bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 Comparing the transmission of carbapenemase-producing and extended-spectrum
2 beta-lactamase-producing *Escherichia coli* between broiler chickens.
3

4 Authors: Natcha Dankittipong[a,‡], Jesse B. Alderliesten[a,‡,1], Jan Van den Broek[a], M.
5 Anita Dame-Korevaar[b], Michael S. M. Brouwer[b], Francisca C. Velkers[a], Alex
6 Bossers[a,b], Clazien J. de Vos[b], Jaap A. Wagenaar[a,b], J. Arjan Stegeman[a], Egil A. J.
7 Fischer[a,$].
8
9 [a] Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, Utrecht, The
10 Netherlands
11 [b] Wageningen Bioveterinary Research, Wageningen University & Research,
12 Houtribweg 39, Lelystad, The Netherlands
13 [1] Current address: Biosystems Data Analysis, Swammerdam Institute for Life
14 Sciences, University of Amsterdam, Sciencepark 904, Amsterdam, The Netherlands
15 [‡] These authors contributed equally
16 [$] Corresponding author: e.a.j.fischer@uu.nl | https://orcid.org/0000-0002-0599-701X
17
18 ORCID IDs
19 Jesse B. Alderliesten: https://orcid.org/0000-0003-1132-4310
20 Michael S. M. Brouwer: https://orcid.org/0000-0002-1067-7555
21 Francisca C. Velkers: https://orcid.org/0000-0002-2923-3233
22 Alex Bossers: https://orcid.org/0000-0002-6586-717X
23 Clazien J. de Vos: https://orcid.org/0000-0002-9280-7041
24 Egil A. J. Fischer: https://orcid.org/0000-0002-0599-701X
25

26 Abstract

27 The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is a threat to public health,
28 because of their resistance to clinically important carbapenem antibiotics. The emergence of CPE in
29 meat-producing animals is particularly worrying because consumption of meat contaminated with
30 resistant bacteria similar to CPE, such as extended-spectrum beta-lactamase (ESBL)-producing
31 Enterobacteriaceae, contributed to colonization in humans worldwide. Currently, no data on the
32 transmission of CPE in livestock is available. We performed a transmission experiment to quantify the
33 transmission of CPE between broilers to fill this knowledge gap and to compare the transmission rates
34 of CPE and other antibiotic-resistant *E. coli*.
35

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

A total of 180 Ross 308 broiler chickens were distributed on the day of hatch (day 0) over 12 pens. On day 5, half of the chickens in each pen were orally inoculated with $5 \cdot 10^2$ colony-forming units of CPE, ESBL, or chloramphenicol-resistant *E. coli* (catA1). Amoxicillin drinking water treatment was given twice daily in 6 of the 12 pens from days 2 to 6 to evaluate the effect of antibiotic treatment on the transmission rates. Cloacal swabs of all animals were taken to determine the number of infectious broilers. We used a Bayesian hierarchical model to quantify the transmission of the *E. coli* strains. *E. coli* can survive in the environment and serve as a reservoir. Therefore, the susceptible-infectious transmission model was adapted to account for the transmission of resistant bacteria from the environment. In addition, the caecal microbiome was analyzed on day 5 and at the end of the experiment on day 14 to assess the relationship between the caecal microbiome and the transmission rates.

The transmission rates of CPE were 52 – 68 per cent lower compared to ESBL and catA1, but it is not clear if these differences were caused by differences between the resistance genes or between the *E. coli* strains. Differences between the groups in transmission rates and microbiome diversity did not correspond to each other, indicating that differences in transmission rates were probably not caused by major differences in the community structure in the caecal microbiome. Amoxicillin treatment from day 2 to 6 increased the transmission rate more than three-fold in all inoculums. It also increased alpha-diversity compared to untreated animals on day 5, but not on day 14, suggesting only a temporary effect.

Future research could incorporate more complex transmission models with different species of resistant bacteria into the Bayesian hierarchical model.

Keywords

antibiotic treatment
carbapenem-resistant *Enterobacteriaceae*
extended-spectrum beta-lactamase
indirect transmission
Bayesian inference
microbiome analysis

Abbreviations

ASV: amplicon sequence variant
BSL: Biosafety level
CPE: carbapenemase-producing *Enterobacteriaceae*
E. coli: *Escherichia coli*
ESBL: extended-spectrum beta-lactamase
HPDI: highest posterior density interval
MAP: maximum a posteriori estimate
PCoA: Principal coordinate analysis
SI-model: susceptible-infectious model

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

76 WBVR: Wageningen Bioveterinary Research
77
78 # 1. Introduction

79 Carbapenemase-producing *Enterobacteriaceae* (CPE; also referred to as carbapenem-resistant
80 *Enterobacteriaceae*) are potentially life-threatening bacteria because of their resistance to clinically
81 important carbapenem antibiotics (Brink, 2019; World Health Organization, 2019; Zhou et al., 2021).
82 CPE are detected worldwide in farm animals, wild animals, companion animals, fish, and the
83 environment (Köck et al., 2018; Bonardi and Pitino, 2019). The emergence of CPE in meat-producing
84 animals is particularly worrying because consumption of meat contaminated with resistant bacteria
85 similar to CPE, such as extended-spectrum beta-lactamase (ESBL)-producing bacteria or plasmid-
86 encoded AmpC (pAmpC)-producing bacteria, contributes to colonization in humans worldwide
87 (Leverstein-van Hall et al., 2011; Rousham et al., 2018; Mughini-Gras et al., 2019). Consequently, it is
88 crucial to assess the transmission dynamics of CPE in livestock farms. We looked at transmission
89 between broilers because the prevalence of ESBL-producing bacteria in broilers is high compared to
90 other livestock (European Food Safety Authority and European Centre for Disease Prevention Control,
91 2022).
92
93 The transmission rate parameter β is a key parameter to describe the transmission dynamics in
94 populations and is here defined as the rate of successful transmission per time unit following contact
95 with an infectious source such as bacteria carrying resistance genes (Keeling and Rohani, 2007).
96 Transmission of ESBL-producing *Escherichia coli (E. coli)* in poultry has been investigated extensively
97 (Huijbers et al., 2016; Dame-Korevaar et al., 2019; Robé et al., 2019; Dame-Korevaar et al., 2020a;
98 Dame-Korevaar et al., 2020b), showing among others that 2 strains of beta-lactamase-producing
99 bacteria (carrying $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CMY\text{-}2}$, respectively) colonized broilers at the same rate (Dame-
100 Korevaar et al., 2019). In contrast, no data on the transmission of CPE in livestock is available. Although
101 poultry is at risk of CPE introduction (Dankittipong et al., 2022), the prevalence of CPE in animals is
102 much lower than the prevalence of ESBL/pAmpC-producing bacteria (European Centre for Disease
103 Prevention and Control, 2018). The difference in the prevalence of CPE and ESBL-producing bacteria
104 could be explained by differences in the transmission dynamics of the resistance genes and the
105 plasmids that carry them (Rozwandowicz et al., 2018; Wilson and Török, 2018). Differences in selective
106 pressure caused by historical use in livestock of third-generation cephalosporins that co-select for
107 carbapenemase-producing genes (Ogunrinu et al., 2020) compared to the use of carbapenems having
108 worldwide never been allowed in livestock (Madec and Haenni, 2018) might also contribute to the
109 difference in prevalence.
110
111 Conventional methods to quantify the transmission of bacteria assume direct transmission between
112 animals (Velthuis et al., 2007). However, *E. coli* can survive for a considerable amount of time in the
113 environment (Table S14) and is commonly transmitted between animals through the faecal-oral route
114 (Lister and Barrow, 2008; van Elsas et al., 2011; van Bunnik et al., 2014). Previous transmission
115 experiments of ESBL-producing bacteria in broilers, wildtype nalidixic-resistant *E. coli* in broilers, and

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

116 *Salmonella Dublin* in young dairy calves highlighted the excretion of these bacteria into the environment
117 and subsequent acquisition of excreted bacteria from the environment as a key mechanism of
118 transmission (Nielsen et al., 2007; van Bunnik et al., 2014; Dame-Korevaar et al., 2017).
119
120 Antibiotic usage is a primary driver of resistant bacteria in clinical and non-clinical settings (Knobler et
121 al., 2003; Davies and Davies, 2010; Holmes et al., 2016) and is widespread in livestock worldwide
122 (Mathew et al., 2007; Aarestrup, 2015). Twenty-two per cent of the conventional broiler farms in the
123 Netherlands did not use antibiotics in 2020, but 44% had a persistently high antibiotic usage exceeding
124 the action threshold defined by the Netherlands Veterinary Medicines Institute and 5% had a persistently
125 high antibiotic usage exceeding the sector-negotiated action threshold (Bonten et al., 2021). Treatment
126 with antibiotics generally temporarily decreases the number of bacterial species in the gut microbiome
127 and lowers the abundance of some common taxa, allowing the abundance of some low-abundant taxa
128 or opportunistic pathogens to increase (Kim et al., 2017; Rochegüe et al., 2021). This might affect the
129 transmission of bacteria, because a more diverse gut microbiome hinders colonization by exogenous
130 bacteria (Kim et al., 2017; Sorbara and Pamer, 2019), thereby reducing the excretion of these bacteria
131 (Dame-Korevaar et al., 2020b).
132
133 We performed a transmission experiment to quantify the transmission of CPE between broilers and to
134 quantitatively compare the transmission rates of CPE and ESBL-producing *E. coli* to determine if the
135 difference in the prevalence of CPE and ESBL in broilers might have been caused by differences in the
136 transmission dynamics of the resistance genes and the plasmids that carry them. Groups with and
137 without amoxicillin treatment were compared to investigate if and how antibiotic treatment affects the
138 transmission, and relations between differences in transmission rates and the caecal microbiome were
139 assessed.
140

141 2. Material and Method

142 **2.1. Transmission experiment**

143 The study protocol was approved by the Dutch Central Authority for Scientific Procedures on Animals
144 and the Animal Experiments Committee of Utrecht University (Utrecht, the Netherlands) under
145 registration number AVD108002015314 and all procedures were performed in full compliance with all
146 legislation. All broilers were observed daily, and any abnormality and mortality were recorded.
147

148 2.1.1. Inoculums

149 Three inoculums were prepared for this experiment, referred to as the CPE-strain, ESBL-strain, and
150 catA1-strain throughout the paper (Table 1). All strains were *E. coli* obtained from broilers in
151 conventional farms in Europe. Before inoculation, all strains were streaked on heart infusion agar with
152 5% sheep blood (Becton Dickinson GmbH, Heidelberg, Germany), transferred to LB medium, and
153 cultured overnight. The *E. coli* cultures were diluted in phosphate-buffered saline with 0.5 McFarland
154 standards resulting in $1.10 \cdot 10^8$ bacteria suspension per mL. Prepared inoculums were enumerated in
155 duplicate counts and each contained $0.55 \cdot 10^3 – 1.0 \cdot 10^3$ colony-forming units per mL.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

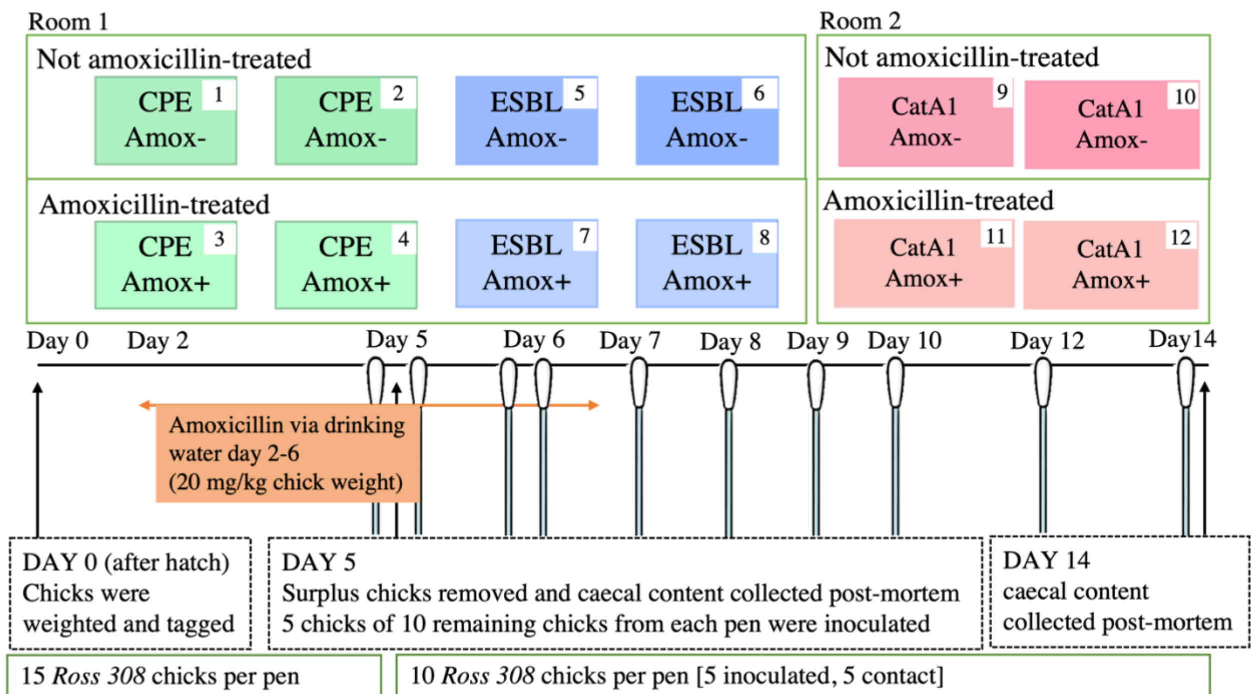
156 *Table 1: Characteristics of the CPE, ESBL, and catA1 isolates used as inoculums. Abbreviations: Inc-group:*
157 *incompatibility group; MLST: multi-locus sequence type.*

Inoculum	*E. coli* isolate	MLST	Selected resistance	Gene	Plasmid Inc-group	Host's country of origin	Reference
CPE-strain	CFSAN083827	4980	Carbapenem	OXA-162	HI2	Romania	(Bortolaia et al., 2021)
ESBL-strain	SafeFoodEra-230	101	Extended-spectrum beta-lactam	CTX-M2	HI2	Germany	(Wu et al., 2013)
catA1-strain	EFFORT 102803008	10	Chloramphenicol	catA1	FIB/FII	The Netherlands	(Leekitcharoenphon et al., 2021)

158
159 2.1.2. Sampling scheme and experimental design
160 The experiment was conducted in human Biosafety level 3 (BSL-3) facilities at Wageningen
161 Bioveterinary Research (WBVR), Lelystad. Before the experiment, samples from the parent stock and
162 environmental samples from the incubator (BSL-1) and experimental facilities were taken which
163 confirmed the absence of ESBL-producing *E. coli*. Two hundred and forty eggs were collected from a
164 conventional Ross 308 broiler parent stock, individually disinfected with 3% hydrogen peroxide and
165 incubated for 21 days at BSL-1 experimental facilities of WBVR. On the day of hatch, day 0, 180
166 hatchlings were transported to the BSL-3 animal facilities of WBVR, where they were weighted, neck
167 tagged with an individual number and randomly distributed over 12 pens, with 15 unsexed broilers per
168 pen (see Table S3 for an overview of the distribution of the sexes in the different groups). Broilers of
169 both sexes were used because a mixed group reflects the practical situation in terms of group dynamics
170 and the prevalence of ESBL or CPE is not known to differ by gender. Pens had a surface area of 1.35
171 m^2, with a bedding of sterilized wood shavings, and were separated from each other by fences of 70 –
172 80 cm high such that no direct contact was possible between pens. Broilers had *ad libitum* access to
173 feed and water and a standard lighting and temperature scheme for broiler chickens was used. The feed
174 should have been a standard broiler diet without antibiotics or coccidiostats, but accidentally feed for
175 layer pullets, free of antibiotics and coccidiostats, was provided. The feed was based on wheat, maize,
176 and soybean meal and contained 2,563 kcal of apparent metabolizable energy per kg and 20% of crude
177 protein heated to 90 °C. From days 2 to 6, amoxicillin was provided via drinking water twice a day at the
178 suppliers' recommended dose of 20 mg/kg live weight to the broilers in pens 3, 4, 7, 8, 11, and 12 (Figure
179 1). Amoxicillin was used as an example of a broad-spectrum antibiotic commonly used in broilers
180 (Ventola, 2015) to compare the transmission of all inoculums in the absence and presence of antibiotic
181 treatment.
182
183 On day 5, cloacal swabs were taken from all broilers using sterile dry Eswabs (MW100, Medical Wire &
184 Equipment, England) to confirm the absence of CPE and ESBL-producing *E. coli*. 10 broilers per pen
185 were kept for the transmission experiment and surplus broilers (at most 5 per pen) were euthanized and
186 their caecal content was collected for microbiome analysis. Five broilers randomly chosen out of the 10
187 remaining broilers per pen were separated from the other broilers and orally inoculated (using a syringe

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

188 with a crop needle) with 0.5 mL PBS which per mL containing approximately 10^3 colony-forming units of
189 *E. coli*, i.e., the CPE-strain (pens 1 – 4), the ESBL-strain (pens 5 – 8), or the catA1-strain (pens 9 – 12).
190 One hour after inoculation, inoculated broilers were returned to their pen where they resided with contact
191 broilers (i.e., broilers that were not inoculated). Cloacal swabs were taken from all broilers at
192 approximately 8 hours after inoculation on day 5, twice on day 6 (8 hours apart), and once per day on
193 days 7 to 10, 12, and 14 (Figure 1) (Dame-Korevaar et al., 2020a). All broilers were euthanized on day
194 14 and their caecal content was collected for microbiome analysis.



195
196 *Figure 1: Setup of the pens (top) and timeline of the experimental design from the moment of hatch to*
197 *the end of the experiment on day 14, with the sampling time points indicated by the swabs (bottom).*
198 *Abbreviations: Amox-: non-amoxicillin-treated; Amox+: amoxicillin-treated.*
199
200 2.1.3. Resistance gene detection
201 All cloacal swabs were enriched overnight in 3 mL buffered peptone water at 37 °C. Thereafter they
202 were inoculated onto selective MacConkey plates supplemented with 0.5 mg/L ertapenem (swabs from
203 pens 1 – 4), 1 mg/L cefotaxime (swabs from pens 5 – 8), or 64 mg/L chloramphenicol (swabs from pens
204 9 – 12) using a sterile loop and incubated overnight at 37 °C. A broiler was defined as positive when
205 colonies were detected on MacConkey plates after overnight incubation. The pen, used inoculum,
206 antibiotic treatment, and the test results of the cloacal swabs (i.e., positive or negative for CPE-strain,
207 ESBL-strain, or catA1-strain) at each sampling time point were recorded for all inoculated and contact
208 broilers (Table S1).
209
210 2.1.4. Microbiome sequencing
211 Microbial DNA was isolated from 0.2 g caecal content according to the manufacturer's instructions using
212 the PureLink microbial DNA isolation kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).
213 Negative controls spiked with a low concentration of microbial community DNA standard

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

214 (ZymoBIOMICS; Zymo Research Corporation, Irvine, CA) were used in the batches of DNA isolation
215 and amplification thereafter as control of performance and sanity throughout the processing (see Figure
216 S1 for a comparison of the theoretical and obtained composition of the negative controls). Following
217 extraction, the DNA extracts were quantified with an InvitrogenTM QubitTM 3.0 Fluorometer and stored
218 at -20 °C for further processing. The hypervariable regions V3+V4 of the 16S rRNA gene were amplified
219 in triplicate using a limited-cycles PCR with the primers CVI_V3-forw CCTACGGGAGGCAGCAG and
220 CVI_V4-rev GGACTACHVGGGTWTCT. The following amplification conditions were used as previously
221 described (Jurburg et al., 2019): 98 °C for 2 minutes, followed by 20 cycles of 98 °C for 10 s, 55 °C for
222 30 s, and 72 °C for 10 s, and finally by 72 °C for 7 minutes. Triplicate PCR products were pooled per
223 sample and checked on a TapeStation (Agilent, USA) and after barcode indexing subsequently
224 sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA) using a version 3 paired-end 300 bp
225 kit.
226

227 **2.2. Data analysis**

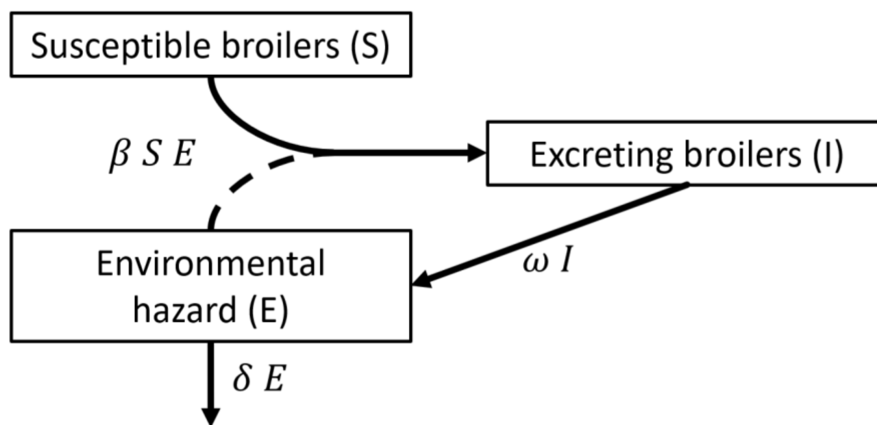
228 2.2.1. SI-model

229 The transmission of *E. coli* between broilers was modelled using a compartmental susceptible-infectious
230 model (SI-model; Figure 2). Previous research identified excretion and subsequent acquisition of *E. coli*
231 from the environment as a key mechanism of transmission (Lister and Barrow, 2008; van Bunnik et al.,
232 2014). We incorporated this in our SI-model by assuming excreting broilers (I) excrete viable bacteria
233 into the environment of their pen at a constant rate of ω units per hour from the moment they start to
234 excrete, and these excreted bacteria will decay at a rate δ. The unknown excretion rate (ω) was scaled
235 such that the hazard produced by 1 broiler during 1 time unit is 1 (Gerhards et al., 2022). The
236 environmental hazard at time t is denoted as E_t. A detailed description of the model including the scaling
237 is given in Section 3.1 of the supplementary information.

238 When negative contact broilers were colonized through contact with bacteria in the environment at rate
239 $\beta\, S_t\, E_t$, they were denoted as cases and incorporated in the SI-model as excreting for the next time
240 interval. Negative inoculated broilers were assumed to start excreting through inoculation instead of
241 through contact with bacteria in the environment and were therefore not denoted as cases.

242 In the SI-model it is assumed that contact broilers are either susceptible (S) or excreting (I). Once broilers
243 start excreting, it is assumed they will continue to excrete until the end of the experiment. To adhere to
244 this structure, a negative test result in a broiler that previously tested positive was assumed to be false
245 negative (see section 1.2 with Table S2 in the supplementary material). In pens 3, 4, 11 and 12, the first
246 positive tests for inoculated and contact broilers occurred at the same time point. However, at least one
247 inoculated broiler must start excreting before colonization of contact broilers can occur. Therefore, we
248 assumed inoculated broilers started excreting halfway between the first time point they tested positive
249 and the previous sampling time point, and contact broilers were assumed to start excreting slightly
250 slower, from the time point they tested positive.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



251

252 *Figure 2: compartmental SI-model of indirect transmission of E. coli between broilers. Excreting broilers*
253 *(I; positive inoculated broilers and positive contact broilers) excrete bacteria into the environment at rate*
254 *ω. Only negative contact broilers are counted as susceptible broilers (S) because negative inoculated*
255 *broilers are assumed to start excreting through inoculation instead of through colonization after contact*
256 *with the environmental hazard (E). Environmental hazard decays at rate δ (h^{-1}). Susceptible contact*
257 *broilers become colonized through contact with bacteria in the environment at transmission rate*
258 *parameter β (h^{-1}), thus becoming excreting broilers. The dashed line connecting environmental hazard*
259 *and excreting broilers indicates bacteria in the environment facilitate colonization but are not converted*
260 *to excreting broilers.*

261 2.2.2. Bayesian hierarchical inference

262 A Bayesian hierarchical model was used to infer the parameters of the SI-model (see section 3.2 in the
263 supplementary material), which requires prior probability distributions for the parameters, observed data
264 (i.e., the number of positive and negative broilers at each sampling time point in each pen), and a
265 likelihood function. The transmission rate parameter (β), which indicates the infectivity and susceptibility
266 of animals, was estimated for each pen separately from the number of susceptible broilers and the force
267 of infection by estimating the average transmission rate parameter over all pens ($\bar{a}$) and the between-
268 pen variation of the transmission rate parameter (z_i). Consequently, transmission in pen i occurs at rate
269 parameter β_i that is the product of the individual transmission rate parameter in that pen ($\bar{a} + z_i$) and the
270 environmental hazard in that pen (E_t). Posterior distributions of the transmission rate parameter for the
271 different clusters (i.e., inoculum and antibiotic treatment) were obtained by combining the posterior
272 distributions of $\bar{a} + z_i$ of all pens in that specific cluster.
273

274 We used results from a previous transmission study in broilers (Dame-Korevaar et al., 2020a) to define
275 prior probability distributions (priors) for the average transmission rate parameter ($\bar{a}$) and its standard
276 deviation (σ). In contrast to (Gerhards et al., 2022) we fixed the decay rate to 0.04 h^{-1} because the
277 broilers remain excreting until the end of the experiment (Table S1) such that no information on the
278 decay of bacteria in the environment was available.
279

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

280 Using the prior probabilities of the parameters and the likelihood function, parameter values were drawn
281 using the Markov chain Monte Carlo simulated process. Four independent Markov chains (Figure S1)
282 were initiated in the model. The transmission rate of each inoculum was extracted from the posterior
283 distribution and transmission rates were compared using the 95% highest posterior density interval
284 (HPDI) and the point estimate at the highest density (maximum a posteriori estimate, MAP). Differences
285 in transmission rates between inoculums and antibiotic treatments were compared by calculating the
286 posterior distribution of the ratio of the transmission rates.
287
288 2.2.3. Microbiome analysis
289 The amplicon sequences were demultiplexed using *bcl2fastq* (Illumina Inc., San Diego, CA) and
290 subsequently filtered, trimmed, error-corrected, dereplicated, chimaera-checked, and merged using R
291 package dada2 1.16.0 (Callahan et al., 2016) with the standard parameters except for TruncLength =
292 (270, 220), trimLeft = (25, 33), maxEE = 2 and minOverlap = 10, using a pseudo-pooling strategy. Reads
293 were classified against the SILVA database version 138 (Quast et al., 2012). The data, the phyloseq
294 object containing the sequence data, and the R code used for the modelling and analyses are provided
295 at https://zenodo.org/ (DOI:xxxxxxxxx).
296
297 The number of reads in the samples (excluding negative controls) ranged from 1363 to 320392 and was
298 standardized to 9071 reads per sample (7th least number of reads; rarefy_even_depth, seed = 314;
299 Figure S2) before alpha-diversity analysis. The final dataset contained 9540981 reads and 7952 different
300 amplicon sequence variants (ASVs). Sequences are deposited in NCBI's Sequence Read Archive under
301 BioProject accession number PRJNAXXXXX.
302
303 DNA sequences isolated from caecal material obtained on days 5 and 14 were analysed separately.
304 Non-bacterial sequences were discarded. Rarefaction curves on genus- and ASV-level were created to
305 check if all genera and ASVs in the samples were recovered (Figure S3). Observed richness, Shannon's
306 index and Pielou's evenness were used to measure alpha-diversity (Finotello et al., 2018). Kruskal-
307 Wallis rank sum test and post hoc Dunn's test with Benjamini-Hochberg correction were used to test for
308 the effects of the inoculums, antibiotic treatment, and their interaction, using a significance level of 0.05.
309 Beta-diversity, a measure of dissimilarity between communities regarding shared taxa, was analysed
310 on non-rarefied data using Bray-Curtis dissimilarity (measuring the fraction of the community specific to
311 either group) and Jaccard distance (measuring the fraction of taxa specific to either group, i.e.,
312 comparing presence and absence) (Schmidt et al., 2017) and visualized using the first 2 axes of the
313 principal coordinate analysis (PCoA). Permutational multivariate analysis of variance was performed
314 using the adonis2 function from the vegan package in R to test for effects of inoculum, antibiotic, and
315 their interaction, and the betadisper function from the vegan package was used to test for homogeneity
316 of group dispersions. The simper function from the vegan package was used to determine which genera
317 contribute most to the Bray-Curtis dissimilarity between groups without and with antibiotic treatment.
318
319 2.2.4. Used software

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

320 Transmission data were analysed with R version 4.1.2 (R Core Team, 2021) with package rstan 2.21.5
321 (Stan Development Team, 2020) using a tree depth of 14, an acceptance rate of 0.99 and 4 chains with
322 4000 iterations, and packages rethinking 2.21 (McElreath, 2020), cmdstanr 0.5.2 (Gabry and Cešnovar,
323 2022), StanHeaders 2.21.0-7 (Stan Development Team, 2018) and bayestestR 0.12.1 (Makowski et al.,
324 2019). Sequence processing and statistical analyses related to the sequencing were performed with R
325 4.0.2 (R Core Team, 2020) with package dada2 1.16.0 (Callahan et al., 2016). Subsequent analyses of
326 the microbiome data were performed with R 4.1.2 (R Core Team, 2021) with packages phyloseq 1.38.0
327 (McMurdie and Holmes, 2013), microbiome 1.16.0 (Lahti and Shetty, 2019), vegan 2.6.2 (Oksanen et
328 al., 2022), and dunn.test 1.3.5 (Dinno, 2017), using packages tidyr 1.2.0 (Wickham and Girlich, 2022),
329 dplyr 1.0.9 (Wickham et al., 2021), and Biostrings 2.62.0 (Pagès et al., 2022) for data handling, and
330 ggplot2 3.3.6 (Wickham, 2016) and cowplot 1.1.1 (Wilke, 2020) for plotting.
331

332 3. Results

333 3.1. Transmission experiment

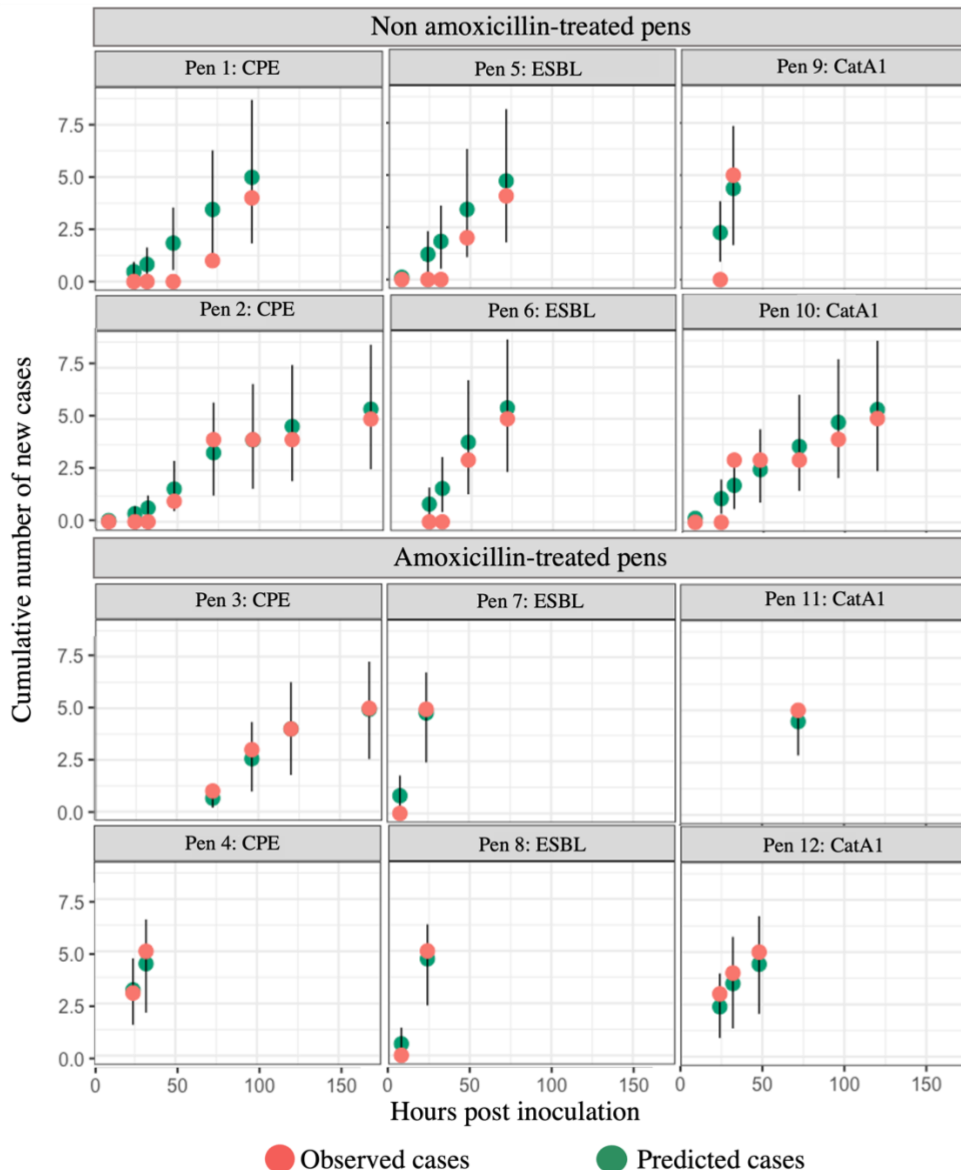
334 The 111 out of 120 inoculated and contact broilers that survived until the end of the experiment all
335 became colonised by the *E. coli* strain used for inoculation (i.e., CPE-strain, ESBL-strain, or catA1-
336 strain). Four broilers from the CPE-strain group, 4 broilers from the ESBL-strain group, and 1 broiler
337 from the catA1-strain group died (Table S1). The majority of the broilers gained weight slower and
338 reached 20% lower final weights than typical Ross 308 broilers, probably because they received feed
339 for laying pullets instead of broilers. No other abnormalities were observed.
340

341 3.2. Transmission rates

342 3.2.1. Predicted versus observed cases

343 The number of cases predicted by the hierarchical model is higher than the number of observed cases
344 in non-antibiotic-treated pens and lower than the number of observed cases in antibiotic-treated pens
345 because of the shrinkage caused by the hierarchical modelling (Figure 3). Shrinkage is a key feature of
346 a hierarchical model because the measurements of different clusters (i.e., inoculum and antibiotic
347 treatment) inform one another such that the predicted result shrinks towards the overall mean. The
348 number of cases increased over a longer period in non-amoxicillin-treated pens (top rows) than in
349 amoxicillin-treated pens (bottom rows) because the larger transmission rate in amoxicillin-treated pens
350 led to the depletion of susceptible broilers.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



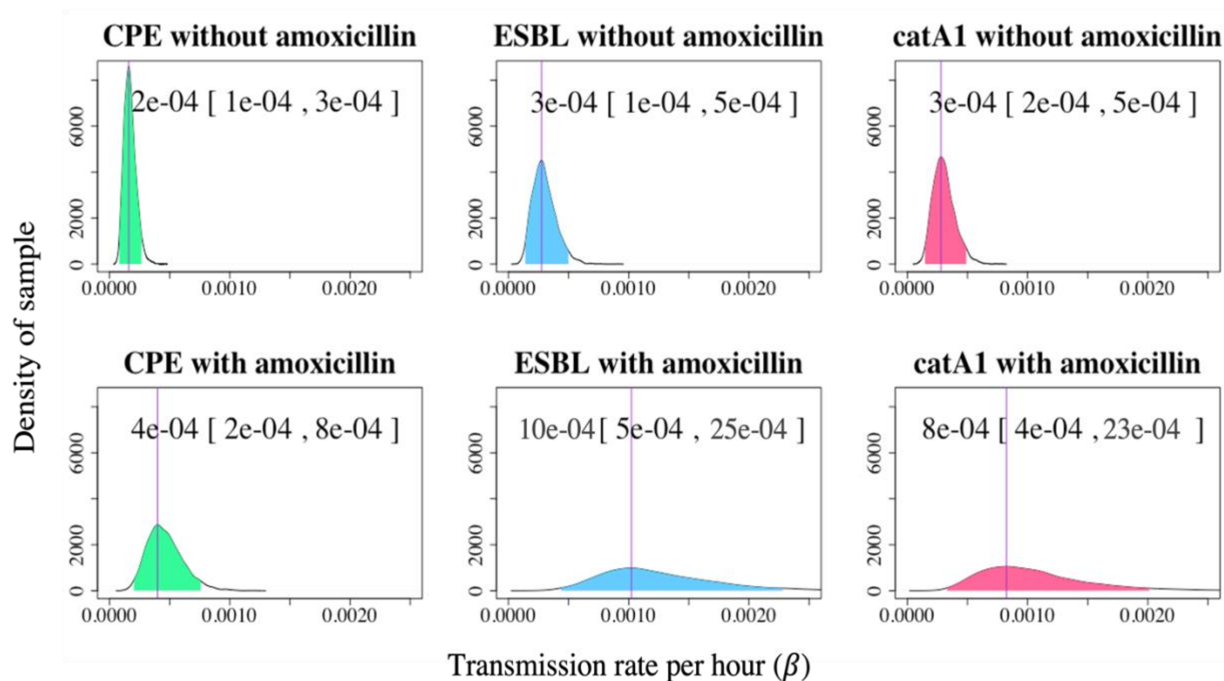
351

352 *Figure 3: number of cases over time. Observed (orange) and predicted (green) cumulative number of*
353 *new cases among the 5 susceptible broilers (i.e., susceptible contact broilers that became colonized) in*
354 *each of the 12 pens (vertical axis) until the sampling time point in hours after inoculation (horizontal*
355 *axis). For the predicted numbers the maximum a posteriori estimates are given, with the whiskers*
356 *indicating 95% highest posterior density intervals. Transmission cannot occur when none of the broilers*
357 *is excreting yet or when all broilers are excreting. No data is shown at those time points.*

358 3.2.2. Effect of inoculums

359 The estimated transmission rates for broilers inoculated with the CPE-strain, ESBL-strain, and catA1-
360 strain were compared using the 95% HPDI and the MAP (shaded area and purple vertical line in Figure
361 4, Figure 5). In the non-amoxicillin-treated groups as well as in the amoxicillin-treated groups, the 95%
362 HPDIs of the transmission rates of the CPE-strain, the ESBL-strain, and the catA1-strain overlap,
363 suggesting their transmission rates are similar (Figure 4). Still, the MAP suggests that CPE-strain has
364 the lowest transmission rate of the 3 inoculums.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

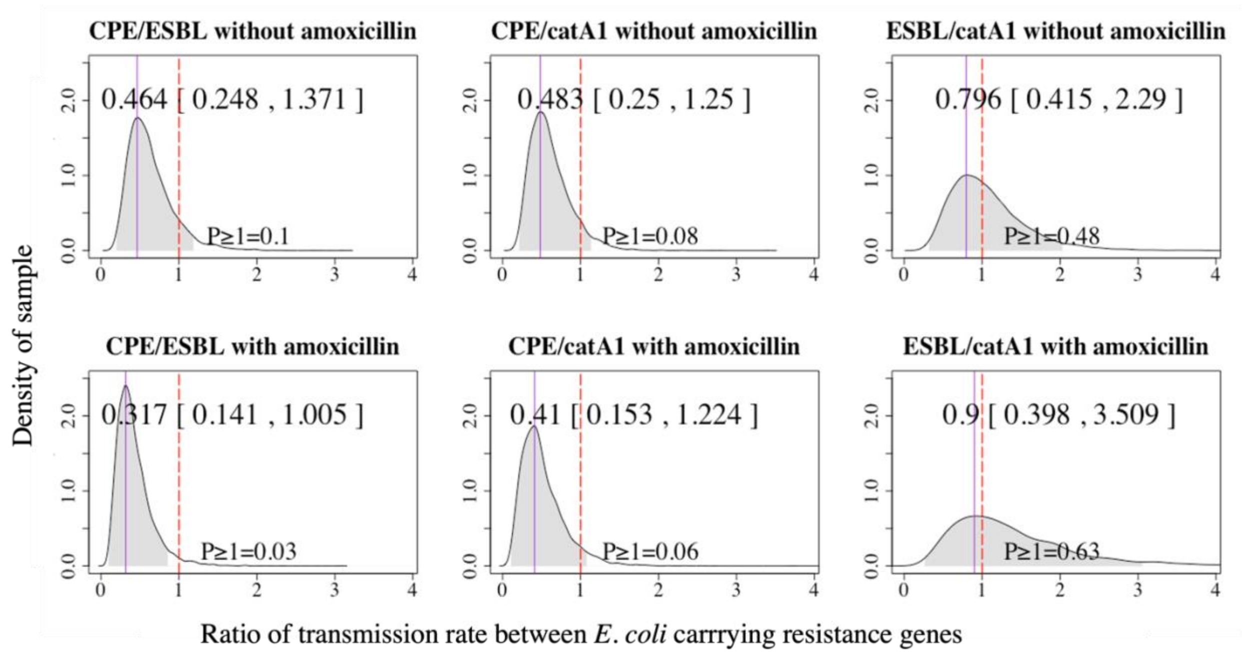


365

366 *Figure 4: Density (vertical axis) of the posterior distribution of the transmission rate per hour (horizontal*
367 *axis) for the CPE-strain, ESBL-strain and catA1-strain. The top and bottom row show plots for the pens*
368 *without and with amoxicillin treatment, respectively. Purple vertical lines indicate the point estimate at*
369 *the highest density and shaded areas are the 95% highest posterior density intervals of the posterior*
370 *distribution; the estimated values of both are shown at the top of the plot.*

371 The MAP of the estimated transmission rate of the CPE-strain is 46% and 48% of the transmission rate
372 of the ESBL-strain and the catA1-strain in the non-amoxicillin-treated groups, respectively, and 32% and
373 41% of the transmission rate of ESBL-strain and catA1-strain in the amoxicillin-treated groups,
374 respectively (Figure 5). HPDIs of the ratio of the transmission rates indicate the probability that
375 transmission of the CPE-strain is faster than the transmission of the ESBL-strain or catA1-strain is 8%
376 – 10% in non-amoxicillin-treated groups, and 3% – 6% in amoxicillin-treated groups (Figure 5). The MAP
377 of the ratio of the ESBL-strain transmission rate to catA1-strain transmission rate is 0.80 without
378 amoxicillin treatment and 0.90 with amoxicillin treatment, and the probability of a ratio equal to or larger
379 than 1 is 0.48 and 0.63 for the groups without and with amoxicillin, respectively. This indicates the
380 transmission rates of ESBL-strain and catA1-strain were similar in this experiment.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



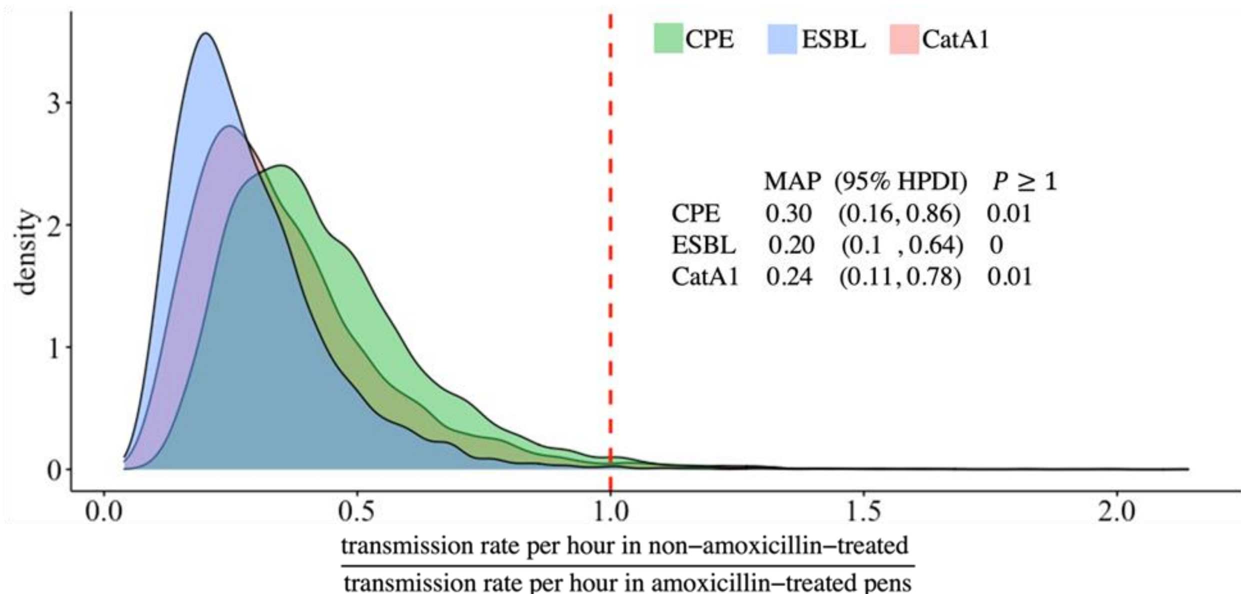
381

382 *Figure 5: Density (vertical axis) of the posterior distribution of the ratio of the transmission rates*
383 *(horizontal axis) for different inoculums: CPE-strain to ESBL-strain, CPE-strain to catA1-strain, and*
384 *ESBL-strain to catA1-strain. The top and bottom row show plots for the pens without and with amoxicillin*
385 *treatment, respectively. Purple vertical lines indicate the point estimate at the highest density and*
386 *shaded areas are the 95% highest posterior density intervals of the posterior distribution; the estimated*
387 *values of both are shown at the top of the plot. Dotted vertical red lines indicate a ratio of 1 and the*
388 *probability of a ratio equal to or larger than 1* $(P \geq 1)$ *is shown at the bottom of the plot.*

389 3.2.3. Effect of amoxicillin

390 The transmission rates of all inoculums are smaller in the non-amoxicillin-treated groups than in the
391 amoxicillin-treated groups (Figure 6). The difference between amoxicillin-treated groups and non-
392 amoxicillin-treated groups is slightly larger for the ESBL-strain and catA1-strain than for the CPE-strain.

393



394

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

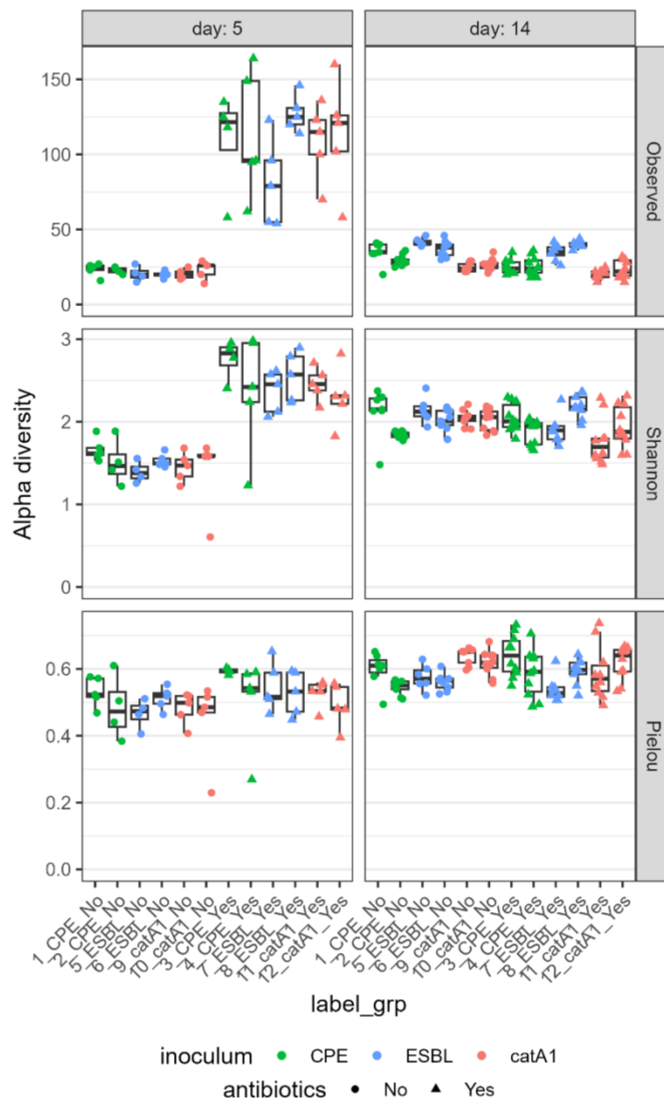
395 *Figure 6: Density (vertical axis) of the ratio of the transmission rates in non-amoxicillin-treated pens over*
396 *amoxicillin-treated pens (horizontal axis) for CPE-strain (green), ESBL-strain (blue) and catA1-strain*
397 *(pink). The dotted red vertical line indicates a ratio of 1 (i.e., the transmission rates of amoxicillin-treated*
398 *and non-amoxicillin groups are the same). The point estimate at the highest density (MAP) and 95%*
399 *highest posterior density intervals (95% HPDI), and the probability of a ratio equal to or larger than 1*
400 *($P \geq 1$) are also shown in the plot.*

401 **3.3. Microbiome analysis**

402 3.3.1. Alpha-diversity

403 Observed richness measuring the observed number of taxa, Shannon's index which takes evenness
404 into account (with higher values if more taxa are present or taxa are more evenly distributed), and
405 Pielou's evenness which is not influenced by richness (with a value between 0 and 1, with higher values
406 if taxa are more evenly distributed), were used to measure alpha-diversity. All alpha-diversity measures
407 of the caecal microbiome at genus level on day 5 (i.e., before inoculation) were similar in the groups
408 inoculated with the different inoculums (i.e., CPE-strain, ESBL-strain, catA1-strain; Figure 7). On day 14
409 various small differences in observed richness and Pielou's evenness were found at genus level.
410 Repeating these analyses at the level of individual ASVs mostly gave the same results (Figure S8;
411 Tables S5 – S8).
412
413 Observed richness and Shannon's index at genus level on day 5 were lower in the non-amoxicillin-
414 treated groups than in the amoxicillin-treated groups, but Pielou's evenness was not different (Figure 7),
415 indicating fewer genera were present in the non-amoxicillin-treated groups but the distribution of their
416 abundances was similar to the distribution of their abundances in the amoxicillin-treated groups. By day
417 14, 8 days after finishing amoxicillin treatment, alpha-diversity was similar in the amoxicillin-treated and
418 non-amoxicillin-treated groups. Repeating these analyses at the level of individual ASVs mostly gave
419 the same results (Figure S8; Tables S5 – S8).

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



420

421 *Figure 7. Alpha-diversity (vertical axis) by inoculum and antibiotic treatment (horizontal axis) at genus*
422 *level. Colours indicate different inoculums (CPE-strain: green; ESBL-strain: blue; catA1-strain: red)*
423 *and symbols indicate the absence (circles) or presence (triangles) of antibiotic treatment. The panels*
424 *show the different alpha-diversity measures (rows) and different days (columns).*

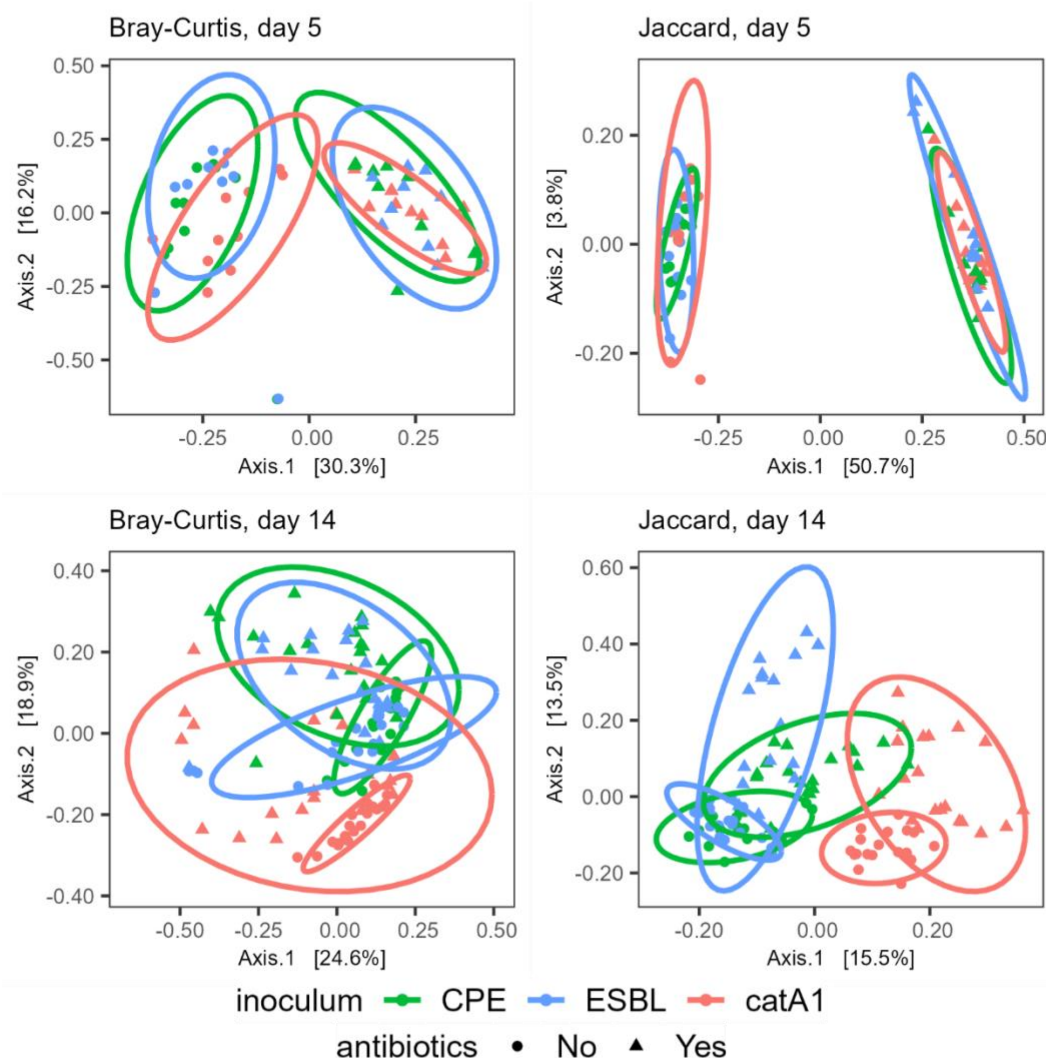
425 3.3.2. Beta-diversity

426 The inoculums explained 6% and 3% of the variation between the groups in Bray-Curtis dissimilarity
427 and Jaccard distance at genus level on day 5, antibiotic treatment explained 27% and 50% of the
428 variation, and their interaction explained 5% and 3% of the variation (Table S9). Only groups without
429 and with antibiotics were separated in the PCoA-plot (Figure 8). Repeating these analyses at the level
430 of individual ASVs mostly gave the same results (see sections 2.5 and 2.6 of the supplementary
431 material). Similarity percentage analyses showed the Bray-Curtis dissimilarities on day 5 between
432 groups without and with antibiotic treatment are driven by the same genera in the groups inoculated with
433 the different inoculation strains. Most of these genera belonged to the classes Bacilli and Clostridia, and
434 some to the class Gammaproteobacteria (Tables S11 – S13).

435

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

436 The inoculums explained 16% and 17% of the variation between the groups in Bray-Curtis dissimilarity
437 and Jaccard distance at genus level on day 14, antibiotic treatment explained 9% of the variation for
438 both measures, and their interaction explained 4% and 6% of the variation (Table S10). For both beta-
439 diversity measures, CPE-strain and ESBL-strain overlapped much with each other in the PCoA-plots,
440 whereas catA1 without antibiotics separated from CPE-strain and ESBL-strain without antibiotics.
441 Groups without and with antibiotics were not separate from each other on genus level (Figure 8) but
442 separated on ASV level with Bray-Curtis dissimilarity (Figure S9).



443

444 *Figure 8. Principal coordinate plots based on Bray-Curtis dissimilarity (left) and Jaccard distance (right) for day 5*
445 *(top) and day 14 (bottom) at genus level. Colours indicate different inoculums (CPE*-strain*: green; ESBL*-strain*:*
446 *blue; catA1*-strain*: red) and symbols indicate the absence (circles) or presence (triangles) of antibiotic treatment.*
447 *Ellipses represent 95% confidence regions assuming a multivariate t-distribution.*
448

449 ## 4. Discussion

450 To our knowledge, this is the first transmission experiment with CPE *E. coli* in livestock. In addition,
451 although the use of a Bayesian hierarchical model as presented in this study is well-recognized in
452 epidemiology, its use in analysing animal transmission experiments is not common (Hu et al., 2017).

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

453 Furthermore, we extended previous work on the relationship between the microbiome and the
454 transmission of intestinal antibiotic-resistant bacteria (Dame-Korevaar et al., 2020b).
455
456 **4.1. Indirect environmental transmission**
457 *E. coli* is an enteric bacterium that is excreted in faeces and propagated in the environment (Conway
458 and Cohen, 2015; Ramos et al., 2020), from where it can spread to other animals and humans (Rwego
459 et al., 2008; Hussain et al., 2017; Rousham et al., 2018; Lepper et al., 2022). The environment can
460 serve as a reservoir for the transmission of resistant bacteria when no excreting animals are present
461 anymore (Dame-Korevaar et al., 2017). Therefore, we adapted the likelihood function to reflect
462 environmental transmission with its prolonged possibility of transmission from accumulated bacteria in
463 the environment.
464
465 It was not possible to estimate the decay rate and the transmission rate parameter with the Bayesian
466 model simultaneously in our study, because a given number of cases can be explained equally well by
467 a higher transmission rate or a lower decay rate. We reviewed the literature on decay rates (Table S14)
468 to find a suitable range of decay rates and ran the hierarchical model with several fixed decay rates
469 ranging from 0.04 – 55 h^{-1} (Table S15). This entire range of decay rates could be fitted well with low
470 Watanabe–Akaike information criterion and divergence transition. Multiple studies in various
471 environments suggest a very low level of *E. coli* decay in the first few days (see section 3.3 of the
472 supplement), therefore we selected the lowest fixed decay rate (δ) of 0.04 h^{-1} in the final model.
473
474 The transmission rates of $3 \cdot 10^{-4}$ h^{-1} and $1 \cdot 10^{-3}$ h^{-1} for the ESBL-strain derived from our model assuming
475 indirect environmental transmission are much lower than the transmission rate of 0.055 (0.045 – 0.066)
476 h^{-1} calculated from a direct model (Dame-Korevaar et al., 2020b). A lower transmission rate is expected
477 because resistant bacteria excreted into the environment were the only source of transmission
478 considered in our SI-model and they decayed at a low rate. Using a higher decay rate would result in
479 higher estimates for the transmission rates (Table S15), with a decay rate of 7.4 h^{-1} giving a transmission
480 rate of 0.04 h^{-1} for ESBL without antibiotics, comparable to the value obtained by (Dame-Korevaar et
481 al., 2020b). Using higher decay rates still results in transmission rates of CPE being lower than the
482 transmission rates of the other inoculums.
483
484 **4.2. Bayesian hierarchical inference**
485 The Bayesian hierarchical model quantifies the transmission rate parameter of each pen using the mean
486 transmission rate parameter and its variation simultaneously, instead of conventionally averaging the
487 variation of all pens. This improves the estimates for each pen, especially when transmission events
488 occur between sampling time points such that some pens have less information (McElreath, 2020). This
489 was relevant for pens 7, 8, and 11 in which new cases were only observed at a very limited number of
490 time points, because multiple transmission events occurred within the first few days (Figure 3), leading
491 to wide HPDIs indicating a wide range of possible transmission rates. The hierarchical structure of the
492 model led to shrinkage of the predicted data. Thus, we did not expect the predicted data to be equivalent

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

493 to the observed data but instead expected systematic differences between the predicted and observed
494 data (Figure 3).
495
496 The actual moment of transmission is rarely observed in transmission experiments because of logistic
497 and ethical limitations to the number of animals and the sampling frequency (Cauchemez et al., 2004).
498 A Bayesian approach in the analysis of transmission experiments can be used to incorporate the
499 uncertainty that is inherent to the data in the statistical model, and to clearly present the uncertainty in
500 the outcomes in the form of the posterior distribution (Hiura et al., 2021). These characteristics make
501 Bayesian hierarchical modelling very suitable to quantify transmission between animals.
502

503 **4.3. Effect of antibiotic resistance and *E. coli* strains on transmission**

504 Resistance genes carried on plasmids generally impose fewer fitness costs on their bacterial hosts than
505 chromosomal mutations resulting in resistance (Vogwill and MacLean, 2015). The fitness costs imposed
506 by plasmids, lowering the population growth of resistant bacteria, can decrease when the number of
507 plasmids within bacteria increases (Lee et al., 2020), increase when the number of resistance gene
508 families on a plasmid increases and are also affected by host factors (Vogwill and MacLean, 2015). The
509 CPE inoculum used in the animal experiment contained 3 plasmids carrying resistance genes from 6
510 families, while the ESBL strain contained 6 plasmids carrying resistance genes from 4 families, and the
511 catA1 strain contained 4 plasmids carrying resistance genes from 4 families (Tables S16 – S18). The
512 CPE-strain had more resistance gene families per plasmid and fewer plasmids than the other strains,
513 both of which could increase fitness costs and thereby lower the transmission rate of CPE-strain
514 compared to the other strains. On the other hand, in vitro conjugation experiments with multiple plasmids
515 and resistance genes showed ESBL genes to be the costliest (Rajer and Sandegren, 2022) and the
516 transmission rate of the CPE-strain was also lower in the presence of amoxicillin (Figure 5) when fitness
517 costs are not expected to limit the transmission rate. This suggests the lower transmission rate of the
518 CPE-strain is more likely caused by differences between the used *E. coli*-strains than by differences in
519 plasmids and resistance genes.
520
521 The CPE, ESBL, and catA1 resistance genes used in the animal experiment were carried by different
522 *E. coli* strains isolated from chickens between 2004 and 2009, so we cannot separate the effect of the
523 different plasmids and the resistance genes they carried from the effect of the different *E. coli* strains. In
524 addition, the resistance genes were located on conjugative plasmids and resistant colonies were not
525 tested to identify the *E. coli* type. As such, part of the transmission might also be explained by plasmid
526 transfer between *E. coli*, rather than by colonisation of the chicken gut by the *E. coli* strains that were
527 present in the inoculums.
528

529 **4.4. Effect of amoxicillin on transmission**

530 Antibiotic treatment alleviates fitness costs of resistance genes and leads to resistant bacteria having a
531 higher growth rate than susceptible bacteria, such that resistant bacteria would be expected to colonize
532 the gut more easily and be transmitted faster in the amoxicillin-treated groups. Indeed, the transmission

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

533 rates of all inoculums were higher in the amoxicillin-treated groups than in the non-amoxicillin-treated
534 groups (Figure 6). Similarly, the relative abundance of the *E.coli/Shigella* genus was lower in amoxicillin-
535 treated pens than in non-amoxicillin-treated pens on day 5 (i.e., before inoculation) but similar on day
536 14 (Figure S7), suggesting there was more ability for the inoculum to grow in antibiotic-treated pens.
537 Nevertheless, the differences in transmission rates observed between the CPE-strain versus the ESBL-
538 strain and the catA1-strain were also observed in amoxicillin-treated pens. This suggests intrinsic
539 differences in the capability for transmission were present in these bacterial strains, which are
540 independent of the antibiotic resistance itself, as we already stated above.
541
542 **4.5. Microbiome analysis**
543 Observed richness and evenness on day 14 differed between inoculums, but those differences were
544 mainly caused by less within-group variation and were small compared to the effect of antibiotics on day
545 5 (Figure 7). The separation between the catA1-strain versus the CPE-strain and the ESBL-strain in
546 beta-diversity on day 14 can be explained by broilers inoculated with the catA1-strain being housed in
547 a room separate from broilers inoculated with the CPE-strain and ESBL-strain, in addition to the effect
548 of being inoculated with a different *E. coli* strain. This room effect was also reflected in the caecal
549 composition of the non-amoxicillin-treated catA1 groups being more similar to the composition of the
550 amoxicillin-treated catA1 groups than to the composition of the non-amoxicillin-treated CPE-groups and
551 ESBL-groups at family level (Figure S5).
552
553 The differences in alpha-diversity and beta-diversity between the different inoculums do not
554 correspond to the differences in the transmission rates between the inoculums. The ESBL-strain
555 contained slightly more observed taxa on day 14, the catA1-strain contained a slightly more even
556 distribution of taxa on day 14, and the catA1-strain separated somewhat from CPE and ESBL on
557 genus level and clearly on ASV level. The transmission rates of the ESBL-strain and the catA1-strain
558 were, however, very similar and the transmission rate of the CPE-strain was lower. This indicates the
559 differences in transmission between the inoculums are most likely not caused by differences in the
560 caecal microbiome.
561
562 Observed richness on day 5 was lower in non-amoxicillin-treated pens than in amoxicillin-treated pens,
563 but Pielou's evenness was similar (Figure 7), suggesting amoxicillin treatment allowed more taxa to
564 increase in abundance but did not lead to differences in the proportion of dominating taxa. The lower
565 richness in non-amoxicillin-treated pens was the opposite of the higher richness expected based on the
566 literature mentioned in the introduction, which might have been caused by the depletion of some major
567 abundant taxa by the amoxicillin treatment, leaving more room for rare taxa to be detected by the
568 sequencing depth that became available. Amoxicillin treatment did affect the microbiome composition
569 at class, family level and genus level (Figure S4; Figure S5; Figure S6). On day 14, only few differences
570 in alpha-diversity and evenness between the non-amoxicillin-treated and amoxicillin-treated groups
571 were found (Figure 7). Similarity percentage analyses indicated the effects of antibiotic treatment on
572 Bray-Curtis dissimilarity on day 5 were driven by the same genera in the groups inoculated with the

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

573 different inoculation strains (Tables S11 – S13). Amoxicillin treatment explained less variation in beta-
574 diversity on day 14 than on day 5, and the non-amoxicillin-treated and amoxicillin-treated groups did not
575 separate clearly in the PCoA plot at genus level on day 14. This indicates differences in the genera
576 present in the caecal microbiome on day 5 caused by antibiotic treatment did not last until day 14.
577 Amoxicillin is cleared quickly from chickens when administration ceases and decays quickly in the
578 environment (Peng et al., 2016), such that the effect of amoxicillin might have been reduced by day 14
579 because it was last administered on day 6. Although other clinically important antibiotics such as
580 cephalosporins are cleared slower and could last longer in the environment such that they could have
581 an effect on day 14, we did not incorporate them in our study because their use in livestock is subject
582 to legal restrictions (Bonten et al., 2021). The higher alpha-diversity in amoxicillin-treated groups on day
583 5 might be related to the higher transmission rates in amoxicillin-treated groups because transmission
584 events mainly occurred within a few days after inoculation (Figure 3). The microbiome of broilers evolves
585 in steps to a more or less stable state in 35 days (Jurburg et al., 2019; Kers et al., 2022). We hypothesize
586 that the dysbiosis of the microbiome caused by antibiotic treatment allows for easier colonization and
587 more rapid growth of new *E. coli* strains such as the inoculums, which is reflected in a more rapid
588 transmission. The opposite, e.g., quicker maturation of the gut microbiome by applying a probiotic, has
589 been shown to slow down transmission (Ceccarelli et al., 2017; Dame-Korevaar et al., 2020b).
590
591 **4.6. Suggestions for further research**
592 The uncertainty and variability of the transmission rates of the 3 *E. coli* strains provide a good range of
593 transmission rates needed to model the transmission of resistance genes carried by commensal bacteria
594 in poultry. Future research could expand the Bayesian hierarchical framework adopted in this study by
595 incorporating data together with their uncertainty from other experiments on bacterial transmission
596 between broilers to capture the influence of differences in environments, chickens' feed, and different
597 species of resistant bacteria. This would result in a transmission model that reflects the situation on
598 broiler farms more realistically.
599
600 In a clean environment, inoculated broilers should start excreting before contact broilers can be
601 colonized. However, in some pens in this experiment, the first excretion of resistant bacteria by both
602 inoculated and contact broilers was detected at the same sampling time point. This is caused by
603 limitations to the sampling frequency. We could use the model by assuming that inoculated broilers
604 started excreting half a time interval earlier. This assumption has previously been used in the analysis
605 of a transmission experiment in broilers where the moment of excretion was similar for inoculated and
606 contact animals (Dame-Korevaar et al., 2020a). In future research, estimation of the exact time point of
607 colonization could be incorporated, e.g., by applying the Bayesian approach described for a model of
608 direct transmission (Hu et al., 2017) to a model of environmental transmission. Taking more frequent
609 samples could also help, although more frequent sampling of the caeca is limited by ethical
610 considerations.
611

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

612 Although the presence of multiple plasmids in a bacterium reflects the situation that is common in nature
613 (Davies and Davies, 2010; MacLean and San Millan, 2015), future research should compare the
614 transmission rates of different resistance genes using a single *E. coli* strain that only contains the
615 plasmid of interest for the different inoculums. We were not able to use that approach because of a lack
616 of the necessary permits to work with genetically modified organisms in animal experiments, but here
617 we showed the difference in transmission rates between strains could be substantial (up to 68%) and is
618 thus relevant. Using that same *E. coli* strain with chromosomal resistance instead of plasmids as
619 inoculum would allow for the comparison of the transmission of plasmid-mediated and chromosomal
620 resistance. Such research can build on this paper by applying the same methodology and determining
621 their sampling schemes based on our results.
622

623 # 5. Summary

624 From our study, we conclude early amoxicillin treatment increases the transmission rate of *E. coli* strains
625 carrying different resistance genes between broilers up to five-fold. Amoxicillin treatment increased
626 alpha-diversity of the caecal microbiome on day 5, but no effects of amoxicillin treatment on the caecal
627 microbiome were found on day 14, suggesting it only has a temporary effect on the caecal microbiome.
628 In addition, the effects of amoxicillin on the transmission rates were most likely not caused by differences
629 in the caecal microbiome, because differences in the microbiomes of the different inoculums did not
630 correspond to the differences in the transmission rates of the different inoculums. The transmission rates
631 of $2 \cdot 10^{-4}$ h^{-1} and $4 \cdot 10^{-4}$ h^{-1} for the CPE-strain were 54 – 68 per cent lower than the transmission rates of
632 the ESBL-strain and 52 – 59 per cent lower than the transmission rates of the catA1-strain. This was
633 reflected in the longer time needed for the CPE-strain to colonize all broilers than for the ESBL-strain
634 and catA1-strain. Such delays might be relevant in the field, especially if competition between different
635 antibiotic-resistant strains occurs. The consistent difference in transmission rates with and without
636 antibiotic treatment indicates the differences in transmission rates were more likely caused by
637 differences between the used *E. coli* strains than by differences in plasmids and resistance genes. The
638 methodology applied in this experiment and the obtained transmission rates can be used to improve
639 models of the transmission of resistant bacteria between broilers.
640

641 # 6. Acknowledgements

642 The authors gratefully acknowledge the assistance provided during this project by the animal caretakers,
643 pathologists and technicians at Wageningen Bioveterinary Research.
644

645 # 7. Funding

646 This work was supported by ZonMW [grant numbers 50-54100-98-229, 50-54100-98-119].
647

648 # 8. References

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

649 Aarestrup, F.M., 2015. The livestock reservoir for antimicrobial resistance: a personal view on
650 changing patterns of risks, effects of interventions and the way forward. Philos Trans R Soc
651 Lond B Biol Sci 370, 20140085.
652 Bonardi, S., Pitino, R., 2019. Carbapenemase-producing bacteria in food-producing animals, wildlife
653 and environment: a challenge for human health. Ital J Food Saf 8, 7956-7956.
654 Bonten, M.J.M., van Geijlswijk, I.M., Heederik, D.J.J., Mevius, D.J., P., S., 2021. Usage of antibiotics
655 in agricultural livestock in the Netherlands in 2020 - appendix.
656 Bortolaia, V., Ronco, T., Romascu, L., Nicorescu, I., Milita, N.M., Vaduva, A.M., Leekitcharoenphon,
657 P., Kjeldgaard, J.S., Hansen, I.M., Svendsen, C.A., Mordhorst, H., Guerra, B., Beloeil, P.A.,
658 Hoffmann, M., Hendriksen, R.S., 2021. Co-localization of carbapenem (blaOXA-162) and
659 colistin (mcr-1) resistance genes on a transferable IncHI2 plasmid in Escherichia coli of
660 chicken origin. J Antimicrob Chemother 76, 3063-3065.
661 Brink, A.J., 2019. Epidemiology of carbapenem-resistant Gram-negative infections globally. Curr Opin
662 Infect Dis 32, 609-616.
663 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2:
664 High-resolution sample inference from Illumina amplicon data. Nature Methods 13, 581-583.
665 Cauchemez, S., Carrat, F., Viboud, C., Valleron, A.J., Boëlle, P.Y., 2004. A Bayesian MCMC approach
666 to study transmission of influenza: application to household longitudinal data. Stat Med 23,
667 3469-3487.
668 Ceccarelli, D., van Essen-Zandbergen, A., Smid, B., Veldman, K.T., Boender, G.J., Fischer, E.A.J.,
669 Mevius, D.J., van der Goot, J.A., 2017. Competitive exclusion reduces transmission and
670 excretion of extended-spectrum-β-lactamase-producing Escherichia coli in broilers. Appl
671 Environ Microbiol 83.
672 Conway, T., Cohen, P.S., 2015. Commensal and pathogenic Escherichia coli metabolism in the gut.
673 Microbiol Spectr 3.
674 Dame-Korevaar, A., Fischer, E.A.J., Stegeman, A., Mevius, D., van Essen-Zandbergen, A., Velkers,
675 F., van der Goot, J., 2017. Dynamics of CMY-2 producing E. coli in a broiler parent flock. Vet
676 Microbiol 203, 211-214.
677 Dame-Korevaar, A., Fischer, E.A.J., van der Goot, J., Velkers, F., Ceccarelli, D., Mevius, D.,
678 Stegeman, A., 2020a. Early life supply of competitive exclusion products reduces colonization
679 of extended spectrum beta-lactamase-producing Escherichia coli in broilers. Poult Sci 99,
680 4052-4064.
681 Dame-Korevaar, A., Fischer, E.A.J., van der Goot, J., Velkers, F., van den Broek, J., Veldman, K.,
682 Ceccarelli, D., Mevius, D., Stegeman, A., 2019. Effect of challenge dose of plasmid-mediated
683 extended-spectrum β-lactamase and AmpC β-lactamase producing Escherichia coli on time-
684 until-colonization and level of excretion in young broilers. Vet Microbiol 239, 108446.
685 Dame-Korevaar, A., Kers, J.G., van der Goot, J., Velkers, F.C., Ceccarelli, D., Mevius, D.J.,
686 Stegeman, A., Fischer, E.A.J., 2020b. Competitive exclusion prevents colonization and
687 compartmentalization reduces transmission of ESBL-producing Escherichia coli in broilers.
688 Front Microbiol 11, 566619.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

689 Dankittipong, N., Fischer, E.A.J., Swanenburg, M., Wagenaar, J.A., Stegeman, A.J., de Vos, C.J.,
690 2022. Quantitative risk assessment for the introduction of carbapenem-resistant
691 Enterobacteriaceae (CPE) into dutch livestock farms. Antibiotics (Basel) 11.
692 Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74,
693 417-433.
694 Dinno, A., 2017. dunn.test: Dunn's test of multiple comparisons using rank sums.
695 European Centre for Disease Prevention and Control, 2018. Surveillance of antimicrobial resistance in
696 Europe – Annual report of the European Antimicrobial Resistance Surveillance Network
697 (EARS-Net) 2017. Stockholm.
698 European Food Safety Authority, European Centre for Disease Prevention Control, 2022. The
699 European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria
700 from humans, animals and food in 2019–2020. EFSA Journal 20, e07209.
701 Finotello, F., Mastrorilli, E., Di Camillo, B., 2018. Measuring the diversity of the human microbiota with
702 targeted next-generation sequencing. Brief Bioinform 19, 679-692.
703 Gabry, J., Cešnovar, R., 2022. cmdstanr: R Interface to 'CmdStan'.
704 Gerhards, N.M., Gonzales, J.L., Vreman, S., Ravesloot, L., van den Brand, J.M.A., Doekes, H.P.,
705 Egberink, H.F., Stegeman, A., Oreshkova, N., van der Poel, W.H.M., de Jong, M.C.M., 2022.
706 Efficient direct and limited environmental transmission of SARS-CoV-2 lineage B.1.22 in
707 domestic cats. bioRxiv, 2022.2006.2017.496600.
708 Hiura, S., Abe, H., Koyama, K., Koseki, S., 2021. Bayesian generalized linear model for simulating
709 bacterial inactivation/growth considering variability and uncertainty. Front Microbiol 12,
710 674364.
711 Holmes, A.H., Moore, L.S.P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P.J.,
712 Piddock, L.J.V., 2016. Understanding the mechanisms and drivers of antimicrobial resistance.
713 The Lancet 387, 176-187.
714 Hu, B., Gonzales, J.L., Gubbins, S., 2017. Bayesian inference of epidemiological parameters from
715 transmission experiments. Sci Rep 7, 16774.
716 Huijbers, P.M.C., Graat, E.A.M., van Hoek, A., Veenman, C., de Jong, M.C.M., van Duijkeren, E.,
717 2016. Transmission dynamics of extended-spectrum β-lactamase and AmpC β-lactamase-
718 producing Escherichia coli in a broiler flock without antibiotic use. Prev Vet Med 131, 12-19.
719 Hussain, A., Shaik, S., Ranjan, A., Nandanwar, N., Tiwari, S.K., Majid, M., Baddam, R., Qureshi, I.A.,
720 Semmler, T., Wieler, L.H., Islam, M.A., Chakravortty, D., Ahmed, N., 2017. Risk of
721 transmission of antimicrobial resistant Escherichia coli from commercial broiler and free-range
722 retail chicken in India. Front Microbiol 8, 2120.
723 Jurburg, S.D., Brouwer, M.S.M., Ceccarelli, D., van der Goot, J., Jansman, A.J.M., Bossers, A., 2019.
724 Patterns of community assembly in the developing chicken microbiome reveal rapid primary
725 succession. Microbiologyopen 8, e00821.
726 Keeling, M.J., Rohani, P., 2007. Modeling Infectious Diseases in Humans and Animals Princeton
727 University Press.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

728 Kers, J.G., Velkers, F.C., Fischer, E.A.J., Stegeman, J.A., Smidt, H., Hermes, G.D.A., 2022.
729 Conserved developmental trajectories of the cecal microbiota of broiler chickens in a field
730 study. FEMS Microbiology Ecology 98.
731 Kim, S., Covington, A., Pamer, E.G., 2017. The intestinal microbiota: antibiotics, colonization
732 resistance, and enteric pathogens. Immunol Rev 279, 90-105.
733 Knobler, S., Marian, N., Stacey, L.K., Stanley, M.L., Tom, B., Health, B.o.G., Burroughs, T., Infections,
734 F.o.E., Knobler, S.L., Lemon, S.M., Najafi, M., 2003. The resistance phenomenon in microbes
735 and infectious disease vectors: implications for human health and strategies for containment
736 (workshop summary). National Academies Press 2003.
737 Köck, R., Daniels-Haardt, I., Becker, K., Mellmann, A., Friedrich, A.W., Mevius, D., Schwarz, S., Jurke,
738 A., 2018. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and
739 companion animals: a systematic review. Clin Microbiol Infect 24, 1241-1250.
740 Lahti, L., Shetty, S., 2019. microbiome R package.
741 Lee, H., Shin, J., Chung, Y.J., Park, M., Kang, K.J., Baek, J.Y., Shin, D., Chung, D.R., Peck, K.R.,
742 Song, J.H., Ko, K.S., 2020. Co-introduction of plasmids harbouring the carbapenemase
743 genes, bla(NDM-1) and bla(OXA-232), increases fitness and virulence of bacterial host. J
744 Biomed Sci 27, 8.
745 Leekitcharoenphon, P., Johansson, M.H.K., Munk, P., Malorny, B., Skarżyńska, M., Wadepohl, K.,
746 Moyano, G., Hesp, A., Veldman, K.T., Bossers, A., Zając, M., Wasyl, D., Sanders, P.,
747 Gonzalez-Zorn, B., Brouwer, M.S.M., Wagenaar, J.A., Heederik, D.J.J., Mevius, D., Aarestrup,
748 F.M., 2021. Genomic evolution of antimicrobial resistance in Escherichia coli. Sci Rep 11,
749 15108.
750 Lepper, H.C., Woolhouse, M.E.J., van Bunnik, B.A.D., 2022. The role of the environment in dynamics
751 of antibiotic resistance in humans and animals: a modelling study. Antibiotics.
752 Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van
753 Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J.,
754 Bonten, M.J., Mevius, D.J., 2011. Dutch patients, retail chicken meat and poultry share the
755 same ESBL genes, plasmids and strains. Clin Microbiol Infect 17, 873-880.
756 Lister, S.A., Barrow, P., 2008. Chapter 8 - Enterobacteriaceae. In: Pattison, M., McMullin, P.F.,
757 Bradbury, J.M., Alexander, D.J. (Eds.), Poultry diseases (sixth edition). W.B. Saunders,
758 Edinburgh, 110-145.
759 MacLean, R.C., San Millan, A., 2015. Microbial evolution: towards resolving the plasmid paradox. Curr
760 Biol 25, R764-767.
761 Madec, J.Y., Haenni, M., 2018. Antimicrobial resistance plasmid reservoir in food and food-producing
762 animals. Plasmid 99, 72-81.
763 Makowski, D., Ben-Shachar, M.S., Lüdecke, D., 2019. bayestestR: describing effects and their
764 uncertainty, existence and significance within the Bayesian framework. Journal of open source
765 software 4.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

766 Mathew, A.G., Cissell, R., Liamthong, S., 2007. Antibiotic resistance in bacteria associated with food
767 animals: a United States perspective of livestock production. Foodborne Pathog Dis 4, 115-
768 133.
769 McElreath, R., 2020. Statistical rethinking: a bayesian course with examples in R and Stan. Chapman
770 & Hall.
771 McMurdie, P.J., Holmes, S., 2013. phyloseq: An R package for reproducible interactive analysis and
772 graphics of microbiome census data. PLOS ONE 8, e61217.
773 Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierikx, C.M., Bonten,
774 M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., de Koeijer, A., van Pelt, W.,
775 Franz, E., Mevius, D.J., Heederik, D.J.J., 2019. Attributable sources of community-acquired
776 carriage of Escherichia coli containing β-lactam antibiotic resistance genes: a population-
777 based modelling study. Lancet Planet Health 3, e357-e369.
778 Nielsen, L.R., van den Borne, B., van Schaik, G., 2007. Salmonella Dublin infection in young dairy
779 calves: transmission parameters estimated from field data and an SIR-model. Prev Vet Med
780 79, 46-58.
781 Ogunrinu, O.J., Norman, K.N., Vinasco, J., Levent, G., Lawhon, S.D., Fajt, V.R., Volkova, V.V., Gaire,
782 T., Poole, T.L., Genovese, K.J., Wittum, T.E., Scott, H.M., 2020. Can the use of older-
783 generation beta-lactam antibiotics in livestock production over-select for beta-lactamases of
784 greatest consequence for human medicine? An in vitro experimental model. PLoS One 15,
785 e0242195.
786 Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B.,
787 Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B.,
788 Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H.B.A.,
789 FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Leo Lahti, L., McGlinn, D.,
790 Ouellette, M., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. Vegan:
791 community ecology package.
792 Pagès, H., Aboyoun P., Gentleman R., DebRoy S., 2022. Biostrings: efficient manipulation of
793 biological strings.
794 Peng, P.C., Wang, Y., Liu, L.Y., Zou, Y.D., Liao, X.D., Liang, J.B., Wu, Y.B., 2016. The excretion and
795 environmental effects of amoxicillin, ciprofloxacin, and doxycycline residues in layer chicken
796 manure. Poult Sci 95, 1033-1041.
797 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O.,
798 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-
799 based tools. Nucleic Acids Research 41, D590-D596.
800 R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for
801 Statistical Computing, Vienna, Austria.
802 R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for
803 Statistical Computing, Vienna, Austria.
804 Rajer, F., Sandegren, L., 2022. The role of antibiotic resistance genes in the fitness cost of
805 multiresistance plasmids. mBio 13, e0355221.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

806 Ramos, S., Silva, V., Dapkevicius, M.L.E., Caniça, M., Tejedor-Junco, M.T., Igrejas, G., Poeta, P.,
807 2020. Escherichia coli as commensal and pathogenic bacteria among food-producing animals:
808 health implications of extended spectrum β-lactamase (ESBL) production. Animals (Basel) 10.
809 Robé, C., Blasse, A., Merle, R., Friese, A., Roesler, U., Guenther, S., 2019. Low dose colonization of
810 broiler chickens with ESBL-/AmpC-producing Escherichia coli in a seeder-bird model
811 independent of antimicrobial selection pressure. Front Microbiol 10, 2124.
812 Rochegüe, T., Haenni, M., Mondot, S., Astruc, C., Cazeau, G., Ferry, T., Madec, J.-Y., Lupo, A., 2021.
813 Impact of antibiotic therapies on resistance genes dynamic and composition of the animal gut
814 microbiota. Animals 11, 3280.
815 Rousham, E.K., Unicomb, L., Islam, M.A., 2018. Human, animal and environmental contributors to
816 antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and
817 One Health approaches. Proceedings of the Royal Society B: Biological Sciences 285,
818 20180332.
819 Rozwandowicz, M., Brouwer, M.S.M., Fischer, J., Wagenaar, J.A., Gonzalez-Zorn, B., Guerra, B.,
820 Mevius, D.J., Hordijk, J., 2018. Plasmids carrying antimicrobial resistance genes in
821 *Enterobacteriaceae*. J Antimicrob Chemother 73, 1121-1137.
822 Rwego, I.B., Gillespie, T.R., Isabirye-Basuta, G., Goldberg, T.L., 2008. High rates of Escherichia coli
823 transmission between livestock and humans in rural Uganda. J Clin Microbiol 46, 3187-3191.
824 Schmidt, T.S., Matias Rodrigues, J.F., von Mering, C., 2017. A family of interaction-adjusted indices of
825 community similarity. Isme j 11, 791-807.
826 Sorbara, M.T., Pamer, E.G., 2019. Interbacterial mechanisms of colonization resistance and the
827 strategies pathogens use to overcome them. Mucosal Immunol 12, 1-9.
828 Stan Development Team, 2018. StanHeaders: headers for the R interface to Stan.
829 Stan Development Team, 2020. RStan: the R interface to Stan.
830 van Bunnik, B.A., Ssematimba, A., Hagenaars, T.J., Nodelijk, G., Haverkate, M.R., Bonten, M.J.,
831 Hayden, M.K., Weinstein, R.A., Bootsma, M.C., De Jong, M.C., 2014. Small distances can
832 keep bacteria at bay for days. Proc Natl Acad Sci U S A 111, 3556-3560.
833 van Elsas, J.D., Semenov, A.V., Costa, R., Trevors, J.T., 2011. Survival of Escherichia coli in the
834 environment: fundamental and public health aspects. Isme j 5, 173-183.
835 Velthuis, A.G., Bouma, A., Katsma, W.E., Nodelijk, G., De Jong, M.C., 2007. Design and analysis of
836 small-scale transmission experiments with animals. Epidemiol Infect 135, 202-217.
837 Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. P t 40, 277-283.
838 Vogwill, T., MacLean, R.C., 2015. The genetic basis of the fitness costs of antimicrobial resistance: a
839 meta-analysis approach. Evol Appl 8, 284-295.
840 Wickham, H., 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.
841 Wickham, H., François, R., Henry, L., Müller, K., 2021. dplyr: a grammar of data manipulation.
842 Wickham, H., Girlich, M., 2022. tidyr: tidy messy data.
843 Wilke, C.O., 2020. cowplot: streamlined plot theme and plot annotations for 'ggplot2'.
844 Wilson, H., Török, M.E., 2018. Extended-spectrum β-lactamase-producing and carbapenemase-
845 producing Enterobacteriaceae. Microb Genom 4.

bioRxiv preprint doi: https://doi.org/10.1101/2023.02.21.529369; this version posted February 21, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

846 World Health Organization, 2019. Critically important antimicrobials for human medicine, 6th revision.
847 World Health Organization, Geneva.
848 Wu, G., Day, M.J., Mafura, M.T., Nunez-Garcia, J., Fenner, J.J., Sharma, M., van Essen-Zandbergen,
849 A., Rodríguez, I., Dierikx, C., Kadlec, K., Schink, A.K., Chattaway, M., Wain, J., Helmuth, R.,
850 Guerra, B., Schwarz, S., Threlfall, J., Woodward, M.J., Woodford, N., Coldham, N., Mevius,
851 D., 2013. Comparative analysis of ESBL-positive Escherichia coli isolates from animals and
852 humans from the UK, The Netherlands and Germany. PLoS One 8, e75392.
853 Zhou, R., Fang, X., Zhang, J., Zheng, X., Shangguan, S., Chen, S., Shen, Y., Liu, Z., Li, J., Zhang, R.,
854 Shen, J., Walsh, T.R., Wang, Y., 2021. Impact of carbapenem resistance on mortality in
855 patients infected with Enterobacteriaceae: a systematic review and meta-analysis. BMJ Open
856 11, e054971.
857