European-wide antimicrobial resistance monitoring in commensal *Escherichia coli* isolated from healthy food animals between 2004 and 2018

Anno DE JONG[1*], Farid EL GARCH[1,2] , Didier HOCQUET[3], Ellen PRENGER-BERNINGHOFF[4], Jeroen DEWULF[5], Lourdes MIGURA-GARCIA[6], Agnès PERRIN-GUYOMARD[7], Kees T. VELDMAN[8], Szilard JANOSI[9], Magdalena SKARZYNSKA[10] , Shabbir SIMJEE[1,11], Hilde MOYAERT[1,12], Markus ROSE[1,13] and on behalf of the EASSA Study Group.¶

[1]EASSA Study Group, c/o CEESA, 168, Av de Tervueren, Brussels, Belgium; [2]Vetoquinol S.A., Lure, France; [3]Infection Control Unit, University hospital of Besançon, CHU Besançon, France; UMR 6249, Chrono-environnement, Université de Bourgogne Franche-Comté, Besançon, France; [4]Institute of Hygiene and Infectious Diseases of Animals, Department of Veterinary Medicine, Justus Liebig University Giessen, Giessen, Germany; [5]Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; [6]IRTA-UAB Mixed Research Unit in Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Barcelona, Spain; IRTA, Animal Health Programme, CReSA, OIE Collaborating Centre, Campus de la Universitat Autònoma de Barcelona, Spain; [7]Anses, Fougères Laboratory, France; [8]Wageningen Bioveterinary Research, Lelystad, The Netherlands; [9]Laboratory of Bacteriology, National Food Chain Safety Office, Budapest, Hungary, [10]Department of Microbiology, National Veterinary Research Institute, Puławy, Poland; [11]Elanco Animal health, Basingstoke, UK.; [12]Zoetis, Zaventem, Belgium; [13]MSD Animal Health Innovation GmbH, Schwabenheim, Germany.

*Correspondence author.

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Objectives: To describe the susceptibility of *Escherichia coli* to medically important antibiotics, collected over four periods (2004-2006, 2008-2009, 2013-2014, 2017-2018), from food-producing animals at slaughter.

Methods: Intestinal contents from cattle, pigs, and broilers were randomly sampled (5-6 countries/host; ≥4 abattoirs/country; 1 sample/animal/farm) for isolation of *E.coli*; antimicrobial susceptibilities were centrally determined by CLSI agar dilution. Clinical breakpoints (CLSI) and epidemiological cut-off values (EUCAST) were applied for data interpretation.

Results: Totally 10,613 *E.coli* strains were recovered. In broilers, resistance percentages were the lowest ($P \leq 0.01$) in the latest time period. A significant decrease in MDR over time was also observed for broilers and a tendency for a decrease for pigs. Resistance to meropenem and tigecycline was absent, and resistance to azithromycin was 0.2-2.0%. Also low resistance to third-generation cephalosporins (1.1-1.6%) was detected in broilers. Resistance to colistin varied between 0.1-4.8%. *E.coli* from broilers showed high resistance to ciprofloxacin (7.3-23.3%) whereas for cattle and pigs this was 0.2-2.5%. Low/moderate resistance to chloramphenicol (9.3-21.3%) and gentamicin (0.9-7.0%) was observed in pigs and broilers. The highest resistance was noted for ampicillin (32.7-65.2%), tetracycline (41.3-67.5%), trimethoprim (32.1-36.2%), and trimethoprim/sulfamethoxazole (27.5-49.7%) from pigs and broilers, with marked country differences. MDR peaked in pigs and broilers with 24 and 26 phenotypes, with 21.9-26.2% and 18.7-34.1% resistance, respectively.

Conclusions: In this pan-EU survey antibiotic susceptibility of commensal *E.coli* varied largely between antibiotics, animal species, and countries. Resistance to critically

important antibiotics for human medicine was absent or low, except for ciprofloxacin in broilers, and ampicillin in pigs and broilers.

Introduction

During the last decades, antimicrobial resistance (AMR) has emerged globally, and poses a significant threat to animal and human health.[1,2] The potential for transfer of AMR from enteric bacteria in animals to humans is a global public health concern.[3] The AMR reservoir of enteric bacteria from livestock has been increasingly investigated for its potential to transfer AMR to humans via direct contact, the environment or contaminated food.[4] These reservoirs are clearly interconnected, but the extent of transmission between these reservoirs remains uncertain.[5,6,7] AMR is problematic not only for pathogenic bacteria but also for the commensal intestinal microbiota. The WHO has identified Enterobacterales to be of critical importance, due to the dissemination of ESBLs, cephalosporinase (AmpC), and carbapenemases.[8,9] From a list of antimicrobial-resistant “priority pathogens” that pose a major threat to public health and for which there is an urgent need for new treatments, WHO categorized Enterobacterales as a priority 1 (critical) pathogen.[10] Additionally, emerging resistance determinants such as mobile colistin resistance have led to increased numbers of reports of multi-drug resistant isolates.[11,12] Various international organizations have addressed the issue of AMR. For instance, the EU has set up an EU-wide AMR control strategy by specific action plans.[13] Among the Enterobacterales, *Escherichia coli* is commonly used in monitoring programmes as an indicator of the Gram-negative gut microbiota.[3] Livestock carries *E. coli* as a commensal organism in their intestine and thus can be regarded as a potential reservoir of acquired resistance determinants.

To address AMR, several European countries have established national monitoring in healthy production animals around the turn of the century, e.g., Denmark (DANMAP),[14] Netherlands (MARAN),[15] Norway (NORM-VET),[16] and Sweden (SVARM).[17] Importantly, since the advent of this century the European Food Safety Authority (EFSA) analyses and reports annually or biannually information on AMR in zoonotic and indicator bacteria from food animals submitted by various EU Member States.[18] These data are further used for investigations of associations between antibiotic consumption and AMR.[3] Furthermore, the veterinary pharmaceutical industry has conducted periodic monitoring of zoonotic and commensal bacteria from European countries through the Executive Animal Health Study Centre (CEESA).[19] Additionally, multiple one-off studies on AMR and mechanisms of resistance of *E. coli* are available.[20-23]

The present study is part of the ongoing European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme, which is coordinated by CEESA. This pan-EU programme collects intestinal bacteria from healthy food animals sampled at slaughter employing a protocol with uniform procedures of sampling and bacterial isolation. MICs to a panel of antimicrobials commonly used in human medicine were determined in a central laboratory.[19] This allows comparison of results across time periods, animal species and countries,[24] which is crucial.[25,26] The organisms of interest are zoonotic *Salmonella* and *Campylobacter* species, and commensal *E. coli* and *Enterococcus* species as indicator organisms. This paper provides antimicrobial susceptibility data and trend analysis over time for *E. coli* collected from beef cattle, slaughter pigs, and broiler chickens between 2004 and 2018 from four EASSA studies (2004-2005; 2008-2009; 2013-2014; 2017-2018). Detailed results of the initial sampling

periods (1999-2003) have been reported previously.[27-29] Resistance mechanisms of *E. coli* of the EASSA 2004-2014 collections such as characterization of the ESBL, AmpC and *mcr* genes of ESBL/AmpC-producing or colistin-resistant strains, respectively, have been published elsewhere.[12,30,31] For EASSA 2017-2018, *mcr* presence of colistin-resistant isolates is included in this paper.

Materials and methods

Sampling procedures, microbiological isolation and identification

The design of the EASSA programme including collection criteria such as animal population and sampling procedures were described previously.[27,32] In brief, samples of intestinal contents of healthy animals at slaughter were randomly collected by participating countries from 4 to 24 abattoirs per country in 5 or 6 EU countries per host species (see Tables S1-4 for the countries per host). From each herd or flock, one animal was randomly selected for sampling. The number of samples was typically between 100 or 200 samples per host and per country. Only one *E. coli* isolate was retained from each sample. Isolation and phenotypic identification of *E. coli* was performed using standardized procedures in each national microbiology laboratory.[28] From the 2013-14 survey onwards, *E. coli* identification was confirmed by MALDI-TOF MS (MALDI-Biotyper, Bruker Daltonics GmbH, Bremen, Germany). Cultures were stored in a central laboratory at -70°C in growth medium with glycerol as cryo-preservative until testing.

Antimicrobial susceptibility testing

Agar dilution MIC testing was performed according to CLSI VET01-A4 (or preceding) standards.[33] Up to 15 antimicrobials/antimicrobial combinations comprising 10 antimicrobial classes as classified to their importance for human medicine by WHO[8]

and recommended by the European Commission[34] and EFSA[35], were tested: 11 Critically Important Antibiotics (CIAs) i.e., ampicillin, azithromycin, ciprofloxacin, cefotaxime, ceftazidime, cefepime, colistin, gentamicin, meropenem, nalidixic acid, tigecycline, and four Highly Important Antibiotics, chloramphenicol, trimethoprim, trimethoprim/sulfamethoxazole (TS) and tetracycline. Azithromycin, ceftazidime, and trimethoprim were only included in the fourth survey; meropenem only in the last two surveys; nalidixic acid and tigecycline in the last three surveys. *E. coli* ATCC 25922 was used as reference strain for quality assurance in each MIC run. MDR of an isolate was defined as clinical resistance to at least one agent in three or more antimicrobial classes.[11] Isolates conferring non-wild type (NWT) and resistance to both cefotaxime and ciprofloxacin were analysed as well.

Detection of mcr *genes*

Colistin-resistant *E. coli* isolates (MIC >2 mg/L)[36] were PCR-screened for the presence of *mcr-1* to *mcr-10* genes using two multiplex PCR primers previously described.[37-39]

Data analyses

Epidemiological cut-off values (ECOFFs) and clinical breakpoints (CBPs) were applied as interpretive criteria for the MIC data. Percentage of clinical resistance, percentage of NWT, MIC_{50} and MIC_{90} values were determined for each antimicrobial, host species and country. Clinical resistance was determined according to M100-S30 breakpoints,[40] except that for tigecycline which was interpreted according to EUCAST guidelines.[36] NWT population was based on ECOFFs.[35,40] CLSI clinical breakpoints and ECOFFs are identical for chloramphenicol, colistin, nalidixic acid, and tetracycline; ECOFFs have not been set for TS. For the other antimicrobials, breakpoints and ECOFFs differ at least by one doubling dilution. Resistance breakpoints and ECOFFs are presented

157 in Table 1 as well as Tables S1-4 available as Supplementary data. The terms to
158 describe the AMR percentages are “rare” (<0.1%), “very low” (0.1-1.0%), “low” (1-
159 10%), “moderate” (10-20%), “high” (20-50%), “very high” (50-70%), and “extremely
160 high” (>70%) and correspond to the criteria applied by EFSA/ECDC.[18]

161 Two-sided χ^2 tests were used for an overall comparison of resistance
162 percentages. In case of a significant difference, pairwise comparisons of resistance
163 prevalence between countries of each animal species and between animal species
164 were used. Two-sided χ^2 tests were used to compare the time period 2017-18 with the
165 other time periods. In all tests, a *P* value of ≤0.05 was considered as significant.
166

167 **Results**

168 A total of 10,613 *E. coli* isolates were recovered in the four EASSA surveys; the
169 numbers per survey amounted to 1496, 2712, 2993, and 3412, respectively. The total
170 number of *E. coli* isolates per host and survey varied between 404 and 1207. The
171 results are summarized in Table 1; the results for the individual countries are
172 presented for each host species in Tables S1-4 available as Supplementary data.

173 Generally, the occurrence of resistance and NWT was markedly lower among
174 cattle isolates than among pig and broiler isolates (Table 1; Figure 1). AMR
175 proportions were highest against ampicillin, tetracycline, trimethoprim and TS, ranging
176 from 3.3–65.3, 7.0–67.5, 0.5–58.2 and 2.8–49.7%, respectively, across all hosts.
177 Significant differences of resistance proportions of above five
178 antimicrobials/antimicrobial combinations were frequently observed among the three
179 hosts. Clinical resistance to the quinolones in broilers was low to high (7.3-23.3%) for
180 ciprofloxacin and very high (51.8-58.1%) for nalidixic acid; NWT proportions were very

high for both compounds. In contrast, in cattle and pigs resistance proportions to these two antimicrobials were very low or low, whereas the ciprofloxacin NWT proportions were slightly higher, albeit low. Resistance proportions to chloramphenicol were primarily moderate for pigs and broilers (14.2-21.3 and 9.3-18.0%), whereas resistance to gentamicin was at a comparatively low level and only significantly higher for poultry as compared to cattle and pigs (3.7-7.0 versus 0.2-2.2%; $P<0.001$). Of the cephalosporins, resistance to cefotaxime was essentially very low in cattle and pigs (0.0-0.5 and 0.2-1.2%), and low in broilers (1.6-7.4%). Resistance to cefepime was virtually absent. Similarly, resistance to colistin was very low, but in the surveys 2008-2009 and 2013-2014 it amounted to 3.1% (cattle), 4.8% (pigs) and 4.4% (broilers). Out of 16 colistin-resistant *E. coli* isolates recovered in the EASSA 2017-2018 survey, 9 (56.3%) harboured *mcr-1* genes. Eight of these isolates originated from broilers (France, Germany); one isolate was from a pig (Spain). Clinical resistance to meropenem and tigecycline was absent for all three hosts, and NWT was only encountered for one avian isolate each. For azithromycin and ceftazidime, data are only available for the time period 2017-2018. Resistance to both molecules was very low or low in all three species (Table 1).

Marked country differences were noted for most antibiotics (Tables S1-4). With regard to cattle, in the first survey resistance to ampicillin, chloramphenicol, tetracycline and TS of French isolates was higher ($P<0.01$) than those of Germany, Italy and UK, but this was not apparent in the last survey, where the highest resistance percentages were recorded for Italy ($P<0.01$). In all four surveys, the level of resistance of porcine isolates was the highest for Spanish isolates: For ampicillin, chloramphenicol, gentamicin, tetracycline and TS, AMR was always higher ($P<0.01$)

than that from Denmark, France, Germany, The Netherlands or UK. The same tendency was observed for the CIAs, although significant differences were usually absent. Porcine isolates from Denmark showed frequently the lowest prevalence of resistance to ampicillin, chloramphenicol, tetracycline and TS. A slightly different picture was seen for the broiler isolates: Whereas the percentages of CIAs resistance in broiler isolates from UK were particularly low, we found extremely high levels of resistance/NWT to quinolones in Hungary and Spain. In general for several antimicrobials including the CIAs, Spanish AMR percentages were among the highest, the proportion of resistant isolates to TS was significantly lower than in the isolates from other countries (P<0.01).

Several changes of resistance or NWT in time were identified (Table 1). The lowest frequencies of resistant isolates were usually found in 2017-2018 for pigs and broilers, e.g., for tetracycline and TS, the frequency was significantly lower compared to the preceding periods (P≤0.05). In broilers, the same holds true for ampicillin, cefotaxime, chloramphenicol, and nalidixic acid (P≤0.05). For broilers, the percentage NWT for both cephalosporins also decreased markedly in the last survey. This contrasts with cattle, where the frequency of the resistant isolates was the highest in 2017-2018 for ampicillin, chloramphenicol, tetracycline and TS (P≤0.05).

MDR results are summarized in Table 2. Overall pooled values were 3.5% for cattle, 23.7% for pigs and 25.9% for broilers, and were significantly lower in cattle for all four time periods (Table 2). For cattle, we found no consistent MDR trend over time, whereas we noticed a tendency for a decrease for pigs. For broilers a significant decrease in MDR (P<0.01) over time was observed. Table 3 presents the overall data of various MDR phenotypes; MDR data for the individual time periods are in

Supplementary Table S5. MDR (based on ten classes) amounted to 2.1-5.1%, 21.9-26.2% and 18.7-34.1% for cattle, pigs and broilers, respectively. For cattle 16 MDR phenotypes were detected (Table 3), with two major MDR patterns: amp/tet/TS (n=36, 1.1%) and amp/chl/tet/TS (n=23, 0.7%). For pigs, 24 MDR phenotypes were observed with amp/tet/TS (n=442, 12.1%) and amp/chl/tet/TS (n=271, 7.4%) as major patterns. For broilers (26 phenotypes) the most frequent MDR phenotype was the combination amp/tet/TS (n=487, 12.9%) followed by amp/cip/tet/TS (n=124, 3.3%) and amp/chl/tet/TS (n=114, 3.0%). Overall, 167 isolates (1.6%) of all 10,613 isolates were resistant to five compounds, and 51 isolates (0.5%) to six compounds. Six isolates were resistant to seven compounds.

Finally, the combined resistance to ciprofloxacin and cefotaxime was analysed (Table 4). In cattle and pigs, combined NWT or combined clinical resistance was either not observed or detected at very low levels. In broilers both percentages NWT and clinical resistance were higher (1.6-7.2% and 0.4-3.4%, respectively). Significant changes over time were only observed for broilers; the percentage of the combined resistance in 2017-18 was lower than the levels of the two preceding periods (P<0.001).

Discussion

Studying AMR in commensal indicator *E. coli* from intestinal content of healthy food-producing animals provides information on the reservoirs of resistant bacteria that can potentially be transferred between animals and between animals and humans. It also provides indirect information on the reservoirs of resistant determinants in animals. The ongoing threat of AMR is a looming public health concern.[41] AMR monitoring in

253 food-producing animals, therefore, has relevance to both public and animal health.
254 AMR exhibited by indicator *E. coli* likely depends on several factors such as the
255 selective pressure from the use of antimicrobials in animals, co-selection of bacteria
256 with MDR, clonal spread of resistant bacteria and dissemination of particular genetic
257 elements, such as resistance plasmids and integrons in Gram-negatives.

258 The study design allowed comparisons to be made between host species,
259 countries and antimicrobial agents. Resistance to ampicillin, trimethoprim, tetracycline
260 and TS were the most common resistance traits observed (Figure 1), with large
261 differences between countries. The frequent occurrence of these resistances likely
262 reflects an extensive use of antimicrobial agents in veterinary field over many years.
263 Also, resistance to ciprofloxacin, a CIA of the Highest Priority, was common in broilers.
264 This contrasted with the very low or low resistance proportions to ciprofloxacin in cattle
265 and pigs. A similar observation was made for the combined resistance to ciprofloxacin
266 and cefotaxime: very low in cattle and pigs, whereas higher in broilers. Among the
267 other CIAs tested, resistance to colistin and azithromycin (both categorized as CIAs of
268 the Highest Priority[8]) was low. Resistance to cefotaxime, ceftazidime or cefepime was
269 absent or detected at very low or low levels in some countries. Resistance to
270 tigecycline and carbapenems (meropenem) was not detected.

271 Comparison of the time periods revealed that for broilers a marked increase in
272 resistance occurred from 2004-06 to 2008-09 for a few antimicrobials, whereas for the
273 final period 2017-18, AMR for most antimicrobials decreased significantly. These
274 results are compatible with those of the EFSA/ECDC study.[18] In the latter study, the
275 trends of resistance focused on four antimicrobials; ampicillin and tetracycline because
276 these antimicrobials have been the most used in Europe, and the High Priority CIAs

277 ciprofloxacin and cefotaxime. For broilers, in 29 European countries, 51 decreasing
278 and 17 increasing temporal trends were recorded. Overall, resistance to all four
279 antimicrobials has declined significantly over 2009-2019.[18] In pigs, generally
280 decreasing trend of AMR and a statistically significant decrease in tetracycline
281 resistance was observed over the period 2009-19; a similar decrease of tetracycline
282 resistance was seen in our programme. In beef cattle significant trends were absent.

283 The increasing prevalence of resistance to third-generation cephalosporins in
284 the past few decades has resulted in a global health problem and resulted in
285 monitoring of the ß-lactamases responsible for this phenotype (i.e., ESBL and AmpC).
286 This EASSA programme over the 2004-2018 collection periods has recorded this
287 resistance in 288 out of 10,613 isolates (2.7%). Detailed results on the occurrence and
288 characterization of ESBL/AmpC-producing *E. coli* bacteria of the four time periods are
289 reported elsewhere;[31,42-44] hence, here a summary comparison among the four time
290 periods is provided (Table 5). The majority of the detected genes encoded ESBLs
291 (63.1%) and AmpC (25.5%). Interestingly, the occurrence of presumptive
292 ESBL/AmpC-producers was similar in the EFSA/ECDC study, ESBL-producers were
293 also more common than AmpC-producers, and isolates producing both ESBL and
294 AmpC were rare.[18] In our study, *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CMY-2}}$ dominated among ESBL and
295 AmpC genes, respectively.[31,44] Similar results were observed in the preceding time
296 period 2002-03,[30] whereas cephalosporin NWT strains were absent in 1999-2001.[27]

297 Colistin (polymyxin E) has been used extensively in food animals all over the
298 world, including Europe. Use in human medicine has been very restrictive for decades
299 owing to its systemic toxicity. In recent years, however, there is an increased need of
300 last-resort antimicrobials such as colistin, to treat MDR infections caused by Gram-

301 negative bacteria.[45-47] Consequently, colistin is now listed as a CIA of highest priority.[8]
302 The discovery of transferable genetic elements (e.g., *mcr* genes) conferring resistance
303 to colistin underlines the importance of the resistance monitoring study. In the period
304 2017-2018, 9 colistin-resistant *E. coli* isolates (0.3% of all *E. coli*) harboured *mcr-1*
305 genes. In the preceding time periods, these figures were 0% (2004-06), 1.7% (2008-
306 09) and 0.8% (2013-14).[12] From 2004 to 2014 *mcr-1* positive *E. coli* were isolated in
307 broilers (n=45, 2.3%) and pigs (n=23, 1.1%); none of the cattle isolates harboured
308 *mcr-1*.[12] In the EFSA/ECDC study, colistin resistance was also infrequently detected in
309 isolates from pigs and broilers.[18]

310 There was considerable variation between individual countries and hosts in the
311 prevalence of resistance. It is tempting to ascribe such variation to differences in
312 amounts of antimicrobial products used. Several national resistance monitoring
313 surveys in Europe include antimicrobial usage (AMU) data in animals.[14-17] Also,
314 specific *ad hoc* studies are available.[22,23,48-50] For all EU countries, the European
315 Surveillance of Veterinary Antimicrobial Consumption (ESVAC) is recording in a
316 uniform manner national AMU in veterinary medicine.[51] The antimicrobial sales for
317 food animals ranged from 2 to 394 mg/population correction unit between countries;
318 the median was 52 mg/population correction unit.[51] Hence, differences in AMR levels
319 of *E. coli* may well be related to AMU.

320 The reduction of AMR in several countries of our study in broilers, particularly for
321 antimicrobials most commonly used in veterinary medicine such as tetracyclines and
322 penicillins, is likely influenced by the overall decline in AMU since 2011, as noted in
323 the ESVAC report.[51] In addition, the decline of the prevalence of ESBL/AmpC in our
324 study, may be related to the decreased cephalosporin use.[51] A comparison of

consumption per animal species would be very helpful in further elucidating this association but is unfortunately currently not available. Yet two recent studies give first insights in AMU in pigs and broilers across nine European countries and also show marked differences in use within and between countries.[49,50] From 2024 onwards, it will become mandatory for EU countries to provide AMU data by animal species under the framework of EU Regulation 2019/6.

Although AMU differences may explain part of the findings from this study, the association between AMU and AMR is not always so straight forward. A single drug can select resistance to several chemically unrelated agents. Moreover, genes conferring resistance to these compounds are often linked to mobile genetic elements resulting in co-selection.[52,53] In addition, selection of resistance by one compound can lead to resistance against different molecules of the same class, (e.g., enrofloxacin and ciprofloxacin cross-resistance). In the absence of cephalosporin use in broilers, introduction through imported breeding stock of *E. coli* carrying AmpC and vertical transmission through the production pyramid could explain the occurrence of cephalosporin-resistant *E. coli*.[14,54,55] Observations made with regard to chloramphenicol (resistance prevalence moderate in this study in pigs and broilers, as in previous studies, e.g.[27,28]), which is already banned for use for farm animals in Europe for many years, also demonstrate at least some disconnection between AMR and AMU of the same antimicrobial class, as the chloramphenicol resistance of avian *E. coli* isolates can not be explained by the use of the related compound florfenicol because florfenicol has not been approved for poultry in the EU. Co-selection by unrelated compounds is the most likely explanation. In contrast, for cattle and pig *E. coli* isolates, the use of florfenicol can select directly for resistance to chloramphenicol

349 due to floR genes. Similarly, azithromycin, an azalide macrolide, is not used in animals
350 while resistance is observed in this and other studies.[14,22]

351 In the EASSA programme both ECOFFs and CBPs were applied. Application of
352 only CBPs could mask important shifts in MICs towards a less susceptible population.
353 On the other hand, the stand-alone application of ECOFFs, and not including CBPs,
354 can cause confusion, particularly among clinicians who are likely to interpret the term
355 “resistant” as “clinically resistant”, and not, “less susceptible but nevertheless
356 susceptible to the prescribed treatment”.[56] An additional analysis of MDR based on
357 ECOFFs shows that the overall MDR percentages are similar because the ECOFFs
358 and CBPs associated with the major phenotypes are identical (chloramphenicol,
359 tetracycline, TS) or differ at most one dilution (ampicillin). The only exception applies
360 to broilers where amp/cip/tet/TS (8.2%) exceeded that of amp/tet/TS (7.4%). This
361 illustrates the relevance of applying both ECOFFs and CBPs in monitoring
362 programmes such as the present one.

363 Taken together, the results described here, analyse data from 10,613 *E. coli*
364 isolates tested for resistance against up to 15 antimicrobial agents, collected over two
365 decades and analysed and interpreted according to a uniform methodology provides a
366 more extensive database than the previous EASSA studies (e.g.[29]) in which nine
367 agents were tested against considerably less isolates. Another strength is that all MIC
368 testing is performed in a central laboratory,[57,58] which enables comparisons of different
369 EU countries and different EASSA surveys. The data provide many very interesting
370 insights and invites for further research on the complex drivers for selection and
371 weaning of antimicrobial resistance.

372

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Transparency declarations

None to declare. All authors of the EASSA Study Group but AdJ are full time employees of the sponsoring companies. AdJ is a consultant employee of CEESA.

Supplementary data

Tables S1 to S5 are available as Supplementary data at *JAC* Online.

References

1 O'Neill, J Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. 2016. http://amr-review.org/.

2 Holmes AH, Moore LSP, Sundsfjord A *et al*. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 2016; **387**: 176–87.

3 ECDC, European Food Safety Authority EMA. Third Joint Inter-agency Report on Integrated Analysis of Antimicrobial Agent Consumption and Occurrence of Antimicrobial Resistance in Bacteria From Humans and Food-producing Animals in the EU/EEA, JIACRA III. 2016–2018. Stockholm, Parma, Amsterdam. 2021.

4 Scott AM, Beller E, Glasziou P *et al*. Is antimicrobial administration to food animals a direct threat to human health? A rapid systematic review. *Int J Antimicrob Agents* 2018; **52**: 316–23.

5 Lazarus B, Paterson DL, Mollinger JL *et al.* Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systemic review. *Clin Infect Dis* 2015; **60**: 439-52.

6 Mughini-Gras L, Dorado-Garcia A, van Duijkeren E *et al.* Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based model study. *Lancet Planetary Health* 2019; 3: e357-69.

7 Martak D, Guther J, Verschuuren TD *et al.* Populations of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* are different in human-polluted environment and food items: a multicentre European study. *Clin Microbiol Infect* 2022; 28: 447 e7- 14.

8 WHO. *Critically Important Antimicrobials for Human Medicine.* 6th Revision, 2019. Geneva, Switzerland.

9 Salgado-Caxito M, Benavides JA, Adell AD *et al.* Global prevalence and molecular characterization of extended-spectrum ß-lactamase producing-*Escherichia coli* in dogs and cats – a scoping review and meta-analysis. *One Health* 2021; **12**: 100236.

10 WHO. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics.* 2017. https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

11 Magiorakos AP, Srinivasan A, Carey RB *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268-81.

12 El Garch F, de Jong A, Bertrand X *et al. mcr-1*-like detection in commensal *Escherichia coli* and *Salmonella* spp. from food-producing animals at slaughter in Europe. *Vet Microbiol* 2018; **213:** 42-6.

13 European Commission. *Communication from the Commission to the European Parliament and the Council - Action Plan against the Rising Threats from Antimicrobial Resistance 748 – AMR Road Map – Action no. 10. Evaluation of Action Plan at EU level.* 2016. www.europe.eu/dgs/health_food.

14 DANMAP 2020. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark.* 2021. http://www.danmap.org/.

15 MARAN 2020. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2019*. 2020. http://www.cidc-lelystad.nl.

16 NORM/NORM-VET 2019. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2020.* http://www.vetinst.no.

17 SWEDRES--SVARM 2020. *Sales of Antibiotics and Occurrence of Resistance in Sweden.* The National Veterinary Institute (SVA), Uppsala, Sweden, 2021. http://www.sva.se.

18 European Food Safety Authority, ECDC. EU Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2018/2019. *EFSA Journal* 2021 **19**: 6490**.**

19 de Jong A, Thomas V, Klein U *et al.* Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. *Int J Antimicrob Agents* 2013; **41:** 403-09.

20 Wasyl D, Hoszowski A, Zajac M *et al.* Antimicrobial resistance in commensal *Escherichia coli* from animals at slaughter. *Front Microbiol* 2013; **4**: 221.

21 Callens B, Sarrazin S, Cargnel M *et al.* Associations between a decreased veterinary antimicrobial use and resistance in commensal *Escherichia coli* from Belgian livestock species (2011-2015). Prev Vet Med 2017; **157:** 50-8.

22 Ceccarelli D, Hesp A, van der Goot J et al. Antimicrobial resistance prevalence in commensal *Escherichia coli* from broilers*,* fattening turkeys, fattening pigs and veal calves in European countries and association with antimicrobial usage at country level. *J Med Microbiol* 2020; **69**: 537-47.

23 Mesa-Varonaldes O, Boone I, Flor M *et al.* Comparison of consumption data and phenotypical antimicrobial resistance in *E. coli* isolates of human urinary samples and of weaning and fattening pigs from surveillance and monitoring systems in Germany. *Antibiotics* 2021; **11**: 28.

24 Simjee S, McDermott P, Trott DJ *et al.* Present and future surveillance of antimicrobial resistance in animals:principles and practices. In: Aarestrup FM, Schwarz S, Shen J, Cavaco L, ed. *Antimicrobial Resistance in Bacteria from Lifestock and Companion Animals.* ASM, 2018; 595-618.

25 Silley S, de Jong A, Simjee S *et al*. Harmonisation of resistance monitoring programmes in veterinary medicine: an urgent need in the EU? *Int J Antimicrob Agents* 2011; **37:** 504-12.

26 World Organisation for Animal Health (WOAH). Harmonisation of national antimicrobial resistance surveillance and monitoring programmes. In: *Terrestrial Animal Health Code*. 2018.

27 Bywater R, Deluyker H, Deroover E *et al*. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother* 2004; **54**: 744-54.

28 de Jong A, Bywater R, Butty P *et al.* A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother* 2009; **63**: 733-44.

29 de Jong A, Thomas V, Simjee S *et al*. A pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food producing animals. *J Antimicrob Chemother* 2012; **67**: 638-651.

30 Nordmann P, Thomas V, de Jong A *et al.* Prevalence and molecular characterization of extended-spectrum β–lactamases (ESBLs) of *E. coli (Ec)* and *Salmonella* spp. *(Sal)* recovered from cattle, pigs and chickens in 8 EU countries. *49th ICAAC*, San Francisco, USA. 2009. Abstract C1-057.

31 Ewers C, de Jong A, Prenger-Berninghoff E *et al.* Genomic diversity and virulence potential of ESBL- and AmpC-ß-lactamase-producing *Escherichia coli* strains from healthy food animals across Europe. *Front Microbiol* **12**: 626774, 2021.

32 de Jong A, Moyaert H, Simjee S. Antimicrobial susceptibility testing of food-borne bacteria related to national and international resistance monitoring programmes. In: Barros-Velazquez. J. ed. *Antimicrobial Food Packaging,* 2016. Elsevier Science 2016. Chapter 9, 117-128.

33 CLSI. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals - Fifth Edition VET01-A4.* 2018.

34 EC C(2020) 7894 final. Commission Implementing Decision 2020/1729 of 17.11.2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Commission Implementing Decision 2013/652/EU.

35 European Food Safety Authority. Technical specifications on the harmonized monitoring and reporting of antimicrobial resistance in *Salmonella, Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. *EFSA Journal* 2016; **10**: 2742.

36 EUCAST. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 9.0. 2019.*

37 Borowiak M, Baumann B, Fischer J *et al.* Development of a novel *mcr-6* to *mcr-9* multiplex PCR and assessment of *mcr-1* to *mcr-9* occurrence in colistin-resistant *Salmonella enterica* isolates from environment, feed, animals and food (2011-2018) in Germany. *Front Microbiol* 2020; **11**: 80.

38 Rebelo AR, Bortolaia V, Kjeldgaard JS *et al.* Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* for surveillance purposes. *Euro surveill* 2018; **23.**

39 Wang C, Feng Y, Liu L *et al.* Identification of novel mobile colistin resistance gene *mcr-10*. *Emerg Microbes Infect* 2020; **9:** 508-516.

40 CLSI. *Performance Standards for Antimicrobial Susceptibility testing - 30th Edition M100-S30*. 2020.

41 Garcia-Migura L, Hendriksen RS, Fraile L *et al.* Antimicrobial resistance of zoonotic and commensal bacteria in Europe: The missing link between consumption and resistance in veterinary medicine. *Vet.Microbiol* 2014;**170:** 1–9.

42 Thomas V, de Jong A, Butty P *et al.* Characterization of mechanisms of resistance and determination of plasmid incompatibility group of *E. coli (Ec)* and *Salmonella* spp. *(Sal)* strains isolated from healthy animals with decreased susceptibility to cefotaxime. *Clin Microbiol Infect* 18, 2012; Suppl. S3: 308.

43 Thomas V, de Jong A, Butty P *et al.* Identification of resistance mechanisms and determination of plasmid incompatibility group in cefotaxime non-wild type *E. coli* and *Salmonella* isolates recovered from healthy food animals. 23rd *ECCMID,* Berlin, Germany, 2013. Abstract P 1199.

44 Sauget M, Atchon A, Almarcha A *et al.* Genome analysis of third-generation cephalosporin-resistant Enterobacteriales recovered from healthy and diseased food-producing animals in Europe (2015-2018). *31st ECCMID,* online 2021. *Int J Antimicrob Agents* 2022 manuscript submitted.

45 Catry B, Cavaleri M, Baptiste K *et al.* Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *Int J Antimicrob Agents* 2015; **46:** 297-306.

46 Maciuca L, Cummins ML, Cozma AP *et al.* Genetic features of *mcr-1* mediated colistin resistance in CMY-2-producing *Escherichia coli* from Romanian poultry. *Front Microbiol* 2019; **10**: 2263.

47 Schwarz S, Johnson AP. Transferable resistance to colistin: A new but old threat. J. Antimicrob Chemother 2016; **71**: 2066–70.

48 Dorado-Garcia A, Mevius DJ, Jacobs JJH *et al.* Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. *J Antimicrob Chemother* 2016; **71**: 3607–19.

49 Sarrazin S, Joosten P, van Gompel L *et al.* Quantitative and Qualitative Analysis of Antimicrobial usage patterns in 180 selected farrow-to-finish pig farms from nine European countries based on single batch and purchase data. *J Antimicrob Chemother* 2019; **74**: 807–16.

50 Joosten P, Sarrazin S, van Gompel L *et al.* Quantitative and qualitative analysis of antimicrobial usage at farm and flock level on 181 broiler farms in nine European countries. *J Antimicrob Chemother* 2019; **74**: 798–806.

51 EMA. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Sales of veterinary antimicrobial agents in 31 European countries in 2019 and 2020. Trends from 2010 to 2020. Eleventh ESVAC report. 2021. Amsterdam.

52 Sharma P, Haycocks JRJ, Middlemiss AD *et al.* The multiple antibiotic resistance operon of enteric bacteria controls DNA repair and outer membrane integrity. *Nature comm* 2017; **8**: 1444.

53 Partridge SR, Kwong SM, Firth N *et al.* Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev* **31**; e00088-17.

54 Nilsson O, Börjesson S, Landen A *et al.* Vertical transmission of *Escherichia coli* carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. *J Antimicrob Chemother* 2014; **69**: 1497-500**.**

55 Myrenäs M, SchauSlettemeäs J, Thorsteinsdottir TR *et al.* Clonal spread of *Escherichia coli* resistant to cephalosporins and quinolones in the Nordic broiler production. *Vet Microbiol* 2018; **213:** 123-128.

56 Simjee S, Silley P, Werling H-O *et al.* Potential confusion regarding the term 'resistance' in epidemiological surveys. *J Antimicrob Chemother* 2008; **61**: 228-9**.**

57 Kahlmeter G, Brown DFJ. Resistance surveillance studies - comparability of results and quality assurance of methods. *J Antimicrob Chemother* 2002; **50**: 775-7**.**

58 Schwarz S, Simjee S, Woodford N *et al.* Assessing the antimicrobial susceptibility of bacteria obtained from animals. *Vet Microbiol* 2010; **141:** 1-4.

Table 1. Antimicrobial susceptibility of *E. coli* isolates (*n*=10,613) of cattle (*n*=3,164), pigs (*n*=3,660) and broilers (*n*=3,789) in four time periods (2004-2006, 2008-2009, 2013-2014, 2017-2018).

		Cattle				Pigs				Broilers			
Antimicrobial	Interpretation*	2004-06 *n*=404	2008-09 *n*=759	2013-14 *n*=841	2017-18 *n*=1160	2004-06 *n*=529	2008-09 *n*=950	2013-14 *n*=1136	2017-18 *n*=1045	2004-06 *n*=563	2008-09 *n*=1003	2013-14 *n*=1016	2017-18 *n*=1207
Ampicillin	MIC_{50}	2	4	4	4	2	4	4	8	>128	256	256	256
	MIC_{90}	4	4	8	8	>128	>256	>256	>256	>128	>256	>256	>256
	R (≥32)	5.4a	**3.3a**	**5.1a**	**7.8a**	32.7b	36.4b	37.5b	35.3b	56.0c	**65.3c**	**57.6c**	**51.7c**
	NWT (≥16)	5.4	3.8	5.2	8.6	33.3	36.9	37.9	36.6	56.4	67.5	57.8	52.5
Azithromycin	MIC_{50}	-	-	-	4	-	-	-	4	-	-	-	4
	MIC_{90}	-	-	-	8	-	-	-	8	-	-	-	8
	R (≥32)	-	-	-	0.2a	-	-	-	1.1b	-	-	-	2.0b
Cefepime	MIC_{50}	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	MIC_{90}	0.03	0.03	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.12	0.12	0.06
	R (≥16)	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.1
	NWT (≥0.25)	0.0	0.3	0.9	0.7	0.6	0.4	1.2	1.0	6.8	6.9	6.9	2.3
Cefotaxime	MIC_{50}	0.03	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	MIC_{90}	0.06	0.06	0.12	0.12	0.06	0.06	0.12	0.12	0.12	0.25	0.25	0.12
	R (≥4)	0.0a	0.3a	0.5a	0.3a	0.2	0.4	1.2	0.8	**5.7b**	**7.4b**	**6.1b**	**1.6b**
	NWT (≥0.5)	0.7	0.4	0.6	0.5	0.6	0.4	1.5	1.1	6.1	9.8	8.2	2.3
Ceftazidime	MIC_{50}	-	-	-	0.25	-	-	-	0.12	-	-	-	0.12
	MIC_{90}	-	-	-	0.25	-	-	-	0.25	-	-	-	0.25
	R (≥16	-	-	-	0.2a	-	-	-	0.1a	-	-	-	1.1b
	NWT (≥1)	-	-	-	0.4	-	-	-	1.0	-	-	-	2.2
Ciprofloxacin	MIC_{50}	0.008	0.016	0.016	0.016	0.008	0.008	0.016	0.016	0.016	0.12	0.12	0.12
	MIC_{90}	0.016	0.03	0.016	0.016	0.016	0.016	0.016	0.12	0.25	8	8	8
	R (≥1)	0.2a	0.5a	**1.3a**	**0.3a**	0.6a	0.8a	2.5a	1.6b	**7.3b**	22.7b	23.3b	**21.8c**
	NWT (≥0.12)	2.2	1.2	2.3	2.8	4.6	4.3	8.2	8.2	37.6	50.6	57.5	52.5
Chloramphenicol	MIC_{50}	4	8	8	8	4	8	8	8	4	8	8	8
	MIC_{90}	8	8	8	8	64	64	64	128	128	128	32	16
	R (≥32)	3.5a	**1.3a**	**2.4a**	**4.7a**	**14.2b**	**16.4b**	**15.8b**	**21.3b**	**14.6b**	**18.0b**	10.6c	**9.3c**
Colistin	MIC_{50}	0.12	0.5	1	0.5	0.12	0.25	1	0.25	0.25	0.5	1	0.25
	MIC_{90}	0.5	0.5	2	0.5	0.25	0.5	2	0.5	0.25	0.5	2	0.5
	R (≥4)	**0.2**	0.1a	**3.1a**	**0.1a**	**0.6**	0.6a	**4.8a**	**0.2a**	0.5	**4.4b**	0.6b	**1.1b**
Gentamicin	MIC_{50}	0.5	0.5	0.5	1	0.5	0.5	0.5	1	0.5	0.5	0.5	1

	MIC_{90}	1	1	1	1	1	1	1	2	1	1	1	2
	R (≥16)	0.2a	0.4a	0.4a	1.1a	1.7b	**0.9a**	1.6b	**2.2a**	**3.7c**	6.4b	5.1c	**7.0b**
	NWT (≥4)	0.9	0.5	0.4	1.3	2.3	1.3	2.2	3.1	3.9	8.9	6.8	8.0
Meropenem	MIC_{50}	-	-	≤0.016	≤0.016	-	-	≤0.016	≤0.016	-	-	≤0.016	≤0.016
	MIC_{90}	-	-	≤0.016	≤0.016	-	-	≤0.016	≤0.016	-	-	≤0.016	≤0.016
	R (≥4)	-	-	0.0	0.0	-	-	0.0	0.0	-	-	0.0	0.0
	NWT (≥0.25)	-	-	0.0	0.0	-	-	0.0	0.0		-	0.0	0.1
Nalidixic acid	MIC_{50}	-	2	4	4	-	2	4	4	-	64	128	32
	MIC_{90}	-	4	4	4	-	4	8	16	-	>128	>128	>128
	R (≥32)	-	1.1a	2.1a	2.2a	-	**4.2b**	6.0b	**7.3b**	-	54.0c	**58.1c**	**51.8c**
Tetracycline	MIC_{50}	1	2	2	2	128	128	32	32	128	128	32	4
	MIC_{90}	64	4	4	16	>128	256	256	128	>128	256	256	128
	R (≥16)	11.1a	**7.0a**	**7.7a**	**10.4a**	**64.3b**	**67.5b**	**57.5b**	**53.3b**	**67.5b**	**61.1c**	**54.7b**	**41.3c**
Tigecycline	MIC_{50}	-	0.25	0.25	0.25	-	0.25	0.25	0.25	-	0.25	0.25	0.25
	MIC_{90}	-	0.5	0.25	0.5	-	0.5	0.5	0.5	-	0.5	0.5	0.5
	R (≥4)	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.0
	NWT (≥2)	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.1	0.0
Trimethoprim	MIC_{50}	-	-	-	0.5	-	-	-	0.5	-	-	-	0.5
	MIC_{90}	-	-	-	0.5	-	-	-	>512	-	-	-	512
	R (≥16)	-	-	-	6.4a	-	-	-	35.7b	-	-	-	32.0b
	NWT (≥4)	-	-	-	6.6	-	-	-	36.2	-	-	-	32.1
Trimeth-sulfa[$]	MIC_{50}	0.06	0.12	0.06	0.06	0.25	0.25	0.12	0.06	2	1	0.12	0.06
	MIC_{90}	0.06	0.25	0.25	0.12	>128	256	>256	>256	>128	256	>256	>256
	R (≥4/76)	4.2a	**2.8a**	3.9a	**5.6a**	**38.0b**	**39.5b**	**35.8b**	**29.5b**	**49.7c**	**45.6c**	**34.6b**	**27.5b**

[a]The clinical breakpoints and ECOFFs are indicated in parentheses. MIC_{50} and MIC_{90} are expressed in mg/L, R (clinical resistance) and NWT (non-wild type) are expressed in %.
[$]Trimeth/sulfa: Trimethoprim/sulfamethoxazole $MIC_{50/90}$ figures refer to trimethoprim concentrations only.
Different letters indicate statistically significant differences of clinical resistance among the three host species of a given time period.
Percentages of resistance in bold indicate significant differences of the time periods 2004-06, 2008-09 and 2013-14 compared to the time period 2017-18 of a given host species.

Table 2. Prevalence of multi-drug resistance (%) of *E. coli* isolates of cattle, pigs and broilers during the four survey time periods.

	Cattle (3164)*	Pigs (3660)*	Broilers (3789)*
2004-2006	3.0[a]	22.7[b]	**34.1[c]**
2008-2009	**2.1[a]**	**26.2[b]**	**33.3[c]**
2013-2014	**2.6[a]**	23.8[b]	**24.2[b]**
2017-2018	**5.1[a]**	**21.9[b]**	**18.7[c]**

*The numbers in parentheses refer to the total number of isolates included in the entire study; for the numbers per study period see Table 1.
Different letters in the same line indicate statistically significant differences of MDR among the three host species.
Percentages of resistance in bold indicate significant differences of the time periods 2004-06, 2008-09 and 2013-14 compared to the time period 2017-18 of a given host species

Table 3. Proportions of multi-drug resistance of *E. coli* isolates of cattle, pigs and broilers during 2004-2018.

Resistance phenotype	Number of drugs	2004-2018					
		Cattle (3164)		Pigs (3660)		Broilers (3789)	
		n	%	*n*	%	*n*	%
Amp/tet/TS	3	36	1.1	442	12.1	487	12.9
Amp/chl/tet	3	15	0.5	53	1.5	32	0.8
Amp/chl/TS	3	8	0.3	22	0.6	16	0.4
Amp/chl/tet/TS	4	23	0.7	271	7.4	114	3.0
Amp/cip/tet/TS	4	3	0.1	6	0.2	124	3.3
Amp/chl/ctx/Tet	4	0	0.0	1	<0.1	6	0.2
Amp/chl/ctx/TS	4	1	<0.1	0	0.0	0	0.0
Amp/chl/gen/Tet	4	0	0.0	3	0.1	4	0.1
Amp/chl/gen/TS	4	0	0.0	1	<0.1	2	<0.1
Chl/gen/tet/TS	4	4	0.1	0	0.0	5	0.1
Amp/ct/tet/TS	4	0	0.0	8	0.2	5	0.1
Amp/ctx/tet/TS	4	2	0.1	5	0.1	28	0.7
Amp/chl/cip/tet	4	0	0.0	0	0.0	19	0.5
Amp/chl/cip/tet/TS	5	3	0.1	13	0.4	44	1.2
Amp/chl/ctx/tet/TS	5	1	<0.1	2	0.1	2	<0.1
Amp/chl/gen/tet/TS	5	8	0.2	11	0.3	5	0.1
Amp/cip/gen/tet/TS	5	2	0.1	1	<0.1	20	0.5
Amp/chl/cip/gen/TS	5	0	0.0	1	<0.1	5	0.1
Amp/cip/ct/tet/TS	5	0	0.0	2	0.1	1	<0.1
Amp/ctx/ct/tet/TS	5	0	0.0	2	0.1	6	0.2
Amp/ctx/cip/tet/TS	5	0	0.0	2	0.1	4	0.1
Amp/chl/ct/tet/TS	5	1	<0.1	14	0.4	17	0.4
Amp/chl/cip/ctx/tet/TS	6	3	0.1	2	0.1	5	0.1
Amp/chl/cip/ct/tet/TS	6	1	<0.1	2	0.1	8	0.2
Amp/chl/cip/gen/tet/TS	6	1	<0.1	4	0.1	18	0.5
Amp/chl/ctx/cip/gen/tet	6	0	0.0	1	<0.1	0	0.0
Amp/ctx/cip/gen/tet/TS	6	0	0.0	0	0.0	6	0.2
Amp/chl/cip/ct/gen/tet/TS	7	0	0.0	2	0.1	4	0.1
Total		112	3.5	867	23.7	982	25.9

MDR was based on the following ten classes: penicillins, extended-spectrum cephalosporins (cefotaxime), fluoroquinolones, phenicols, polymyxins, aminoglycosides, carbapenems, folate pathway inhibitors, tetracyclines and glycylcyclines. Abbreviations: amp, ampicillin; ctx, cefotaxime; chl, chloramphenicol; cip, ciprofloxacin; ct, colistin; gen, gentamicin; tet, tetracycline; TS, trimethoprim/sulfamethoxazole.

Table 4. Non-wild type and clinical resistance to both ciprofloxacin and cefotaxime based on ECOFFs and clinical breakpoints during four time periods for cattle, pig and broiler isolates.

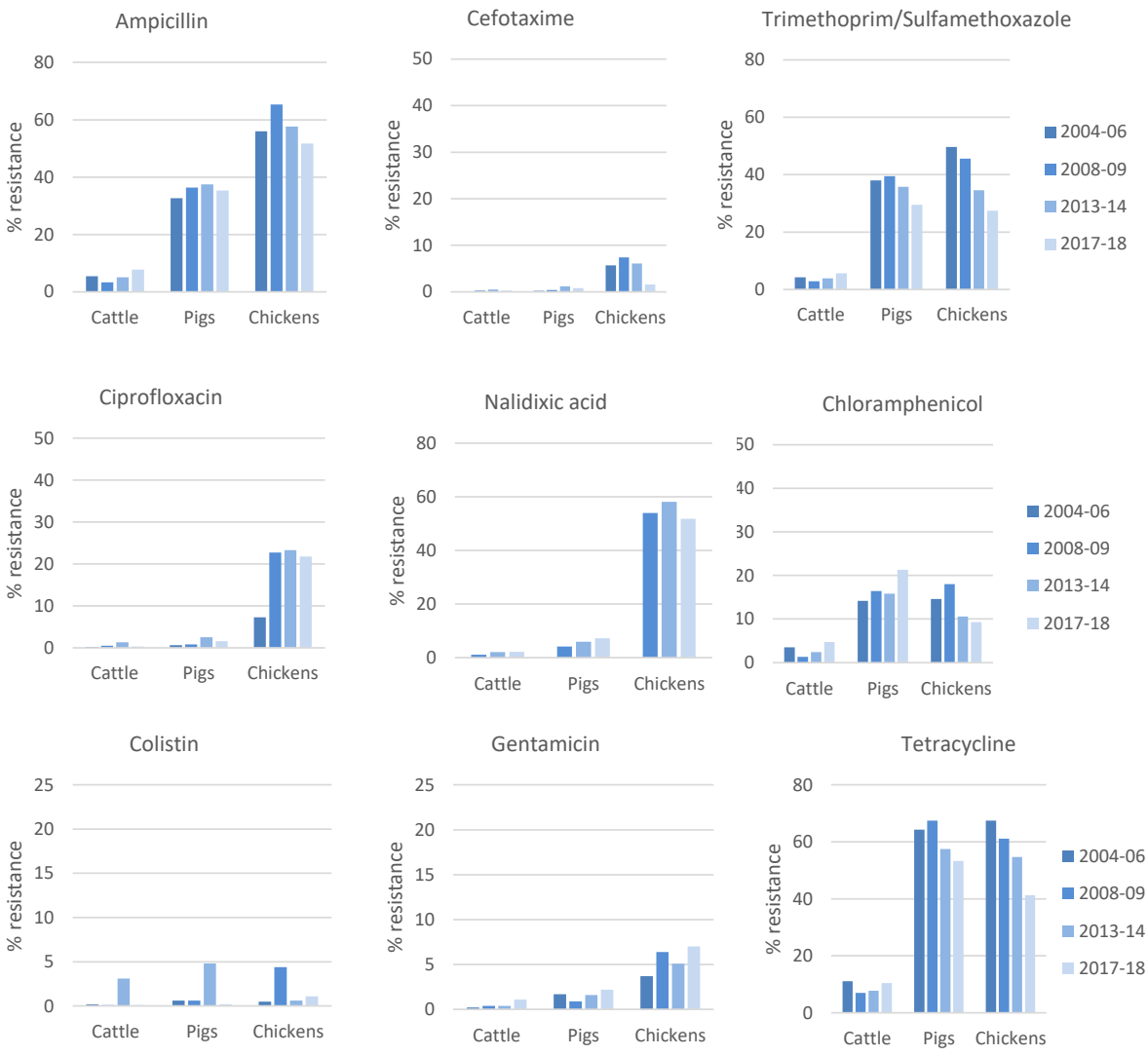
	2004-2006				2008-2009				2013-2014				2017-2018			
	NWT		Resistance		NWT		Resistance		NWT		Resistance		NWT		Resistance	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cattle	0	0	0	0	0	0	0	0[a]	4	0.5	3	0.4[a]	3	0.3	1	0.1[a]
Pigs	0	0	0	0	1	0.1	0	0[a]	7	0.6	5	0.4[a]	2	0.2	1	0.1[a]
Broilers	21	3.7	2	0.4	72	7.2	34	**3.4[b]**	62	6.1	25	**2.4[b]**	19	1.6	11	**0.9[b]**

Different letters in the same column indicate statistically significant differences of combined resistance among the three host species. Percentages of resistance in bold indicate significant differences of the time periods 2008-09 and 2013-14 compared to the time period 2017-18 of a given host species

Table 5. Overview on the occurrence of ESBL- and/or AmpC-producing *E. coli* in food animals during four time periods of the EASSA project.

Numbers	2004-2006	2008-2009	2013-2014	2017-2018	Overall
Total isolates	1,496	2,712	2,993	3,412	10,613
CP-resistant isolates*	45 (3.0%)	109 (4.0%)	100 (3.3%)	34 (1.0%)	288 (2.7%)
ESBLs	35	59	68	27	189
AmpC	2	47	20	7	76
ESBL and AmpC	1	0	2	0	3
Unknown mechanism	7	3	10	0	20

*CP: cefotaxime-and/or ceftazidime-resistant. Percentage CP-resistant isolates is indicated in parentheses.


Ampicillin
% resistance
80
60
40
20
0
Cattle
Pigs
Chickens
Cefotaxime
% resistance
50
40
30
20
10
0
Cattle
Pigs
Chickens
Trimethoprim/Sulfamethoxazole
% resistance
80
60
40
20
0
Cattle
Pigs
Chickens
2004-06
2008-09
2013-14
2017-18
Ciprofloxacin
% resistance
50
40
30
20
10
0
Cattle
Pigs
Chickens
Nalidixic acid
% resistance
80
60
40
20
0
Cattle
Pigs
Chickens
Chloramphenicol
50
40
30
20
10
0
Cattle
Pigs
Chickens
2004-06
2008-09
2013-14
2017-18
Colistin
% resistance
25
20
15
10
5
0
Cattle
Pigs
Chickens
Gentamicin
% resistance
25
20
15
10
5
0
Cattle
Pigs
Chickens
Tetracycline
% resistance
80
60
40
20
0
Cattle
Pigs
Chickens
2004-06
2008-09
2013-14
2017-18