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Antimicrobial use, biosecurity, herd characteristics, and antimicrobial resistance in indicator *Escherichia coli* in ten Finnish pig farms

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Abstract

We investigated connections between antimicrobial use (AMU), biosecurity, and the numbers of pigs and staff in ten Finnish farrow-to-finish herds. Data on AMU in each herd were collected for 12 months. AMU was quantified as treatment incidences per 1000 days at risk (TI) using the consensus defined daily dose calculation. Biosecurity was scored using the Biocheck.UGent™ system. We also examined antimicrobial resistance patterns of indicator *E. coli* isolated from faeces of selected pigs. In each herd, two groups of five pigs were formed: 1) antimicrobial treatment group (ANT: at least one pig in the litter was identified as sick and treated with antimicrobials) and 2) non-antimicrobial

treatment group (NON: the litter was not medicated). Faecal samples were taken from these pigs at 5 and 22 weeks of age, cultured, and indicator *E. coli* isolates were tested for antimicrobial susceptibilities. The AMU varied considerably between the herds. Altogether, most of the antimicrobial treatment courses were assigned to weaned piglets. When AMU was quantified as TIs, suckling piglets had the highest TI (mean 46.6), which was significantly higher ($P < 0.05$) than TIs in fatteners and breeders (9.3 and 7.3, respectively). The difference between TI in suckling and TI in weaned piglets (19.1) was not statistically significant. There was a tendency for a negative correlation between the TI in breeders and the number of sows ($r = -0.56$, $P = 0.09$). Larger herds had higher external biosecurity scores than smaller herds (LS-means; 72 vs. 66, $P < 0.05$). The proportions of *E. coli* isolates resistant to at least one antimicrobial were higher in pigs at 5 weeks than in pigs at 22 weeks of age (Binomial proportion means; 40.5% vs. 15.5%, $P < 0.05$); as well as proportions of isolates resistant to at least three antimicrobial classes (23.0% vs. 3.7%, $P < 0.01$). These proportions did not differ between the ANT and NON groups at either 5 or 22 weeks of age ($P > 0.05$). We found few connections: enhanced external biosecurity levels found in the large herds co-occurred with lower use of antimicrobials and herds with low biosecurity scores - especially in the internal subcategories - appeared to have higher proportions of resistant isolates. Conclusively, we suggest that enhancing internal biosecurity might contribute to a reduction in the spreading of antimicrobial resistance in pig herds.

Keywords: Pig production, Antimicrobial treatment, Technical unit, Herd size, Biocheck, *E. coli* isolate, Finnish herd

1. Introduction

Antimicrobials have been used in pigs for decades mainly for the treatment and prevention of infectious diseases, as well as for growth promotion. The use of antimicrobials in animals has played a crucial role in the development and spread of antimicrobial resistance (AMR) (e.g. Burow and Käsbohrer, 2017; ECDC et al., 2017; Holmes et al., 2016; Ungemach et al., 2006). Consequently,

approaches to prudent use of antimicrobials at the herd-level have become one of the major interests in pig production. Biosecurity is the implementation of measures that aim to prevent and decrease the spreading of infectious agents within production animal farms. External biosecurity aims to prevent the entering of pathogens to a herd and by internal biosecurity measures the dissemination within a herd is reduced (e.g. Laanen et al., 2013; Sahlström et al., 2014). There is some evidence that antimicrobial usage (AMU) can be reduced by improving biosecurity of the pig herds (Collineau et al., 2017; Laanen et al., 2013; Postma et al., 2017, 2016). In Finland, however, the on-farm biosecurity has been reported to be on a low level (Sahlström et al., 2014), while AMU for food producing animals - including pigs - has also been very low compared to other European countries (ESVAC, 2020; Grave et al., 2014). Hence, the association between AMU and biosecurity in Finnish pig herds remains uncertain.

In addition to the observed AMU, the history of antimicrobial use at herd-level can play a significant role in the prevalence of AMR in pig microbiomes. The microbiome and resistome in the piglet gut are most likely passed down from the sow (Belloc et al., 2005; Callens et al., 2015; Mathew et al., 1998). Antibiotic resistance genes are often incorporated into mobile genetic elements that allow resistance gene carriage with low fitness costs to bacteria (Davies and Wales, 2019; Partridge et al., 2018; Vogwill and Maclean, 2015) and also certain chromosomal mutations conferring resistance can be very cost-efficient to bacteria (Luo et al., 2005; Zeitouni and Kempf, 2011). As a consequence, resistance features can be persistent in animal microbiomes irrespective of the current antimicrobial treatment incidences (Andersson and Hughes, 2011; Davies and Wales, 2019). Davies and Wales (2019) have subsequently suggested that in order to reduce the AMR, it would be important to investigate measures such as (internal) biosecurity that could have potential in reducing the dissemination and persistence of resistant bacteria in pig herds.

Although the AMU level is reportedly low in Finland, the national monitoring data from 2017 still showed that resistance against tetracycline (18%), sulfamethoxazole (12%), trimethoprim (11%) and ampicillin (9%) was somewhat common in indicator *Escherichia coli* (*E. coli*) isolated from pig caecal samples upon slaughter (FINRES-Vet 2016-2017, 2018). Similar as well as higher resistance

proportions have been identified in many other European countries (EFSA, 2019). To find additional methods to reduce the prevalence of AMR, also other potential factors than measures for limiting AMU should be examined, such as farming practices.

We investigated the associations between herd-level AMU during the year prior to the initiation of the study, biosecurity statuses, and herd characteristics (i.e. the numbers of pigs and herd staff) of ten Finnish farrow-to-finish pig herds. Furthermore, we conducted a cohort study in each herd, in order to examine the resistance patterns of indicator *E. coli* from faeces of pigs at 5 and 22 weeks of age, and their associations with antimicrobial treatments of the sampled animal groups and herd biosecurity. We hypothesized that improved overall biosecurity of the herds would be associated with lower herd-level AMU, and that the proportions of resistant indicator bacteria would be lower in herds with high biosecurity level. We also expected that proportions of resistant isolates would differ between the sampled pigs depending on the antimicrobial treatments.

2. Materials and methods

The study procedure was approved by the Ethical committee of the Viikki Campus Research, University of Helsinki (7/2016).

2.1. Study herds and design

This was an observational study following two animal cohorts in commercial herds during their normal production. The study included a convenience sample of seven farrow-to-finish herds and three production chains; the latter consisted of three piglet-producing herds, and three finishing herds rearing their piglets until slaughter. In the present study, all ten production chains are considered as farrow-to-finish herds. All finishers were transported to the same slaughterhouse located in Western Finland. The inclusion criteria for the study herds were: 1) piglets were born in herds consisting of 50 to 500 sows, and 2) the farmers reported all AMU data to the National Health Classification Registry (Sikava), the ongoing surveillance program of AMU in Finnish pigs. The herds participating in this

study were abbreviated from A to J in order of the first visiting date. The herds were visited three times between December 2016 and October 2017.

The study aimed to include (in the follow-up) at least one medicated and one non-medicated litter in each of the ten herds. To ensure this, the farmer of each herd selected four litters approximately two weeks of age at the time of the first herd visit. The researcher assigned the chosen litters into one of two groups, ANT (antimicrobial treatment group, one litter per herd) or NON (non-antimicrobial treatment group, three litters per herd). The ANT litters contained at least one piglet that had been identified as sick and subsequently medicated with antimicrobials, whereas no piglets in the NON litters had been medicated with antimicrobials. Three NON litters were initially included in order to have at least one non-medicated litter remaining in each herd at the time of the last sampling. The NON litters were separately housed in pens without the possibility of physical contact with the ANT litters or housed in separate rooms. Up to five female piglets of each litter (one ANT and three NONs) were then indiscriminately selected by the researcher and marked with ear tags for identification in further faecal samplings. At weaning (i.e. approximately four weeks of age), the selected piglets from the NON litters were housed only with pigs weaned from the other NON litters, whereas the selected piglets from the ANT litters were housed with pigs weaned from any litter irrespective of antimicrobial treatment. If antimicrobial treatment was required for any pig in the NON litters, the pig was removed from this litter before medication and excluded from the study. Of the ten study herds, Herds E and H had no pigs treated with antimicrobials before the first sampling. Thereafter, at least one pig of the ANT litter in Herd H was treated with antimicrobials between the first and second samplings. In Herd E, none of the selected groups received antimicrobials throughout the entire sampling period.

2.2. Data collection

2.2.1. Herd characteristics

The numbers of pigs in the study herds were collected through the Finnish Swine Registry system authorized by the Finnish Food Authority (Ruokavirasto, former Finnish Food Safety Authority). The

system provides the number of pigs raised in different age categories and is updated monthly based on the farmer's report. The total number of animals in each age category was calculated by adding up the numbers of the pigs recorded every month in the year prior to the initiation of the study, and dividing by the defined duration of each age category, i.e. suckling piglets = 1 month, weaners = 1.5 months, fatteners = 4.5 months, sows, gilts or boars (hereafter breeders) = 12 months. The durations were set as normal production, following the suggestion of the farmers in the study herds.

2.2.2. Antimicrobial use and quantification

The caretakers of the herds collected the use of antimicrobials for individually ear-marked follow-up pigs in ANT litters in separate sheets for the entire study period and gave them to the researchers. All use of antimicrobials that included all pigs in the study herds was collected for 12 months before the first herd visit through the Sikava program, an online health and welfare register maintained by stakeholders for pig herds in Finland. This data contained information of all pigs in the herds that had received antimicrobials, including medicinal product names, treatment course durations (days), dosage of administered antimicrobials, and the age group of the treated animals. For data analysis, antimicrobials were divided in six different groups: penicillin, other beta-lactams than penicillin, tetracyclines, fluoroquinolones, sulfa-trimethoprim, and macrolide-lincosamide-streptogramin B group (MLSB). Antimicrobial groups and concentrations of active substances were obtained based on the product name. The antimicrobial treatments obtained from the Sikava program could not be linked to individual pigs, because the growing pigs do not have individual ear tags. Thus we considered one antimicrobial treatment course as one medicated pig in calculating the numbers of animals that received antimicrobial treatments (Figure 1), even though the same pig may have been treated more than once. To compare AMU on the herds of different sizes and against other countries, as well as to determine the association of AMU with age groups, herd characteristics, biosecurity and AMR, treatment incidences (TI) using a consensus defined daily dose (DDD) were calculated according to the following formula described by Timmerman et al. (2006).

$$\mathrm{TI} = \frac{\text{Total amout of active substances administered (mg)}}{\mathrm{DDD}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) \times \text{number of days at risk} \times \text{number of animals at risk} \times \text{standard weight (kg)}} \times 1000\ pigs\ at\ risk$$

The TI is an indicator of AMU, which quantifies the number of animals out of a theoretical group of 1000 animals administered daily with antimicrobials within a defined risk period of every age group under consideration (number of days at risk). A consensus DDD list was obtained from Postma et al. (2015) and it takes into account also the long-acting antimicrobial products using a factor that represents the duration of activity of long-acting products. The days at risk for the different age categories were set as suckling piglets = 28 days, weaners = 42 days, fatteners = 130 days, breeders = 365 days. Standard weights of pigs in each age category were set as suckling piglets = 2 kg, weaners = 7 kg, fatteners = 35 kg, breeders = 220 kg. The TI for pigs from birth until slaughter (TI 200) was calculated using a standardized life span of 200 days at risk as in Postma et al. (2016), that is, the data for numbers of animals, their weights and numbers of days at risk were obtained from the data on suckling piglets, weaners and fatteners and these numbers were placed in the formula. In addition to the AMU data collected from the Sikava program, the farmers kept separate records of the antimicrobial medications of the study pigs and the pigs in the same pen in the ANT group (Table 1).

2.2.3 Biosecurity scoring of the herds

The biosecurity of the herds was evaluated using the Biocheck.UGent™ scoring system (available at www.biocheck.ugent.be) during the second herd visit. Briefly, the Biocheck.UGent™ consists of six external and six internal biosecurity subcategories with 109 questions. The subcategories are weighted based on the likelihood of introduction and spread of infectious diseases via different routes. The scale ranges from 0 to 100, indicating 'total absence of biosecurity' to 'perfect biosecurity', respectively. The Biocheck.UGent™ questionnaire was translated into Finnish for the farmers of the study herds to avoid language difficulty. The responses were used for scoring biosecurity statuses of the herds through the Biocheck.UGent™ webpage.

2.3. Faecal sample collection, isolation and antimicrobial susceptibility testing of *E. coli*

Faecal samples (approximately 20 g) were collected from the rectum of the selected pigs at approximately 5 and 22 weeks of age in each herd. At the first sampling, faecal samples were collected from each of the five pigs in the ANT group, if available, and up to the 15 pigs in the NON group. At the second sampling, faecal samples were collected from the same pigs as in the first sampling in the ANT group, and from up to five of the same pigs in the NON group. Of the samples collected from the NON pigs, up to five samples originating from the same pigs were selected for culturing. All samples were transported to the laboratory in a refrigerated box, and stored at -80 ℃ until culturing. The samples were spread on chromogenic agar (Brilliance™ E. coli/coliform Selective agar, Oxoid, United Kingdom). After an overnight incubation at 37℃, up to three typical, lactose-positive purple *E. coli* colonies per pig were selected and sub-cultured on blood agar. Up to three isolates per pig per sampling were stored at -80℃ in Bacto™ Brain Heart Infusion (Becton, Dickinson and Company, France) broth with 15% glycerol until susceptibility testing. If typical purple *E. coli* colonies were not present, pink colonies were selected and the species were later confirmed with MALDI-TOF (Microflex LT, Bruker Daltonic Gmbh, Germany).

The susceptibility testing data of Herd B was excluded since the untreated pigs (NON group) and pigs treated with antimicrobials (ANT group) were not housed separately from each other as was instructed by the researchers. Antimicrobial susceptibility testing of *E. coli* isolates was performed by broth microdilution using Sensititre™ plates (EUVSEC, Trek Diagnostic Systems, UK) following the CLSI standard (CLSI, 2013). The following antimicrobials were included: ampicillin, azithromycin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, tigecycline and trimethoprim. Susceptibility results (minimum inhibitory concentrations, MICs) were interpreted as resistant (non-wild type) or sensitive (wild type) according to the current epidemiological cut-off values (ECOFFs, available in October, 2018) as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The only exception was azithromycin and tigecycline, for which there were no cut-off values available. If an isolate showed resistance to cefotaxime, ceftazidime or meropenem, confirmation of the phenotype was further tested using a Sensititre™ EUVSEC2 plate, which included the following antimicrobials:

cefepime, cefotaxime, cefotaxime/clavulanic acid, cefoxitin, ceftazidime, ceftazidime/clavulanic acid, ertapenem, imipenem, meropenem and temocillin.

2.4. Statistical analysis

SAS v.9.4. (SAS Institute, 2012) was used for statistical processing of the data for herd characteristics (animal data, farm workers and the experience of the farmers), TIs, biosecurity scores, proportions of AMR, and their correlations. The UNIVARIATE procedure with the Shapiro-Wilk test was used to test normality. All correlations in the study were tested using Spearman rank correlation coefficients.

The TTEST procedure was used to test whether the average number of sows in the study herds was different from the Finnish average. A Poisson distribution with a logarithmic link function was fitted to the GLIMMIX procedure to analyse differences between the TIs in different age groups. The age group was used as a fixed effect and the herd as a random effect. Differences in the least squares means of TIs between the age group were tested for significance using the Tukey-Kramer procedure. Differences in average scores between internal and external biosecurity of the study herds were tested using paired TTEST procedures. The GLM procedure was used to determine differences in the biosecurity scores according to the herd size (as the categorical independent variable) in which the herds were divided by the higher or lower median number of total animals.

A binomial distribution with a logit model was fitted to the GLIMMIX procedure to analyse the influences of antibiotic treatment and sampling times on the proportions of AMR *E. coli* isolates from the sampled pigs. The model included the proportions of resistance as a dependent variable (binomial proportion: the number of resistant isolates for at least one antimicrobial agent or at least three antimicrobial classes, divided by the total number of isolates used on susceptibility testing), and the group (ANT vs. NON), sampling period and their interactions as independent variables. When determining the resistance to at least three antimicrobial classes, antimicrobials were classified to different classes as follows: aminopenicillins, 3rd generation cephalosporins, aminoglycosides, amphenicols, quinolones, polymyxins, sulphonamides, trimethoprim and tetracyclines. The herd was included as a random effect. Data for the ANT group in Herd E at both sampling periods, as well as

for the ANT group in Herd H at five weeks of age were processed as missing values, since the pigs from those groups had not been treated with antimicrobials and thus did not meet the study procedure. The Kenward-Rogers estimation of degrees of freedom was used to account for the unequal number of isolates per herd. Significant differences of the least squares means between the ANT and NON pigs at different sampling times were determined by the Tukey-Kramer test. Data are presented as means with SEMs on the binomial proportion scale.

The associations between resistance in indicator *E. coli* isolates, herd, antimicrobial treatment in the ANT pigs (Table 1), sampling time and the overall biosecurity scores were analysed with Permutational Multivariate Analysis of Variance (PERMANOVA) using the function *adonis* in the vegan package (Oksanen et al., 2016) and RStudio (RStudio Team, 2018). The numbers of resistant isolates per tested antimicrobial and herd were collected to a response variable table: the rows had the sample identifier containing information on herd, group and sampling time, and the tested antimicrobials were in the columns. The numbers in the cells were the proportions of resistant isolates. The explanatory variable table had the corresponding sample identifier as the response variable table in rows. The columns included herd, antimicrobial treatment in ANT group (0 = not medicated at all, 1 = medicated before the first sampling, 2 = medicated between the first and second sampling, 3 = medicated before the first and second sampling), group (ANT or NON), sampling time (5 or 22 weeks) and the biosecurity scores (overall external, internal and total, as categorical variables). The biosecurity subcategory scores were not included with the explanatory variables.

3. Results

3.1. Herd characteristics

The average number of sows in the ten study herds (Table 2) was not significantly different from the average of Finnish herds reported by the Finnish Food Authority in 2017 (209 vs. 151, $P = 0.15$). Experience of the farmers (excluding employees) for pig production varied from 5 to 35 years (Table

257 2). The number of pigs per staff correlated with the number of sows ($r = 0.83$, $P < 0.01$) and with the
258 total number of pigs ($r = 0.88$, $P < 0.001$) in the herds.

259 3.2. AMU and antimicrobial treatment incidences

260 Penicillin, beta-lactams other than penicillin, sulfa-trimethoprim and tetracycline were used in all age
261 groups (Figure 1, Figure 2). Most of the antimicrobial treatment courses were administered to weaners
262 (Figure 1). For all animal groups except weaners, the TIs were highest with the use of penicillin or
263 beta-lactams other than penicillin (Figure 2). Treatment incidences varied considerably also between
264 the herds (Table 3). When comparing the TIs between the age groups, the TI for suckling piglets was
265 significantly higher than the TI for fatteners or breeders ($P < 0.05$ for both), whereas the TI for
266 weaners did not significantly differ from the other age groups (Table 3). The TI for suckling piglets
267 tended to correlate with the TI for weaners ($r = 0.60$, $P = 0.07$). There were no correlations in TIs
268 between the other age groups. The TI for breeders tended to correlate negatively with the number of
269 sows in the herds ($r = -0.56$, $P = 0.09$). The number of pigs per staff correlated negatively with the TI
270 for weaners ($r = -0.68$, $P < 0.05$), and tended to correlate negatively with the TI for breeders ($r = -$
271 0.62, $P = 0.05$).

272 3.3. Biosecurity

273 The external biosecurity score was higher than the internal biosecurity score in the study herds ($P <$
274 0.001, Table 4). Of all the subcategories, ‘cleaning and disinfection’ and ‘compartmentalization and
275 use of equipment’ ranked the lowest scores (Table 4). The total, external, and internal biosecurity
276 scores did not correlate with the TIs in different age groups in the herds. The external biosecurity
277 scores were higher in the group of herds with a higher median of total number of pigs than in the one
278 with a lower median of total number of pigs (LS-means ± SE; 72 ± 1.3 vs. 66 ± 1.3, $P < 0.05$).

279 3.4. Antimicrobial resistance of indicator *E. coli* from the selected pigs in two groups

280 All herds except Herd B were included to test for AMR in *E. coli*. Among all indicator *E. coli* isolates
281 studied (n = 500), a total of 366 (73%) isolates were susceptible to the tested antimicrobials.
282 Resistance was the most common against sulfamethoxazole (18%, n = 89), tetracycline (16%, n = 81),

283 trimethoprim (14%, n = 69), and ampicillin (9%, n = 44) (Table S5, supplementary material). The
284 resistance phenotypes somewhat varied between herds, and altogether 23 different resistance
285 phenotypes were found (Table S5, supplementary material). The most common resistance profile was
286 resistance to sulfamethoxazole, tetracycline, and trimethoprim (35/134, 26% of all the resistant
287 isolates). Resistance to only tetracycline was also commonly found (23/134, 17% of all the resistant
288 isolates). Extended-spectrum beta-lactamase (ESBL) phenotype or meropenem resistant *E. coli* were
289 not detected. However, isolates with a presumptive AmpC phenotype (e.g. resistance to cefotaxime or
290 ceftazidime and cefoxitin) were found in two herds (Herds F and H).

291 The ANT and NON group had no significant differences in proportions of *E. coli* isolates resistant to
292 at least one antimicrobial at both 5 weeks ($F_{1,29}$ = 0.86, P = 0.36, Figure 3) and 22 weeks of age ($F_{1,29}$
293 = 1.31, P = 0.26, Figure 3), irrespective of the sampling time (Means ± SEMs; 33.5% ± 7.6 vs. 19.8%
294 ± 5.8, respectively, P = 0.16). However, there were significant differences between the pigs at 5 and
295 22 week of age, irrespective of the treatment group (Means ± SEMs; 40.5% ± 7.2 vs. 15.5% ± 5.2,
296 respectively, $P < 0.05$). The proportion of *E. coli* isolates resistant to at least one antimicrobial from
297 the pigs at 22 weeks tended to decrease in the ANT group ($F_{1,29}$ = 3.57, P = 0.07, Figure 3) and in the
298 NON group ($F_{1,29}$ = 3.34, P = 0.08, Figure 3), when compared with those at five weeks. Similarly, the
299 proportions of *E. coli* isolates resistant to at least three antimicrobial classes did not differ between the
300 ANT and NON groups at both five weeks ($F_{1,29}$ = 0.58, P = 0.45, Figure 3) and 22 weeks of age ($F_{1,29}$
301 = 1.46, P = 0.24, Figure 3), irrespective of the sampling time (Means ± SEMs; 13.6% ± 4.6 vs. 6.8% ±
302 3.3, respectively, P = 0.25). On the other hand, the proportions of *E. coli* isolates resistant to at least
303 three antimicrobial classes from the pigs at 5 weeks were higher than those at 22 weeks of age in both
304 the ANT ($F_{1,29}$ = 5.92, $P < 0.05$, Figure 3) and NON groups ($F_{1,29}$ = 4.40, $P < 0.05$, Figure 3). The
305 difference also existed between the pigs at 5 and 22 weeks of age, irrespective of the treatment groups
306 (Means ± SEMs; 23.0% ± 4.7 vs. 3.7% ± 2.1, respectively, $P < 0.01$). Higher proportions of indicator
307 *E. coli* isolates resistant to ampicillin, sulfamethoxazole and trimethoprim were found at 5 weeks
308 compared to 22 weeks of age, irrespective of the antimicrobial treatment groups ($F_{1,29}$ = 4.95, $P <$
309 0.05, $F_{1,29}$ = 6.13, $P < 0.05$, and $F_{1,29}$ = 9.47, $P < 0.01$, respectively, Table S6, supplementary

material). The ANT pigs only showed a tendency of higher proportions of isolates resistant to sulfamethoxazole, when compared with the NON pigs, irrespective of the sampling period ($F_{1,29}$ = 3.30, P = 0.08, Table S6, supplementary material).

3.5. Associations between antimicrobial resistance in indicator *E. coli*, antimicrobial use, herd characteristics and biosecurity

The biosecurity scores of the herds and the proportions of resistant isolates from the pigs in the ANT and NON groups were visualized using ggplot2 (Wickham, 2009) and RStudio (RStudio team, 2018). The data were organized into a composite figure in the order of increasing AMU (Figure 4). AMU was neither clearly lower in herds that had higher biosecurity scores, nor clearly higher in herds that had lower biosecurity scores (overall external, internal and total or in subcategories, Figure 4A, B). Additionally, higher AMU in herds could not be clearly reflected as the higher proportion of resistant isolates (Figure 1, Figure 4A, C). Despite this, herds that had low scores – especially in internal biosecurity subcategories - appeared to have higher proportions of resistant isolates (Figure 4B, C). The PERMANOVA analysis indicated that associations between observed resistance and the overall internal, external or total biosecurity score were not significant ($P > 0.05$). Since we were not able to use PERMANOVA to analyse the influence of the biosecurity subcategories on the resistance, the possible connection (that was) observed visually could not be confirmed by statistical analyses. The herd explained most of the variance in the proportions of resistant isolates (18%), followed by the sampling time (4%) and whether the pigs in the ANT group were medicated (3%) (Table 7).

4. Discussion

Our results were consistent with previous findings that AMU as TIs was higher for younger pigs (e.g. Sjölund et al., 2016), and that higher proportions of AMR in indicator *E. coli* were found from younger pigs (Burow et al., 2019). However, in contrast to Burow et al. (2019), we did not detect significant differences in the proportions of resistant *E. coli* isolates in response to antimicrobial treatment. Although our study herds showed considerable variation in AMU, we found that larger

336 herds were likely linked to lower AMU for breeding pigs, and they had also enhanced external
337 biosecurity statuses, similarly as previously reported by Laanen et al. (2013). Thus, further
338 examination of the connections between external biosecurity and AMU might provide insight into
339 practices that could reduce AMU in pig herds. Interestingly, our figures implied that there could be a
340 connection between low scores in internal biosecurity subcategories and higher proportions of AMR in
341 indicator *E. coli* from the sampled pigs. Although this finding is based on only visual observation and
342 not on results obtained using statistical analyses, to the best of our knowledge, this is the first report
343 possibly linking higher biosecurity directly to lower prevalence of AMR in pigs

344 Our study design had limitations, such as small sample size (ten commercial herds). A small number
345 of herds and large variation in antimicrobial treatments between the herds could have hindered us from
346 detecting some associations. However, the variation in AMU also suggests that measures aiming to
347 lower the AMU would be more efficient if they would be tailored to those herds that have problems
348 with bacterial infections. In addition, the pigs in the ANT group were medicated with diverse
349 antimicrobials in different herds, and faecal samples from the selected pigs were obtained from only
350 one medicated (ANT) and one non-medicated (NON) group in each herd. Accordingly, we could not
351 elucidate whether the herd-level AMU and biosecurity scores were significantly associated with the
352 proportions of resistant *E. coli* isolates from the sampled pigs.

353 Suckling piglets of our study had the highest TI, which is similar to the Swedish study by Sjölund et
354 al. (2016). The inconsistency between the highest TI in suckling piglets and the observation that most
355 of the treatment courses were assigned to weaned piglets may have been partly caused by the
356 difference in the used antimicrobials for different age groups and the difficulties in administrating the
357 right dosage. Suckling piglets were mostly treated with penicillin or beta-lactams other than penicillin,
358 whereas macrolides or fluoroquinolones were administered to weaned piglets. Beta-lactams are used
359 in larger quantities of active substances than macrolides and fluoroquinolones. Even though the DDD-
360 values of penicillin and other beta-lactams are high, they may not reflect the common situation of
361 overdosing on these drugs to small piglets. Overdosing of especially injectable antimicrobials to
362 suckling piglets can occur frequently, because of high concentrations of the active substances in the

363 commercial products. Despite the inconsistency, the difference in TIs between suckling and weaned
364 piglets was moderate and not statistically significant. Nevertheless, our finding demonstrates that the
365 used antimicrobials can contribute considerably to the results, if TIs between different age groups are
366 compared.

367 Younger animals (suckling and weaned piglets) had higher TIs than older animals (fatteners and
368 breeders). The more immature immune system of young animals can increase the risk of infection and
369 might increase the AMU for younger pigs. We found only a tendency of correlation between TI of
370 suckling and TI of weaners, while Sjölund et al. (2016) demonstrated several positive associations in
371 the TIs between the different age groups. The overall AMU was low in our study herds. We found that
372 the TIs for breeders and growing pigs (i.e. TI 200) were lower than the ones found in Belgian, French,
373 German and Swedish herds (Sjölund et al., 2016). Among the countries participating to the European
374 Surveillance of Veterinary Antimicrobial Consumption (ESVAC) monitoring programme, Finland has
375 commonly placed with the four lowest user countries (ESVAC, 2020), possibly partly because in-feed
376 group prophylaxis with antimicrobials is not implemented in Finland. These factors can explain the
377 overall low AMU in our study herds compared to the data from countries presented by Sjölund et al.
378 (2016).

379 Large herds were linked to lower TI for their breeding pigs. Gardner et al. (2002) suggested that large
380 herds have usually adopted management systems including heightened biosecurity measures
381 associated with lower risk factors of disease transmission. Thus, large herds could also implement
382 measures that would be beneficial for reducing AMU in pigs, such as stringent biosecurity. We indeed
383 noticed that the large herds in our study had also enhanced external biosecurity statuses, as was also
384 previously shown by Laanen et al. (2013). On the other hand, we identified more pigs per farm staff in
385 the larger herds, which could make detecting the sick animals more demanding for the farm staff,
386 potentially leading to decreases in total detection and thereby treatment rates. Our result that the higher
387 ratio of pigs to farm staff correlated with the lower TIs for weaners (and tended to correlate with lower
388 TIs for breeders) would support also this assumption, which therefore cannot be ruled out.

389 Similar to the Swedish study by Backhans et al. (2016), we could not find significant associations
390 between the biosecurity scores and AMU. This is contrary to the Belgian studies, which demonstrate
391 their inverse association (e.g. Laanen et al., 2013; Postma et al., 2016). The overall low AMU in our
392 study herds, compared to those in the Belgian study herds, could be one reason for the different
393 outcomes. The vast majority of the medications in our data were administered parenterally for single
394 pigs only, not for groups. Laanen et al. (2013) explained the negative correlation between AMU and
395 internal biosecurity with the need for in-feed group prophylaxis if the internal biosecurity is poor. It is
396 also worth noting that the biosecurity scores of our study herds were lower than the scores in other
397 European countries, including Belgium (e.g. Postma et al., 2016; Raasch et al., 2018). Especially
398 internal biosecurity scores were low and were attributed to very low scores of 'measures between
399 compartments and the use of equipment' and 'cleaning and disinfection' among the internal
400 biosecurity subcategories. According to our questionnaire results, 80% of the farmers had not applied
401 the disinfection measures after cleaning of the stables. Perhaps both, the relatively low AMU and low
402 implementation of disinfection measures, partially reflect the overall favourable pig disease situation
403 in Finland (Finnish Food Authority, 2017), as suggested by Visschers et al. (2015).

404 We found that isolates from 22-week-old pigs showed less resistance than isolates from 5-week-old
405 pigs and that the herd-level AMU was also lower in fatteners than in suckling piglets. Since it is
406 generally accepted that antimicrobial use selects resistant bacteria in animal microbiomes (e.g. Burow
407 et al., 2014; Van Den Bogaard and Stobberingh, 2000) and there is evidence that the proportion of
408 resistant indicator bacteria can decrease when AMU is reduced (AgersoØ and Aarestrup, 2013; Belloc
409 et al., 2005; Burow et al., 2019; Dorado-García et al., 2016), one could easily conclude that the lower
410 prevalence of resistant isolates would reflect the lower herd level AMU for older animals. However,
411 several studies have demonstrated that the gut microbiome of neonatal subjects harbours more
412 resistant bacteria than older subjects, irrespective of antimicrobial exposure (e.g. Bäckhed et al., 2015;
413 Gibson et al., 2016; Miller et al., 2019; Moore et al., 2015; Pärnänen et al., 2018). It thus seems more
414 likely that our results of younger pigs having higher proportions of resistant isolates to single and
415 multiple antimicrobials could be due to the undeveloped gut microbiome, regardless of the higher

herd-level AMU for this age group. In contrast to Burrow et al. (2019), we could not find significant differences in the proportions of resistant indicator *E. coli* isolates between antimicrobial treatment and non-treatment groups. Only a tendency of higher proportion of isolates resistant to sulfamethoxazole was observed in the medicated pig group. Yet, we may not conclude that AMU would not influence the AMR in the pig microbiomes. Munk et al. (2018) demonstrated that the country-level AMU was more associated with herd-level AMR gene abundance than the current treatment incidences in the herds. It therefore seems that the antimicrobial use history could be a significant factor influencing the prevalence of resistant bacteria in pig herds; its influence is probably long lasting and possibly stronger than the effects of antimicrobial treatments taking place in the herds at the time of the sampling.

The connection between biosecurity and AMR in pig herds has been recently discussed (Davies and Wales, 2019) and our visualizations implied a plausible link between poor biosecurity measures, particularly in internal subcategories, and the higher proportions of AMR in *E. coli.* Unfortunately, due to our study limitations, we could not determine if this association was statistically significant. The connection between internal biosecurity and AMR could be explained in light of the principal objective of internal biosecurity, which aims to limit the spread of infectious agents within the herds. However, to the best of our knowledge, the potential for stringent biosecurity measures to reduce the prevalence of AMR in pigs by decreasing bacterial transmission within the herds has not been investigated. As discussed above, the resistance features are persistent in microbiomes (Andersson and Hughes, 2011; Davies and Wales, 2019), and resistant bacteria can be transferred from the dam to litters (Belloc et al., 2005; Callens et al., 2015; Mathew et al., 1998). Therefore, we believe that our visualizations imply that enhancing biosecurity, particularly internal measures, could contribute to lower resistance levels in pig microbiomes.

5. Conclusions

This was the first study to identify associations between antimicrobial use, biosecurity, herd characteristics, and AMR in indicator *E. coli* in Finnish pig herds. We discovered that large herds had better external biosecurity status, and this could in part lead to reduction of AMU in the herds. We found that the herd-level AMU was higher in younger pigs, while higher proportions of AMR in indicator *E. coli* isolates were also found in the younger pigs. However, contrary to our hypothesis, we could not find significant differences in the proportions of AMR in indicator *E. coli* in response to antimicrobial treatment. We suggest that antimicrobial use history and the persistent nature of AMR in herd microbiomes might explain the prevalence of AMR in pig herds, rather than current antimicrobial treatments. Our results also implied that improvements of internal biosecurity measures could reduce the prevalence of AMR by decreasing the spread of bacteria within the pig herds. Therefore, we propose that the potential of enhanced internal biosecurity in AMR mitigation would be addressed in future research projects.

Declaration of Competing Interest

None.

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607 Table 1. Antimicrobials used in the herds during the year before the study began and in the
608 antimicrobial treatment group (ANT) of each herd during the sampling periods[1].

Herd	Antimicrobial groups* used in the herds during 1 year before selecting the study animals (alphabetical order)	Antimicrobial groups used for pigs in ANT during the sampling periods	
		Before 5 weeks of age	Between 5 and 22 weeks of age
A	B-L, Flu, MLSB, Pen, Sul	Pen	No
B	B-L, MLSB, Pen	NA	NA
C	B-L, Flu, MLSB, Pen, Sul, Tet	MLSB	No
D	B-L, Flu, MLSB, Pen, Sul, Tet	MLSB	No
E	B-L, Flu, MLSB, Pen, Sul	No	No
F	B-L, Flu, Pen, Sul, Tet	Pen	No
G	B-L, Pen, Sul	Sul, B-L or both	No
H	Pen, Sul, Tet	No	Sul
I	B-L, Flu, MLSB, Pen, Tet	Flu	Flu
J	B-L, Pen, Sul, Tet	Pen	No

609 [1] The ANT group had at least one piglet treated with antimicrobials in a pen. Faecal samples were
610 collected from the study pigs at approximately 5 and 22 weeks of age.

611 *B-L: Beta-lactams other than penicillin, Flu: Fluoroquinolone, MLSB: Macrolide, Lincosamide or
612 Streptogramin B, Pen: Penicillin, Sul: Sulfa-trimethoprim, Tet: Tetracycline. No: antimicrobial
613 treatments were not used, NA: data for antimicrobial treatment were not available.

614

615 Table 2. Descriptions of the herds included in this study (n = 10).

	Total sum of pigs	**Mean**	**SD**	**Min.**	**Median**	**Max.**
Sows, n	2087	209	115.2	56	238	380
Suckling piglets, n	55750	5575	2996.4	1702	5256	9994
Weaners, n	55514	5015	3232.6	1809	4841	11263
Fatteners, n	15800	1580	1139.9	169	1639	3832
Total pigs, n	129777	12978	7019.8	4003	12129	24337
Total pigs / staff, n[1]		3838	921.7	2001	4043	4867
Experience of farmers, years		23	8.9	5	25	35

616 [1]The number of pigs per staff: total number of pigs divided by the number of farm staff during normal
617 production.

618

619 Table 3. Descriptive information on the antimicrobial treatment incidences (TI) DDD[1] in different age
620 groups of pigs in one year at ten Finnish farrow-to-finish herds.

	Mean	SD	Min.	Median	Max.
TI suckling piglets	46.6[a]	61.2	0.6	36.8	207.0
TI weaners	19.1[ab]	23.7	0.0	13.0	75.8
TI fatteners	9.3[b]	6.6	2.3	7.4	20.7
TI entire growing pigs (TI 200)[2]	16.5	10.5	2.2	16.1	33.3
TI breeders (gilts, sows, boars)	7.3[b]	6.8	0.2	5.1	18.0

621 [1]Treatment incidence (TI) indicates the number of animals out of a theoretical group of 1000 animals
622 treated daily with antimicrobials (per 1000 days). A consensus defined daily doses (DDD) list by
623 Postma et al. (2015) was used.

624 [2]The TI for pigs from birth until slaughter (TI 200) was calculated by using the data on suckling
625 piglets, weaners and fatteners for obtaining the numbers of animals, days at risk and standard weights.

626 [a,b]Different superscripts within column indicate significant differences.

627

628 Table 4. Descriptive results for external and internal biosecurity subcategory scores evaluated
629 according to the Biocheck.UGent™ in ten Finnish farrow-to-finish pig herds.

Subcategory	**Mean**	**SD**	**Min.**	**Median**	**Max.**
Total biosecurity	57	9	45	54	71
External biosecurity	69	4	65	69	76
Purchase of animals and semen	87	9	68	88	100
Removal of animals, manure, carcasses	67	16	26	69	83
Feed, water and equipment supply	45	15	23	48	62
Personnel and visitors	71	23	24	77	100
Vermin and bird control	71	13	50	70	100
Environment and region	61	29	10	68	90
Internal biosecurity	44	17	24	41	72
Disease management	60	21	40	60	100
Farrowing and suckling period	51	15	29	57	64
Nursery unit	57	20	21	57	86
Fattening unit	59	29	21	68	93
Compartmentalization and use of equipment	40	14	21	37	61
Cleaning and disinfection	22	30	0	6	75

630

631

632 Table 7. Permutational multivariate analysis models and their results used for studying the associations
633 between observed resistance in indicator *E. coli* isolates, use of antimicrobials in ANT pigs, group
634 (ANT or NON)[1], and sampling time (5 or 22 weeks of age). Only significant associations are shown.

Tested variables	R^2	**P-value**
Herd	0.18	< 0.001
Sampling time (5 or 22 weeks of age)	0.04	< 0.001
Use of antimicrobials in ANT group (Table 1)	0.03	< 0.001
Group (ANT or NON)	0.004	< 0.05

635 [1]ANT (antimicrobial treatment group): at least one piglet had been medicated with antimicrobials in a
636 pen, NON (non-antimicrobial treatment group): no pigs were medicated with antimicrobials in a pen.

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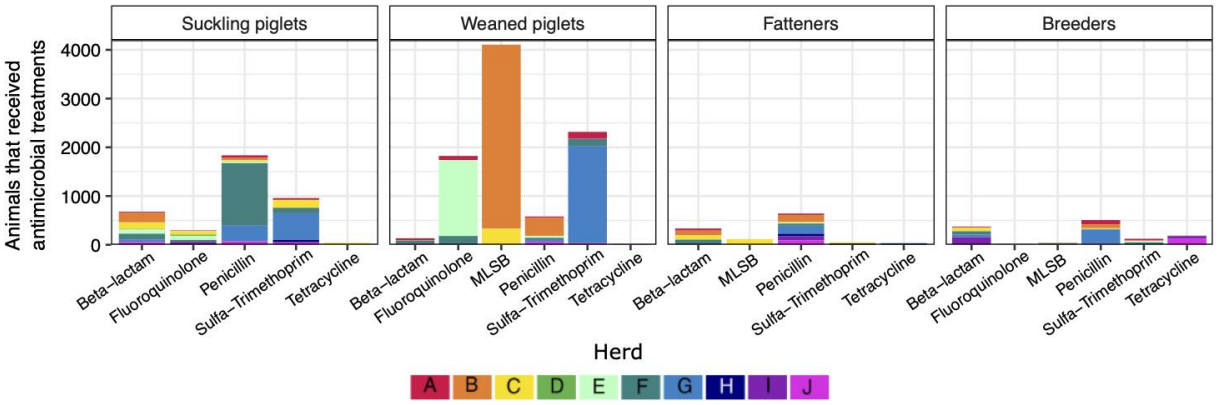
638
639

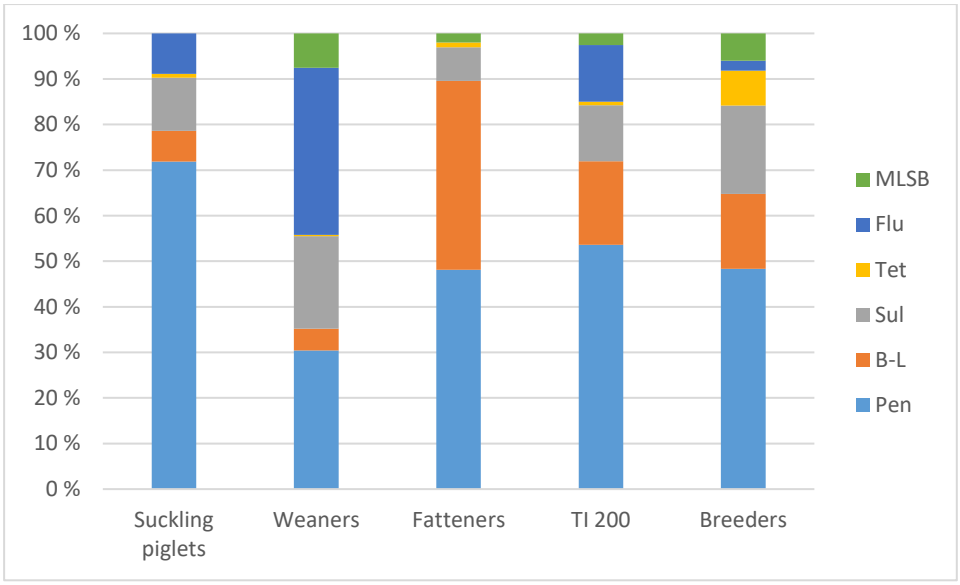


640

641 Figure 1. Numbers of animals that received antimicrobial treatments in each age group and herd.

642 Stacked bars show the herds according to the legend at the bottom.

643



644

645 Figure 2. Proportion of different antimicrobial groups contributing to the total treatment incidence (TI)
646 of different age groups of pigs during one year at ten Finnish farrow-to-finish herds. TI 200 was
647 calculated by using the data on suckling piglets, weaners and fatteners for obtaining the numbers of
648 animals, days at risk and standard weights. MLSB: Macrolide, lincosamide or streptogramin B, Flu:
649 Fluoroquinolone, Tet: Tetracycline, Sul: Sulfa-trimethoprim, B-L Beta-lactams other than penicillin,
650 Pen: Penicillin.

651

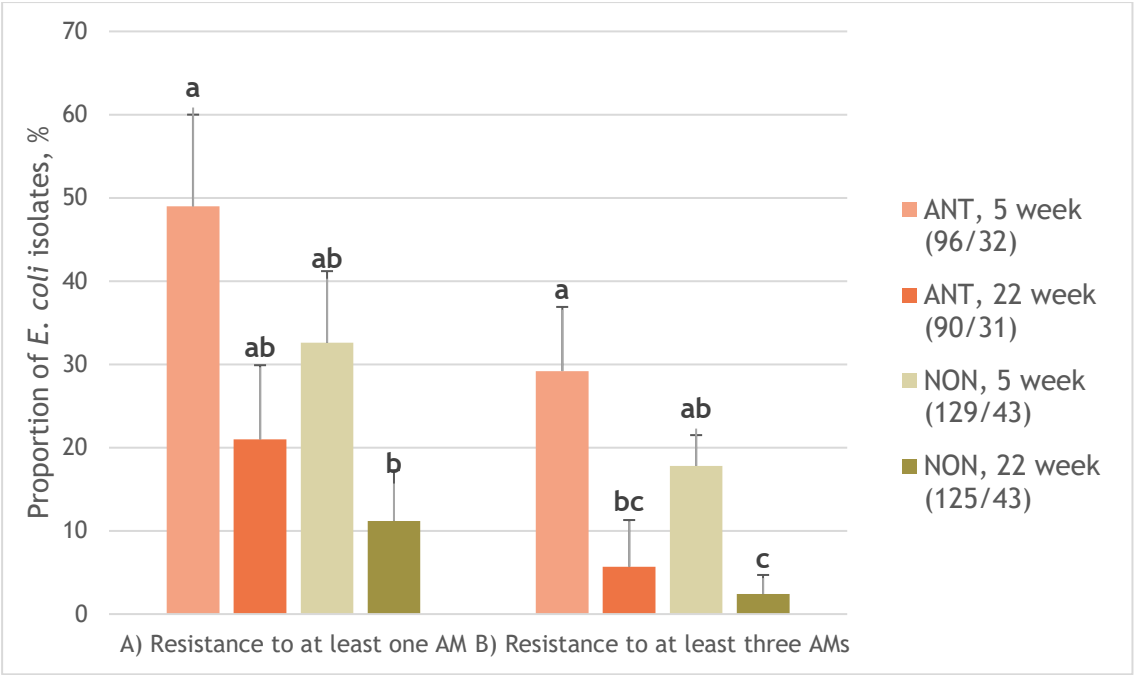
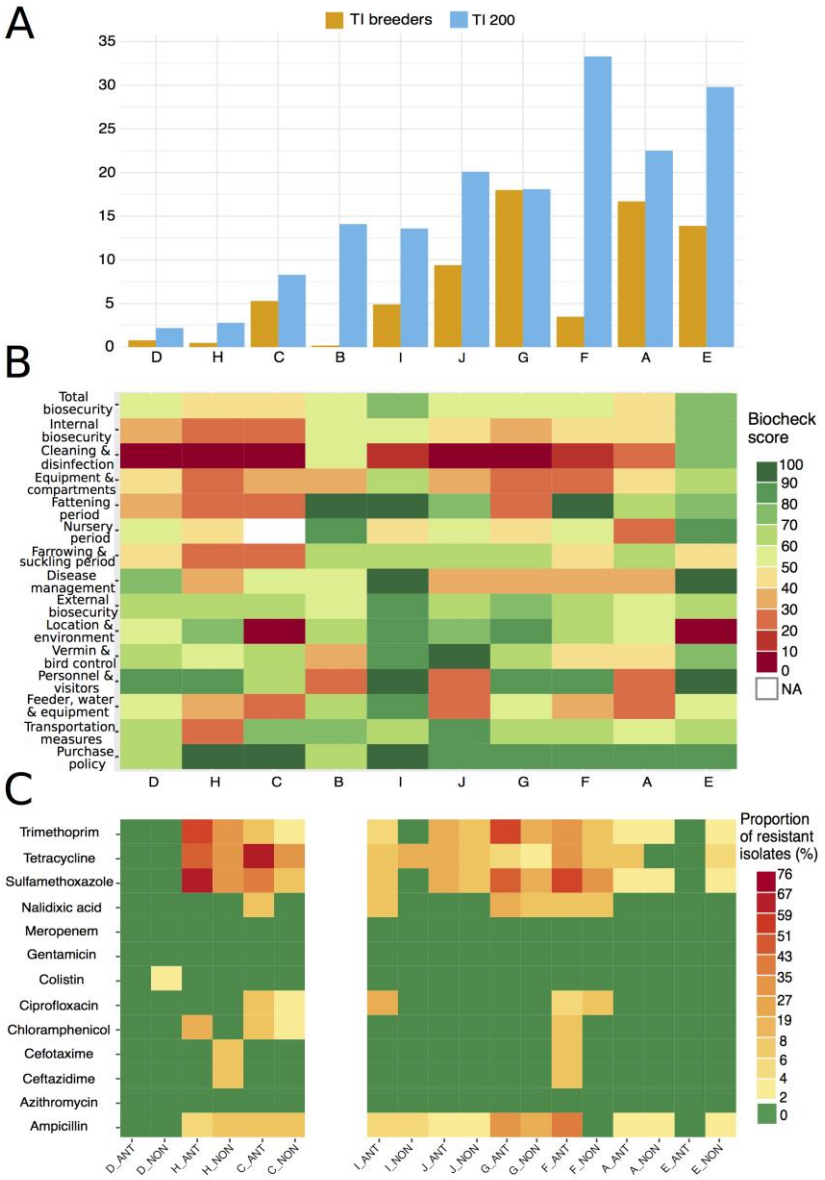


Figure 3. Binomial proportion means of resistant indicator *E. coli* isolates, A) against at least one antimicrobial, B) against at least three antimicrobial classes, in faeces of selected pigs at approximately 5 and 22 weeks of age in nine herds. NON pigs originated from groups that did not receive antimicrobials from birth until slaughter, while ANT pigs were from groups in which at least one pig had been treated with antimicrobials. The numbers in the legend represent the total isolates and the sampled pigs (isolates/pigs). The different letters (a, b, c) indicate that the proportions of resistant isolates were significantly different between variables ($P < 0.05$, for both in A and B).



661

662 Figure 4. A) The treatment incidences (TI) for breeders and pigs from birth until slaughter (TI 200) in
663 each herd, arranged in the order of increasing TI. The TIs were calculated using the data of the pig's
664 antimicrobial use during one year in each of the herds (A-J). B) Heatmap showing the biosecurity
665 scores in each herd. C) Heatmap showing proportions of resistant isolates from the focal pigs
666 originating from groups where at least one pig was treated with antimicrobials (ANT) and from groups
667 receiving no antimicrobials (NON) in each herd. The ANT pigs in herd E had not been medicated, but
668 they were housed with other pigs that might possibly receive antimicrobial treatments after weaning.
669 For all the variability in the treatments of the ANT pigs, see Table 1. The proportions of resistant
670 isolates were calculated using the results from both sampling times (i.e. 5 and 22 weeks of age). Most

671 of the isolates were resistant to two or more antimicrobials. For resistance profiles, see Table S5,

672 supplementary material.

673

674 Supplementary data

675 Table S1. Resistance profiles of all resistant *Escherichia coli* isolates (n = 134) from the nine study
676 herds based on EUCAST ECOFFs.

Resistance profile	**# of isolates (% of all isolates)**	**Herd code (# of isolates)**
SMX-TET-TMP-AMP-CIP-NAL-CHL	3	C(3)
SMX-AMP-CIP-NAL-CHL-FOT-TAZ-FOX	1	F(1)
SMX-TET-TMP-AMP-CIP-CHL	1	C(1)
SMX-AMP-NAL-CHL-FOT-TAZ-FOX	2	F(2)
SMX-TET-TMP-AMP	6	A(1), E(1), F(3), J(1)
SMX-TMP-AMP-NAL	9	G(9)
TET-TMP-AMP-CIP	1	I(1)
SMX-TET-TMP	35 (7%)	C(1), F(6), G(3), H(15), J(10)
SMX-TET-AMP	2	C(1), F(1)
SMX-TMP-AMP	8	A(1), F(1), G(6)
AMP-FOT-TAZ-FOX	5	F(1), H(4)
SMX-TET	8	C(8)
SMX-TMP	3	F(1), G(1), H(1)
SMX-AMP	1	C(1)
SMX-CHL	3	H(3)
TET-TMP	1	C(1)
TET-AMP	1	J(1)
CIP-NAL	6	F(3), I(3)

SMX	8	F(5), I(3)
TET	23 (4.6%)	A(2), C(16), E(1), I(4)
TMP	2	G(2)
AMP	5	C(3), H(1), I(1)
COL	1	D(1)
All resistant	134 (26.8%)	
Susceptible	366 (73.2%)	
All	550	

677 AMP: Ampicillin, TAZ: Ceftazidime, FOT: Cefotaxime, CHL: Chloramphenicol, CIP: Ciprofloxacin,
678 FOX: Cefoxitin, NAL: Nalidixic acid, SMX: Sulfamethoxazole, TET: Tetracycline, TMP:
679 Trimethoprim.

680