



**HAL**  
open science

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

Catherine Mccarthy, Alexis Viel, Chris Gavin, Pascal Sanders, Robin R.L. Simons

## ► To cite this version:

Catherine Mccarthy, Alexis Viel, Chris Gavin, Pascal Sanders, Robin R.L. Simons. Estimating the likelihood of ESBL-producing *E. coli* carriage in slaughter-aged pigs following bacterial introduction onto a farm: A multiscale risk assessment. *Microbial Risk Analysis*, 2022, 20, pp.100185. 10.1016/j.mran.2021.100185 . anses-03441007

**HAL Id: anses-03441007**

**<https://anses.hal.science/anses-03441007>**

Submitted on 15 Mar 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

4  
5 Catherine M<sup>c</sup>Carthy<sup>a\*</sup>, Alexis Viel<sup>b</sup>, Chris Gavin<sup>a</sup>, Pascal Sanders<sup>b</sup>, Robin R.L. Simons<sup>a</sup>

6 <sup>a</sup> Department of Epidemiological Sciences, Animal and Plant Health Agency (APHA), Woodham Lane,  
7 New Haw, Addlestone, KT15 3NB

8 <sup>b</sup> French Agency for Food, Environmental and Occupational Health & Safety (Anses), Laboratoire de  
9 Fougères, 35300 Fougères, France

10

11 **\*Corresponding author:** Catherine M<sup>c</sup>Carthy

12 **Corresponding author address:**

13 APHA Weybridge, New Haw, Addlestone, KT15 3NB, UK

14 +44 (0) 208 7203010

15 [Catherine.McCarthy@apha.gov.uk](mailto:Catherine.McCarthy@apha.gov.uk)

16

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

## 19 Abstract

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

49

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

## 52 1 Introduction

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

59

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

71

72 There is ongoing debate as to the contribution that the food chain plays with regards to the  
73 transmission and spread of AMR bacteria in humans. This is likely to vary widely by bacterial species,  
74 transmission routes, and other factors. For example, the majority of human extended-spectrum beta  
75 ( $\beta$ )-lactamase producing *E. coli* (hereafter, ESBL *E. coli*) carriage, is acquired from other humans  
76 (Mughini-Gras *et al.*, 2019). Recent studies indicate that the attribution from the open population  
77 (defined as clinically healthy individuals who had not travelled to high-risk regions nor engaged in  
78 farming activities) may be as high as 60.1% (Mughini-Gras *et al.*, 2019). However, it is accepted that  
79 humans can acquire zoonotic pathogens such as *Salmonella* and shiga toxin-producing *Escherichia*  
80 *coli* (STEC) from consumption of meat (EFSA, 2019) and the World Health Organisation argues that  
81 food is one of the possible transmission routes of AMR from animals to humans (World Health  
82 Organization, 2015). In the same recent modelling study, food consumption (meat, seafood and  
83 vegetables) accounted for 18.9% of human ESBL carriage cases (Mughini-Gras *et al.*, 2019). In the  
84 United Kingdom, 4.7% of pork products tested at retail were positive for ESBL/AmpC *E. coli*, whereas  
85 2.5% of *Campylobacter* spp. isolates from retail chicken meat displayed multi-drug resistance  
86 profiles (Willis *et al.*, 2018). It is therefore prudent to consider how to limit the acquisition of  
87 antimicrobial resistant bacteria via the food chain. It is recognised that the livestock industry may  
88 play a large role in dissemination of AMR through both direct occupational and non-occupational  
89 contact with farm animals (Mughini-Gras *et al.*, 2019), and indirect methods such as the  
90 dissemination of antibiotics into the environment (World Health Organization, 2015; Casey L. Cazer  
91 *et al.*, 2017; Filippitzi *et al.*, 2019). The need to control resistant bacteria in the livestock industry,  
92 both in order to reduce onwards transmission to humans, and to maintain treatment options for  
93 animals, has been well documented in the literature.

94

95 Many generic models have previously been developed to investigate the transmission of AMR at the  
96 bacterial level from donor bacteria to recipient bacteria. These models indicate that resistant  
97 bacterial populations can persist in the absence of antimicrobial pressure, through horizontal and  
98 vertical gene transfer (Freter *et al.*, 1983; Bergstrom *et al.*, 2000; Volkova *et al.*, 2012). However,  
99 models focusing purely on the biochemical, within-host aspects fail to incorporate the complicated  
100 host population dynamics which occur within a farm environment. For example, heterogeneous  
101 animal mixing and ingestion habits, the movement of animals within and between farms, cleaning  
102 and disinfection protocols, and treatment of animals for a multitude of farm pathogens, may all  
103 impact on the persistence of a population of endemic bacteria. Moreover, biochemical bacterial  
104 growth studies often use broths or growth mediums which replicate ideal growth conditions and can  
105 have quite different results to growth within faeces in a farm environment. A previous mathematical  
106 model comparing the impact of antimicrobial usage with the emergence of resistant bacteria in  
107 finisher pig farms found that both the transmission coefficient between pigs and the spontaneous  
108 clearance rate of drug-resistant bacteria influenced whether the resistant bacteria reached a steady  
109 endemic state on the farm or whether the bacteria could be cleared from the farm (Abatih *et al.*,  
110 2009). The combination of pharmacokinetic-pharmacodynamic (PKPD) models with on-farm models  
111 of bacterial transmission and realistic farm management practices, would offer a unique opportunity  
112 to understand the disease dynamics in a more realistic environment.

113

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

124 **2 Methods**

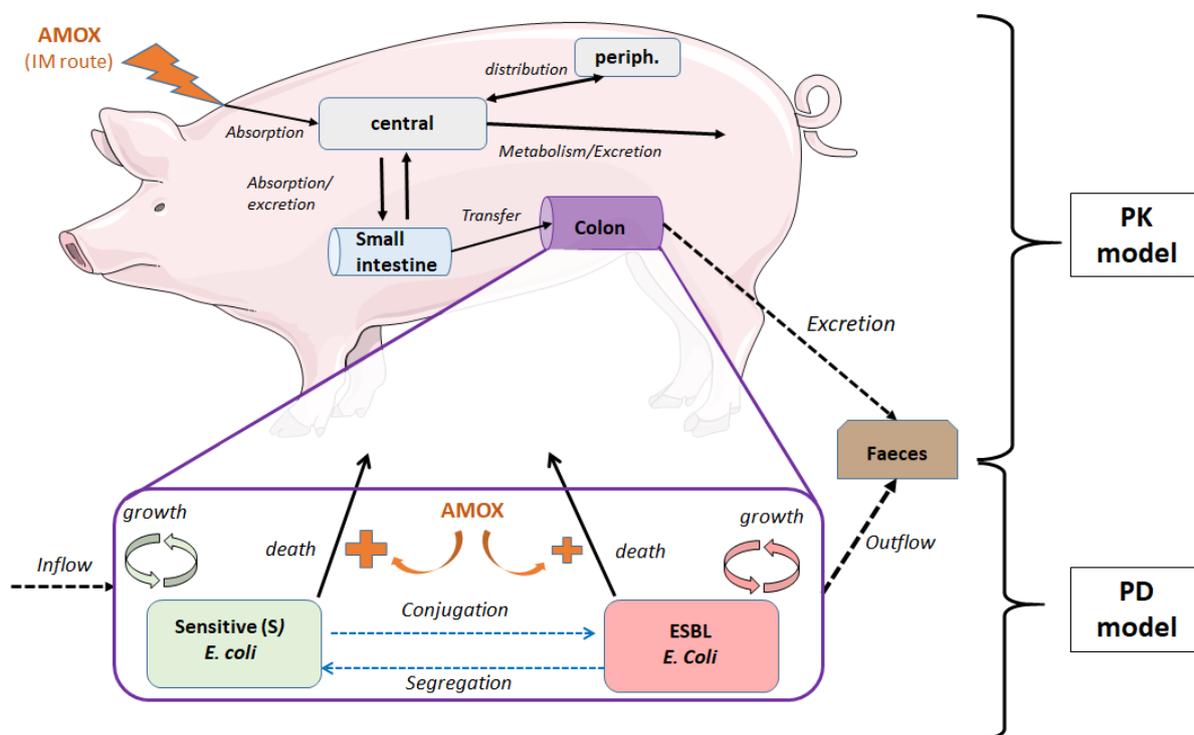
125 This risk assessment combined output from a PKPD model with a farm transmission model. The final  
126 output was the prevalence and concentration of resistant bacteria in the faeces of pigs sent to the  
127 abattoir. While the results are demonstrated using ESBL-producing commensal *E. coli* (hereafter,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

133

134 **2.1 PKPD model**

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

143



144

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

149

150 A hybrid PK model was developed with empirical compartments for the central system (central and  
151 peripheral compartments) based on previous population PK studies of amoxicillin in pigs (Rey *et al.*,  
152 2014; Agersø and Friis, 1998) and more mechanistic compartments for the GIT (with physiological  
153 volumes and transit rates) (see appendix A1.1 for equations and parameter distributions).

154

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

167

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

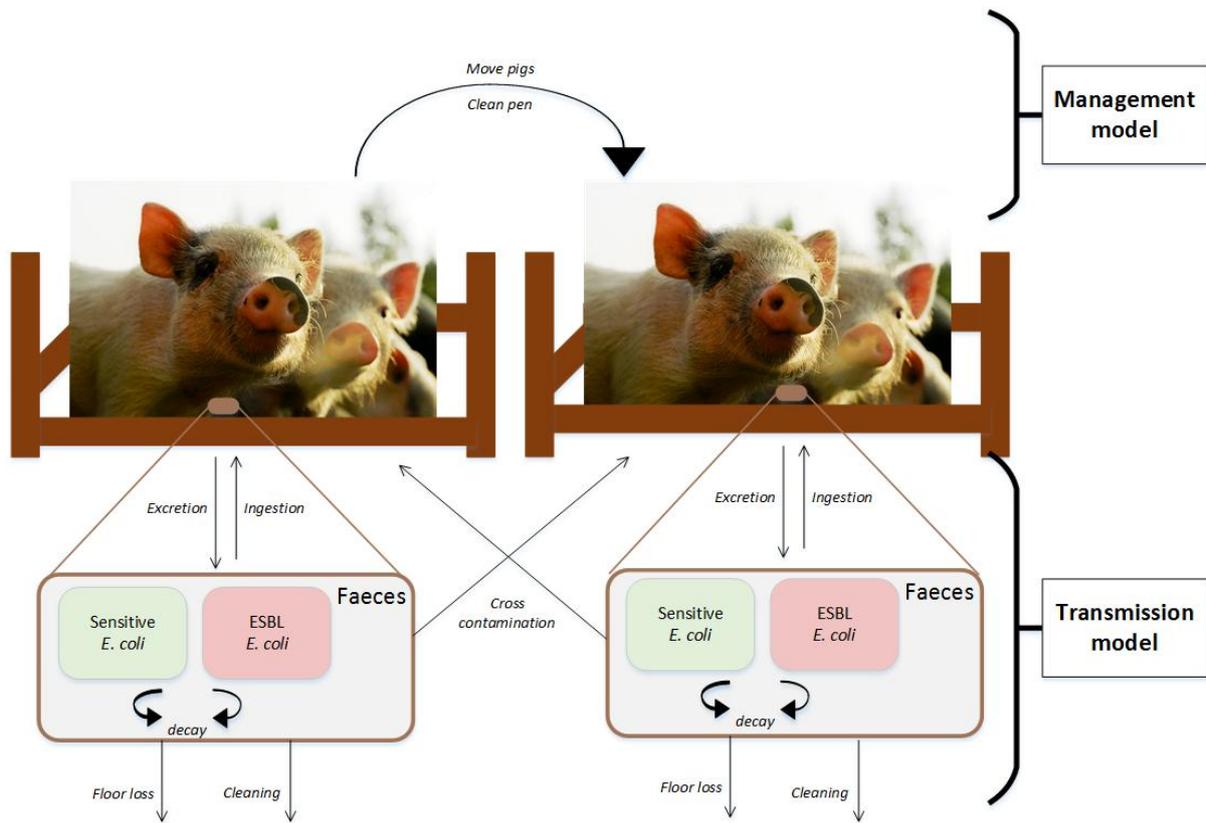
175

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

179

## 180 2.2 Farm transmission model

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



188

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

195

196 The farms were designed as breeder-finisher systems, where pigs stay on the same farm from birth  
 197 until they go to the abattoir. The model incorporated different practices of commercial pig farms.  
 198 Farms may run an all-in-all-out (AIAO) production system (where batches of pigs were kept together  
 199 in one room for each of the weaning, growing and finishing stages, without direct contact with other  
 200 batches all the way through rearing), or a continuous production system which allows mixing of  
 201 different batches of pigs at each stage of development. Farms could also use solid or slatted floor in  
 202 the pig pens. Different production systems were randomly selected for each iteration but the  
 203 probability of selection was weighted towards that of the UK commercial pig industry, as in Hill et al.  
 204 (2016). Therefore, the majority of farms had a continuous system on solid floor (43.2%), followed by  
 205 an all-in-all-out (AIAO) system on solid floor (39.2%), continuous system on slatted floor (10.9%) and  
 206 AIAO on slatted floor (6.6%). The baseline model sums the predicted number of positive pigs over all  
 207 batches/farms to estimate the prevalence of colonised pigs going to slaughter. Comparisons of the  
 208 relative risk in different farm types was also explored.

209

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

214

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

221

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

235

### 236 2.3 Integrating the PKPD output with the farm transmission model

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

247

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

255

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

272

#### 273 2.4 Scenarios for farm-based interventions

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

286

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

293

294

295

296

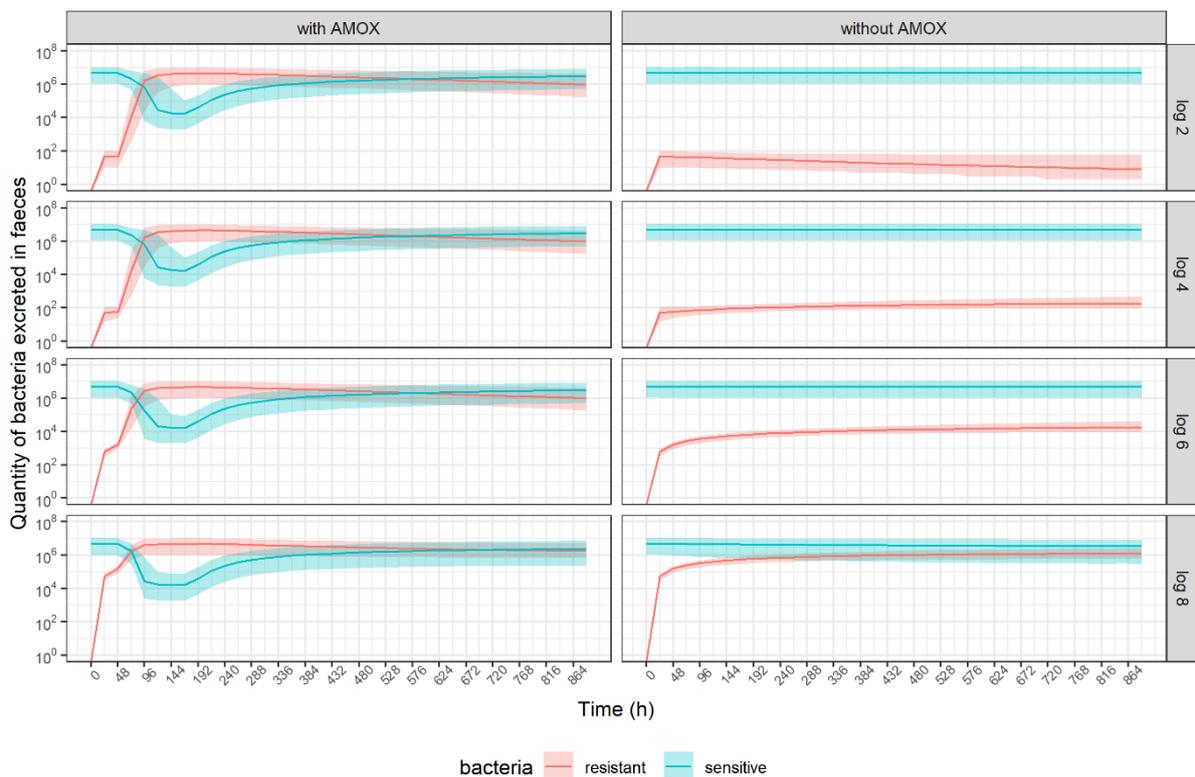
297

298

299

### 300 3 Results

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

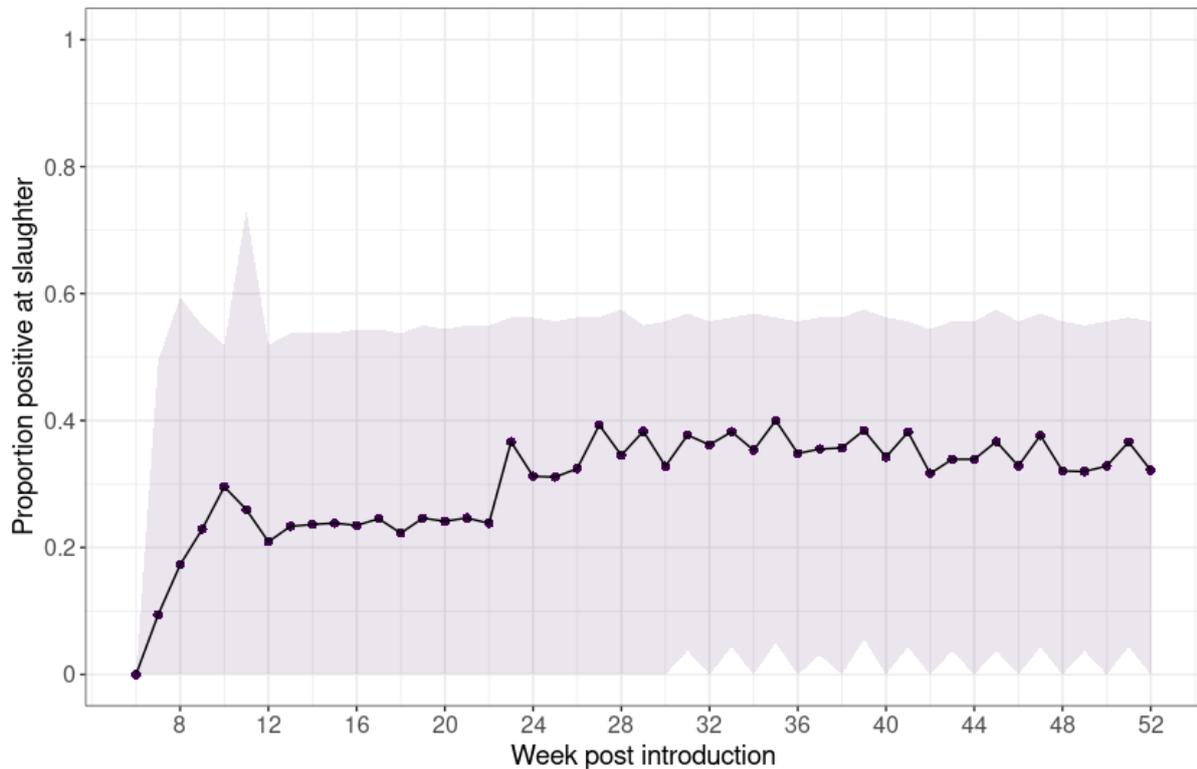


308

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

313

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

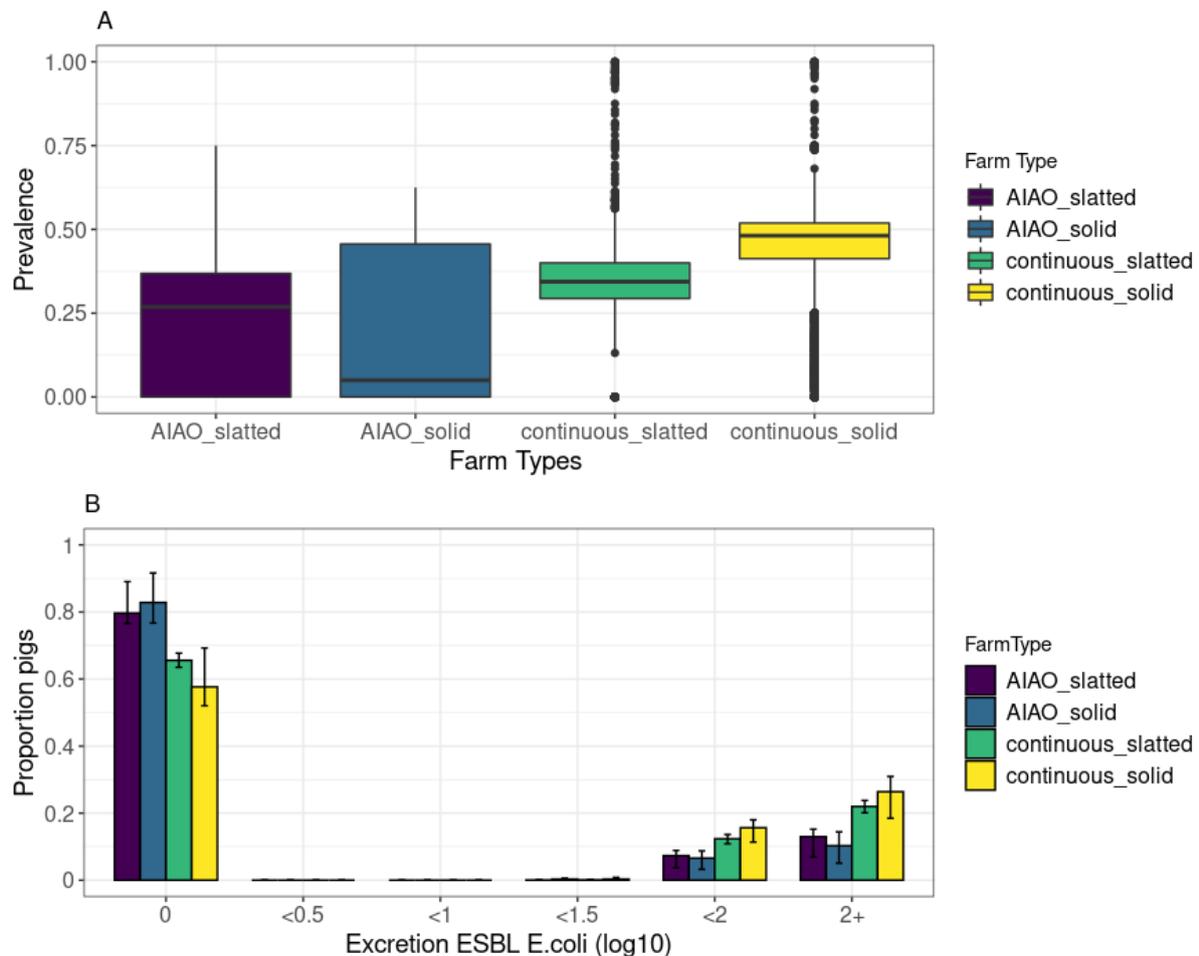


322

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

327

328 A comparison of different farm types suggested that AIAO housing was a protective measure for  
 329 both prevalence in slaughter-aged pigs and faecal shedding rates. The average batch prevalence in  
 330 pigs raised in AIAO systems on slatted floor was 26.9% (5<sup>th</sup> and 95<sup>th</sup> percentiles: 0.0-51.6), whereas  
 331 those on solid floor had a reduced average batch prevalence of 5.0% (5<sup>th</sup> and 95<sup>th</sup> percentiles: 0.0-  
 332 56.3) (Figure 5A). The prevalence was higher in continuous systems, where pigs reared on slatted  
 333 floor had an average prevalence of 34.4% (5<sup>th</sup> and 95<sup>th</sup> percentiles: 0.0-52.5) and those raised on  
 334 solid floor had a prevalence of 48.1% (5<sup>th</sup> and 95<sup>th</sup> percentiles: 0.0-59.4). Similarly, pigs raised in AIAO  
 335 systems had lower faecal shedding rates, with 13.0% pigs (5<sup>th</sup> and 95<sup>th</sup> percentiles: 6.9-15.2) on  
 336 slatted floor and 10.3% pigs (5<sup>th</sup> and 95<sup>th</sup> percentiles: 5.1-14.4) on solid floor shedding over 2 log<sub>10</sub>  
 337 cfu/g (Figure 5B). In comparison, 22.0% pigs (5<sup>th</sup> and 95<sup>th</sup> percentiles: 20.1-23.8) on slatted floor and  
 338 26.4% pigs (5<sup>th</sup> and 95<sup>th</sup> percentiles: 18.5-30.9) on solid floor, raised in a continuous system, shed  
 339 over 2 log<sub>10</sub> cfu/g.

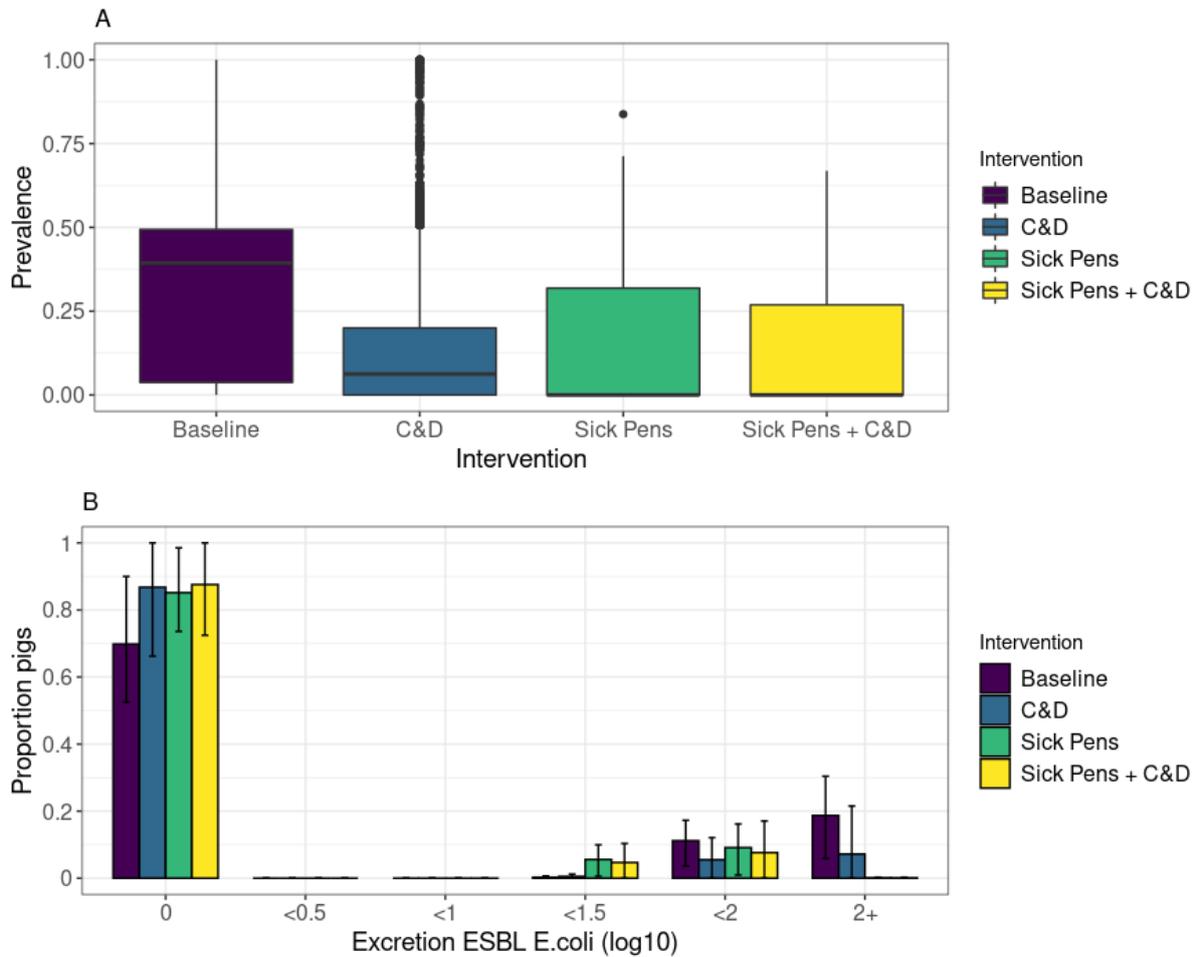


340

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

345

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



357

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

362

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

370

371

372

373

374

375

## 376 4 Discussion

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

390

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

402

403 An additional strength of the current risk assessment is in its ability to assess the comparative impact  
404 of different control measures. In many infectious disease processes, the minority of high-shedding  
405 animals are responsible for the majority of transmission, the so-called “super-shedding” animals  
406 (Chase-Topping *et al.*, 2008). The use of sick pens had the largest effect on this, reducing the  
407 percentage of pigs shedding over 2 log<sub>10</sub> cfu/g from 18.7% in the baseline scenario to 0.1%.  
408 Enhanced C&D also created a beneficial effect, although not to the same extent as the use of sick  
409 pens. The ability for farmers to apply strict biosecurity measures or isolation of sick pens is likely to  
410 vary between commercial establishments. The current risk assessment did not consider whether  
411 applying an enhanced C&D protocol would have an additional indirect benefit for AMR transmission,  
412 by helping to reduce levels of endemic diseases and therefore antimicrobial usage. Additionally, it  
413 did not consider whether heavy disinfectant use would offer a selective advantage to resistant  
414 bacteria by preferentially removing sensitive bacteria. This has previously been suggested for various  
415 antiseptics and heavy metals (Martin and Maris, 1995; Potenski *et al.*, 2003; Braoudaki and Hilton,  
416 2004; Soumet *et al.*, 2012) however, the exact role that the use of disinfectants has on driving AMR  
417 resistance is controversial (Cheng *et al.*, 2019). Further work is needed to investigate this factor  
418 before it can reliably be incorporated into QMRA models. The use of sick pens here was designed as  
419 an initial exploration into whether segregating sick pigs would reduce resistant bacteria transmission  
420 amongst pigs (with the hypothesis that pigs being treated with antibiotics would have the highest

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

428

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

443

444 Despite the importance of the  $\beta$ -lactamase enzyme on the resistance phenomena by protecting the  
445 resistant bacteria, the degradation of AMOX by these enzymes within intestines did not seem to be  
446 an influential factor concerning the excreted quantity of AMOX according to the sensitivity analysis.  
447 This is in agreement with older studies looking at the inactivation of amoxicillin by biological and  
448 non-biological processes in human faeces (de Vries-Hospers *et al.*, 1993; Jansen *et al.*, 1992).  
449 However, recent studies in mice have highlighted a positive influence of these enzymes on the  
450 protection against ampicillin for intestinal bacteria not expressing  $\beta$ -lactamase enzyme, suggesting  
451 the degradation of the  $\beta$ -lactam drug as the main factor explaining these findings (Gjonbalaj *et al.*,  
452 2020). Overall, the importance of the gut microbiota on the metabolism of antimicrobial drug is an  
453 ongoing subject (Zimmermann *et al.*, 2019) and new data may help to refine the PKPD model.

454

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

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

468

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

482

483 Several of the parameters in this risk assessment were extrapolated and therefore associated with  
484 high uncertainty. For instance, farm management parameters, such as the cleaning coefficients,  
485 were taken from Hill *et al.* (2015), and as such, we have assumed that *E. coli* within faeces reacts in a  
486 similar fashion to *Salmonella* within faeces. Concerning the PKPD model, the plasmid transfer rate  
487 was taken from *in vitro* studies with values differing by several orders of magnitude (Chauzy *et al.*,  
488 2019; Kristoffersson *et al.*, 2020), and the inflow and outflow rate of bacteria within the GIT were  
489 taken from previous models in cattle (Cazer *et al.*, 2017; Volkova, Cazer and Gröhn, 2017).  
490 Moreover, these parameters were among the most influential when looking at the results of the  
491 GSA. Therefore, there is reasonable uncertainty regarding the absolute values of the prevalence and  
492 load of resistant bacteria in slaughter-aged pigs. Interestingly, the number of colonised piglets on  
493 day 1 of the model did not produce a very high influence in the sensitivity analysis suggesting that it  
494 had a low effect on the overall prevalence. This is perhaps concerning, and suggests that even small  
495 numbers of colonised piglets are sufficient to cause transmission of the bacteria throughout the  
496 farm. Moreover, results were reasonably robust to the placement of the initial colonised piglets,  
497 with carriage becoming possible in slaughter-aged pigs if piglets were placed together in one pen or  
498 in several different pens.

499

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

508

509

## 510 5 Declaration of interest

511 Declaration of interest: None.

512

## 513 6 CRediT author statement

514 **Catherine M<sup>c</sup>Carthy:** Methodology, Software, Writing – Original Draft. **Alexis Viel:** Methodology,  
515 Software, Writing – Original Draft. **Chris Gavin:** Methodology, Software, Writing – Review & Editing.  
516 **Pascal Sanders:** Conceptualization, Methodology, Writing – Review & Editing, Project administration.  
517 **Robin R.L. Simons:** Conceptualization, Methodology, Writing – Review & Editing, Project  
518 administration

519

520

## 521 7 Acknowledgements

522 This manuscript is part of the European Joint Programme One Health EJP. This project has received  
523 funding from the European Union’s Horizon 2020 research and innovation programme under Grant  
524 Agreement No 773830. Matched funding was provided by the Veterinary Medicines Directorate  
525 (APHA).

526 The authors would also like to thank the following people for their valuable comments and  
527 assistance: Emma Snary, Richard Smith, Ginny Crouch and Rob Dewar at the APHA, UK and Jérôme  
528 Henri and Michel Laurentie at Anses, France.

529

## 530           References

- 531    Abatih, E. N. *et al.* (2009) 'Impact of antimicrobial usage on the transmission dynamics of  
532    antimicrobial resistant bacteria among pigs', *Journal of Theoretical Biology*, 256(4), pp. 561–573. doi:  
533    10.1016/j.jtbi.2008.10.017.
- 534    Avery, S. M., Moore, A. and Hutchison, M. L. (2004) 'Fate of Escherichia coli originating from  
535    livestock faeces deposited directly onto pasture', *Letters in Applied Microbiology*, 38(5), pp. 355–  
536    359. doi: 10.1111/j.1472-765X.2004.01501.x.
- 537    Bergstrom, C. T., Lipsitch, M. and Levin, B. R. (2000) 'Natural selection, infectious transfer and the  
538    existence conditions for bacterial plasmids', *Genetics*, 155(4), pp. 1505–1519.
- 539    Bos, M. E. H. *et al.* (2016) 'Transmission through air as a possible route of exposure for MRSA',  
540    *Journal of Exposure Science and Environmental Epidemiology*, 26(3), pp. 263–269. doi:  
541    10.1038/jes.2014.85.
- 542    Braoudaki, M. and Hilton, A. C. (2004) 'Adaptive resistance to biocides in Salmonella enterica and  
543    Escherichia coli O157 and cross-resistance to antimicrobial agents.', *Journal of clinical microbiology*,  
544    42(1), pp. 73–78. doi: 10.1128/jcm.42.1.73-78.2004.
- 545    Cazer, C. L. *et al.* (2017) 'Monte Carlo simulations suggest current chlortetracycline drug-residue  
546    based withdrawal periods would not control antimicrobial resistance dissemination from feedlot to  
547    slaughterhouse', *Frontiers in Microbiology*, 8(SEP). doi: 10.3389/fmicb.2017.01753.
- 548    Ceccarelli, D. *et al.* (2017) 'Competitive exclusion reduces transmission and excretion of extended-  
549    spectrum-beta-lactamase-producing Escherichia coli in broilers', *Applied and environmental*  
550    *microbiology*, 83(11), pp. 1–13. doi: <https://doi.org/10.1128/AEM.03439-16>.
- 551    Cheng, G. *et al.* (2019) 'Selection and dissemination of antimicrobial resistance in Agri-food  
552    production.', *Antimicrobial resistance and infection control*, 8, p. 158. doi: 10.1186/s13756-019-  
553    0623-2.
- 554    Dame-Korevaar, A. *et al.* (2017) 'Dynamics of CMY-2 producing E. coli in a broiler parent flock',  
555    *Veterinary Microbiology*. Elsevier, 203(September 2016), pp. 211–214. doi:  
556    10.1016/j.vetmic.2017.03.024.
- 557    Dohmen, W. *et al.* (2017) 'Risk factors for ESBL-producing Escherichia coli on pig farms: A  
558    longitudinal study in the context of reduced use of antimicrobials', *PLoS ONE*, 12(3). doi:  
559    10.1371/journal.pone.0174094.
- 560    EC (2015) *EUdraLex - Volume 5 - Pharmaceutical legislation for medicinal products for veterinary use*.  
561    Available at: [https://ec.europa.eu/health/documents/eudralex/vol-5\\_en](https://ec.europa.eu/health/documents/eudralex/vol-5_en).
- 562    EFSA (2011) 'Scientific Opinion on the public health risks of bacterial strains producing extended-  
563    spectrum  $\beta$ -lactamases and/or AmpC  $\beta$ -lactamases in food and food-producing animals', *EFSA*  
564    *Journal*, 9(8), p. 2322. doi: 10.2903/j.efsa.2011.2322.
- 565    EFSA (2019) *The European Union One Health 2018 Zoonoses Report*, *EFSA Journal*. doi:  
566    10.2903/j.efsa.2019.5926.
- 567    Evers, E. G. *et al.* (2017) 'Comparative exposure assessment of ESBL - producing Escherichia coli  
568    through meat consumption', *PLoS ONE*, 12(1). doi: 10.1371/journal.pone.0169589.
- 569    Filippitzi, M. E. *et al.* (2019) 'Quantitative risk model to estimate the level of antimicrobial residues  
570    that can be transferred to soil via manure, due to oral treatments of pigs', *Preventive Veterinary*  
571    *Medicine*, 167(November 2018), pp. 90–100. doi: 10.1016/j.prevetmed.2019.03.022.

- 572 Forum, W. E. (2013) *Global risks 2013: eighth edition*. doi: 10.1007/978-1-349-59857-1.
- 573 Freter, R., Freter, R. R. and Brickner, H. (1983) 'Experimental and mathematical models of  
574 *Escherichia coli* plasmid transfer in vitro and in vivo', *Infection and Immunity*, 39(1), pp. 60–84.
- 575 Frey, H. C., Mokhtari, A. and Zheng, J. (2004) *Recommended practice regarding selection, application  
576 and interpretation of sensitivity analysis methods applied to food safety process risk models*.  
577 Washington, DC.
- 578 FSA (2006) *Project M01028: Cleaning and Disinfection of Lairage-To- Stunning Areas in Abattoirs*.
- 579 Gray, J. T. and Fedorka-Cray, P. J. (2001) 'Survival and infectivity of *Salmonella Choleraesuis* in swine  
580 feces', *Journal of Food Protection*, 64(7), pp. 945–949. doi: 10.4315/0362-028X-64.7.945.
- 581 Hill, A. A. *et al.* (2016) 'A farm transmission model for *Salmonella* in pigs, applicable to E.U. member  
582 states', *Risk Analysis*, 36(3).
- 583 Kliebe, C. *et al.* (1985) 'Evolution of plasmid-coded resistance to broad-spectrum cephalosporins',  
584 *Antimicrobial Agents and Chemotherapy*, 28(2), pp. 302–307. doi: 10.1128/AAC.28.2.302.
- 585 Knothe, H. *et al.* (1983) 'Transferable resistance to cefotaxime, cefoxitin, cefamandole and  
586 cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*', *Infection*, 11, pp.  
587 315–317. doi: 10.1007/BF01641355.
- 588 Köck, R. *et al.* (2018) 'Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and  
589 companion animals: a systematic review', *Clinical Microbiology and Infection*, 24(12), pp. 1241–  
590 1250. doi: 10.1016/j.cmi.2018.04.004.
- 591 Martin, H. and Maris, P. (1995) '[Antiseptic and antibiotic resistance of 310 gram-positive strains  
592 isolated from udders after use of post-milking teat germicides].', *Veterinary research*. England, 26(1),  
593 pp. 43–56.
- 594 Merck (2016) 'Streptococcus suis Infection', in *MSD Veterinary Manual*. Whitehouse Station, NJ:  
595 MSD. Available at: [https://www.msdsvetmanual.com/generalized-conditions/streptococcal-  
596 infections-in-pigs/streptococcus-suis-infection#v3275292](https://www.msdsvetmanual.com/generalized-conditions/streptococcal-infections-in-pigs/streptococcus-suis-infection#v3275292).
- 597 Mughini-Gras, L. *et al.* (2019) 'Attributable sources of community-acquired carriage of *Escherichia*  
598 *coli* containing  $\beta$ -lactam antibiotic resistance genes: a population-based modelling study', *The Lancet*  
599 *Planetary Health*, 3(8), pp. e357–e369. doi: 10.1016/S2542-5196(19)30130-5.
- 600 Munk, P. *et al.* (2018) 'Abundance and diversity of the faecal resistome in slaughter pigs and broilers  
601 in nine European countries', *Nature Microbiology*. doi: 10.1038/s41564-018-0192-9.
- 602 NethMap (2019) *NethMap 2019: Consumption of antimicrobial agents and antimicrobial resistance  
603 among medically important bacteria in the Netherlands*.
- 604 Pitout, J. D. D. and Laupland, K. B. (2008) 'Enterobacteriaceae : an emerging public-health concern',  
605 *The Lancet Infectious Diseases*, 8(March).
- 606 Potenski, C. J., Gandhi, M. and Matthews, K. R. (2003) 'Exposure of *Salmonella* Enteritidis to chlorine  
607 or food preservatives decreases [corrected] susceptibility to antibiotics.', *FEMS microbiology letters*.  
608 England, 220(2), pp. 181–186. doi: 10.1016/S0378-1097(03)00099-5.
- 609 Soumet, C. *et al.* (2012) 'Resistance to phenicol compounds following adaptation to quaternary  
610 ammonium compounds in *Escherichia coli*.', *Veterinary microbiology*. Netherlands, 158(1–2), pp.  
611 147–152. doi: 10.1016/j.vetmic.2012.01.030.
- 612 Tassinari, E. *et al.* (2019) 'Microevolution of antimicrobial resistance and biofilm formation of

- 613 Salmonella Typhimurium during persistence on pig farms', *Scientific Reports*, 9(1), pp. 1–12. doi:  
614 10.1038/s41598-019-45216-w.
- 615 VMD (2018) *UK Veterinary Antibiotic Resistance and Sales Surveillance (VARSS) 2017*. Available at:  
616 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/915743/_1691664-v1-VARSS_2017_Watermark_FINALx-accessible.pdf)  
617 [/915743/\\_1691664-v1-VARSS\\_2017\\_Watermark\\_FINALx-accessible.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/915743/_1691664-v1-VARSS_2017_Watermark_FINALx-accessible.pdf).
- 618 Volkova, V. V. *et al.* (2012) 'Mathematical model of plasmid-mediated resistance to ceftiofur in  
619 commensal enteric escherichia coli of cattle', *PLoS ONE*, 7(5). doi: 10.1371/journal.pone.0036738.
- 620 Willis, C. *et al.* (2018) *Surveillance Study of Antimicrobial Resistance in Bacteria Isolated from Chicken*  
621 *and Pork Sampled on Retail Sale in the UK*. Available at:  
622 [https://www.foodstandards.gov.scot/downloads/AMR\\_in\\_chicken\\_and\\_pork\\_Final\\_Report\\_July\\_20](https://www.foodstandards.gov.scot/downloads/AMR_in_chicken_and_pork_Final_Report_July_2018_Final.pdf)  
623 [18\\_Final.pdf](https://www.foodstandards.gov.scot/downloads/AMR_in_chicken_and_pork_Final_Report_July_2018_Final.pdf).
- 624 World Health Organization (2015) *Global Action Plan on Antimicrobial Resistance*. doi:  
625 10.1128/microbe.10.354.1.