.*, 2016). The current risk assessment assumed that infected feed was unlikely to be a significant entry route for resistant bacteria onto a farm. However, carbapenem-resistant *Enterobacteriaceae* have been found in gulls (Köck *et al.*, 2018) although the directional spread of resistant bacteria between pig farms and environmental reservoirs is currently unknown. Moreover, airborne transmission has been found to be a possible transmission route for livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) on pig farms (Bos *et al.*, 2016), therefore opening up the possibility of spread from nearby farms. Although this may be a less relevant concern for ESBL *E. coli*, in order for this to truly function as a generic risk assessment, more work is needed to understand the relative significance of environmental routes for bringing resistant bacteria onto farms.

Several of the parameters in this risk assessment were extrapolated and therefore associated with high uncertainty. For instance, farm management parameters, such as the cleaning coefficients, were taken from Hill et al. (2015), and as such, we have assumed that *E. coli* within faeces reacts in a similar fashion to *Salmonella* within faeces. Concerning the PKPD model, the plasmid transfer rate was taken from *in vitro* studies with values differing by several orders of magnitude (Chauzy *et al.*, 2019; Kristoffersson *et al.*, 2020), and the inflow and outflow rate of bacteria within the GIT were taken from previous models in cattle (Cazer et al., 2017; Volkova, Cazer and Gröhn, 2017). Moreover, these parameters were among the most influential when looking at the results of the GSA. Therefore, there is reasonable uncertainty regarding the absolute values of the prevalence and load of resistant bacteria in slaughter–aged pigs. Interestingly, the number of colonised piglets on day 1 of the model did not produce a very high influence in the sensitivity analysis suggesting that it had a low effect on the overall prevalence. This is perhaps concerning, and suggests that even small numbers of colonised piglets are sufficient to cause transmission of the bacteria throughout the farm. Moreover, results were reasonably robust to the placement of the initial colonised piglets, with carriage becoming possible in slaughter-aged pigs if piglets were placed together in one pen or in several different pens.

The risk assessment outlined here suggests that after sufficient introduction of ESBL *E. coli* onto a pig farm, the bacteria are likely to persist on the farm for at least a year leading to a high level of carriage and faecal shedding in slaughter age pigs. Both isolating pigs in sick pens for the duration of their antibiotic treatment and an enhanced C&D protocol were effective at reducing the number of positive batches and the number of high-shedding pigs. Future work should concentrate on repeating the analysis for other resistant bacteria (such as livestock-associated methicillin-resistant *Staphylococcus aureus* (LA MRSA) or colistin – resistant *E. coli*) in order to understand interventions which can control multiple resistant pathogens.

## 5 Declaration of interest

Declaration of interest: None.

## 6 CRediT author statement

**Catherine McCarthy:** Methodology, Software, Writing – Original Draft. **Alexis Viel:** Methodology, Software, Writing – Original Draft. **Chris Gavin:** Methodology, Software, Writing – Review & Editing. **Pascal Sanders:** Conceptualization, Methodology, Writing – Review & Editing, Project administration. **Robin R.L. Simons:** Conceptualization, Methodology, Writing – Review & Editing, Project administration

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