**APPENDIX B**

**High-Throughput Robotic Analysis of Antimicrobial Resistance in Porcine *Escherichia coli*: A National Cross-Sectional Study Evaluating Herd-Level Variability in Australia**

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**Abstract**

**Background:** Conventional antimicrobial resistance (AMR) surveillance in livestock typically involves testing only 100–300 *Escherichia coli* isolates per animal sector annually, which often masks herd-level variation and delays the identification of emerging AMR threats. In this study, we employed a high-throughput robotic workflow to evaluate AMR characterization at a national scale while investigating herd-level resistance.

**Methods:** *Escherichia coli* (n=2,703) were isolated from rectal contents (n=300) of slaughtered pigs from 30 Australian pig herds that supplied around 70% of Australian production. Up to 94 colonies per herd were isolated and subjected to antimicrobial suseptability tsting using a robotic antimicrobial suseptability platfrom. Broth microdilution determined MICs to 14 antimicrobials in nine classes. An AMR index that weights drugs by human-health importance summarised phenotypes at the isolate and herd-level. Twenty-one phenotypically concerning isolates underwent Illumina whole-genome sequencing.

**Results:** Resistance to first-line agents was widespread: ampicillin 77% and tetracycline 79%. In contrast, resistance to critically important antimicrobials (CIAs) remained rare (ciprofloxacin 0.11 %, extended spectrum cephalosporins 0.04 %). Resistance to carbapenems and colistin were not detected. Overall, 56.9 % of isolates were multidrug-resistant, most commonly to β-lactams, folate-pathway inhibitors, phenicols and tetracyclines. The herd-level AMR index spanned 1.51–5.76, revealing pronounced between-herd heterogeneity. Three herds were positive for CIA-resistant isolates, which standard low-density sampling would almost certainly have missed. Sequencing identified fluroquinlone resistant isolates in ST10 (*qnrS1*), ST69 (*qnrS1*), and ST744 (QRDR, *bla*CTX-M-27)

**Conclusions:** Testing roughly ten-fold more isolates than conventional AMR surveys uncovered substantial, farm-specific variability and captured very low prevalence CIA resistance in *E. coli*. While resistance to first-line antimicrobials and MDR phenotype was widespread and heterogeneous, resistance to CIA remained low—an encouraging baseline that can still be preserved. The high-throughput, high-density approach therefore delivered an affordable, practical, early-warning system and a spatially resolved benchmark of resistance that is directly actionable at the herd-level. Integrating such robotic platforms and the easily-interpreted AMR index into routine surveillance will empower veterinary stewardship to safeguard the effectiveness of antimicrobials that are critical to human and veterinary medicine.

**Keywords:** AMR, robotics, *Escherichia coli*, pigs, surveillance, public health

**Introduction**

Antimicrobial resistance (AMR) is a major threat to public health, resulting in approximately five million human deaths per year(Murray et al., 2022). The continued emergence and proliferation of resistance to essential antimicrobials, such as carbapenems, extended-spectrum cephalosporins (ESCs), and fluoroquinolones, is particularly concerning (World Health Organization, 2024a). These, and other “critically important antimicrobials” (CIAs) form the final line of defense for treating serious bacterial infections in humans, and their reduced effectiveness represents a significant threat to human health (Murray et al., 2022; World Health Organization, 2024b). Moreover, the ability of AMR to propagate across the “One Health” network involving humans, animals, food and the environment, highlights the need for coordinated strategies to prevent its proliferation (World Health Organization; Food and Agriculture Organization of the United Nations; United Nations Environment Programme; World Organisation for Animal Health, 2023).

Effective management of AMR relies on procurement of data identifying the location and amount of specific forms of resistance (World Health Organization, 2022). Surveillance of food animals is crucial since faecal microflora inevitably transfer to carcasses during processing (Marshall and Levy, 2011; World Health Organization, 2017), so that animal products can act as a vehicle for widespread dispersal of AMR in human populations (World Health Organization, 2017). In addition, there have been longstanding concerns over inappropriate use of antimicrobials in animal production (Ellis et al., 2024; Landers et al., 2012). To be most effective, surveillance must strategically target early detection of resistance to CIAs and support antimicrobial stewardship at the herd and flock levels, where interventions to prevent emergence of resistance to CIAs can be quickly and effectively implemented (World Health Organization, 2024a, b). Commensal *Escherichia coli* form an ideal group to act as an indicator for these purposes because it is a ubiquitous component of the gut microbiomes of both food animals and humans and can readily acquire, carry, and transfer resistance genes (EFSA/ECDC, 2024; van den Bogaard and Stobberingh, 2000). Therefore, monitoring resistance in commensal *E. coli* from livestock is central to national AMR surveillance efforts forming the basis for guidance of management strategies.

National frameworks for AMR surveillance in animals using commensal *E. coli* are now over three decades old and have some serious limitations (DANMAP, 2023; Government of Canada, 2023; Laird et al., 2022; Truswell et al., 2023; U.S. Food and Drug Administration, 2023). Most importantly, sampling error is very high and sensitivity of detection is low due to reliance on examining relatively small numbers of isolates, usually from 100 to 300 on a national scale for any given class of animal. (EFSA/ECDC, 2024; U.S. Food and Drug Administration, 2010). Statistical error in traditional AMR surveillance is exacerbated by a lack of recognition that the underlying variation of resistance in the population is much greater than implied by the simplified approach to sampling of animals and isolates. To emphasise the latter, a recent study of commensal *E. coli* in Australian pigs (*Sus scrofa domesticus*) (Laird et al., 2022), involving ten herds, ten pigs per herd, and eight isolates per pig, revealed substantial variation in phenotypic and genotypic AMR traits in isolates both within and between individual pigs, plus a high level of herd-to-herd variation. To deal with all this variation, the number of isolates examined needs to be increased; however, in traditional laboratory settings this is rarely achieved due to the cost and labour requirements arising from the manual handling of samples, reagents, and data. Consequently, the conventional approach to AMR surveillance needs improvement in three ways. Firstly, it must boost the ability to detect low-abundance forms of AMR to enhance timely recognition of the emergence of resistance to CIAs. Secondly, surveillance must be able to accurately capture shifts in the prevalence of resistance over time so the data becomes useful for monitoring the success or failure of interventions. Thirdly, AMR surveillance must be able to characterize the spatial distribution of AMR in animal populations since herds and flocks are the level where decisions are made that have the greatest and most immediate impact on antimicrobial usage (World Health Organization, 2022). To achieve these improvements, advanced methodologies integrating high-throughput phenotypic and genomic tools are required in the laboratory (Truswell et al., 2021). These include recognized tests such as the broth microdilution assay yielding data on antimicrobial minimum inhibitor concentration (MIC), with follow up of isolates having conspicuous AMR phenotypes using whole genome sequencing (Clinical and Laboratory Standards Institute, 2020; Truswell et al., 2021). Therefore, when high-throughput robotics platforms are used and accompanied by statistically robust sampling, the next-generation of AMR surveillance is achievable.

For AMR surveillance to influence key decisions about the use of antimicrobials in food-animals, the findings must be presented in a way that non-experts can also interpret and act on. While the traditional reporting of isolate sensitivity against panels of 12 to 14 antimicrobials does meet the needs of the microbiology scientific community, it does not provide an easily interpreted quantity that animal owners and veterinarians can use for monitoring and comparison. To achieve this, we have earlier proposed the use of an antimicrobial resistance index, whereby, the occurrence of resistance to each antimicrobial in the test panel is weighted according to that antimicrobials importance rating (ASTAG, 2018; Laird et al., 2022; Truswell et al., 2021). The AMR index for an isolate is then the sum of weights that characterise the phenotype. In this process, weightings are derived from the importance rating scheme endorsed by the Australian Strategic Technical Advisory Group on Antimicrobial resistance (ASTAG, 2018).

The aim of this study was to investigate the effectiveness of an alternative format for AMR surveillance based on high throughput robotics, maintaining reliance on internationally recognized assays for assessing the sensitivity of isolates to antimicrobials (Clinical and Laboratory Standards Institute, 2018, 2020). The study focused on Australian pig herds as the industry is strongly committed to AMS from the perspective of both pigs and public health (Australian Pork Limited, 2020). It was intended to demonstrate the capacity for a ten to twenty fold increase in the number of isolates evaluated in most national programs for a given class of animal, while delivering data specific for individual herds (~ 90 *E. coli/* herd). The intention was to present the results in traditional format (single drug and multiple class sensitivity) for microbiological interpretation, as well as in the form of an AMR index for each isolate and each herd so that interpretation and comparisons could be made more easily in support of antimicrobial stewardship. In this way we anticipated presenting data that would be much more relevant to animal owners and veterinarians compared to present efforts based on small numbers of isolates that yield little momentum for reform of antimicrobial use at the herd-level.

**Methods**

***Sample collection***

A total of 300 faecal samples from finisher pigs, comprised of ten samples from each of 30 commercial pork herds located across five Australian States, were collected at abattoirs between July to November 2022. The number of herds sampled within each State was approximately in proportion to the size of the pig population: four from Western Australia, five from South Australia, six from New South Wales, seven from Victoria and eight from Queensland. The herds sampled in this study are part of the majority of herds comprising those that are all very large and supplying approximately 70% of pigs entering the food chain in Australia.

Samples were collected by veterinarians, or by experienced quality assurance technicians. An incision was made in the rectal wall post-evisceration using sterilised equipment to gather faeces into sterile containers. Individual samples were obtained at ten-minute intervals on the slaughter line until all ten samples were collected for a herd. If a selected pig did not have any rectal contents, a replacement sample was collected from the next available pig in the slaughter sequence. Samples were transported to the laboratory at between 2 to 8 ºC and processed within 48 hours of collection. Each sample was processed for *E. coli* isolation and subsequent antimicrobial sensitivity testing (AST). Ethical approval was not required for this study, as it met exemption criteria due to sampling being conducted post-processing on the abattoir floor.

***Isolation and antimicrobial susceptibility testing***

Samples were obtained using sterile cotton swabs inserted deep into the collected faeces. For each herd, five swabs from five samples were placed into sterile 1 x phosphate buffered saline (PBS, 10mL) and vortexed to acquire one pooled sample. A total of two pooled samples were acquired from each herd, with a total of 60 pooled samples across the 30 herds. Each pooled sample was loaded onto the Robotic Antimicrobial Susceptibility Platform (RASP) for serial dilution and agar inoculation onto CHROMagar™ ECC (MicroMedia, Edwards Group) using two-zone spiral plating with the EVO150™ (Tecan) and Scirobotic spiral plater (Truswell et al., 2021). Plated samples were incubated at 37 ºC for 16 to 20 hours. Presumptive *E. coli* colonies were identified using the chromogenic reaction of the agar, as per the manufacturer's guidelines using Pickolo™ (SciRobotics) colony picking software. Up to 94 presumptive *E. coli* colonies from each pooled sample were robotically selected using machine vision (Truswell et al., 2021) and inoculated by spiral plating into Luria-Bertani (LB) broth, then incubated at 37 ºC for 16 to 20 hours for storage and subsequent assays. After incubation, isolates selected from agar plates were identified via Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Biotyper Microflex; Bremen Germany) and then subjected to AST (Truswell et al., 2021).

The broth microdilution method was used to determine antimicrobial susceptibility and was performed on the RASP according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2020; Truswell et al., 2021). *E. coli* ATCC 25922 and an in-house positive control *E. coli* ST131 were used as quality control strains. Susceptibility to 14 antimicrobials from nine classes was assessed across the dilutions shown in Table 1. All AST plates were imaged using the SensititreTM Vizion TM Digital MIC Viewing System.

Wildtype and non-wildtype interprations from MIC values were based on the epidemiological cut-off (ECOFF) values from the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2023). Clinical resistance status (sensitive or clinically resistant) was obtained using breakpoints set by the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2018). In the case of apramycin and florfenicol, CLSI clinical resistance breakpoints were not available and these were substituted with NARMS breakpoint for apramycing (U.S Food and Drug Administration, 2023) and a breakpoint derived from literature (Singer et al., 2004). Isolates with clinical resistance to three or more antimicrobial classes were classified as multi-class resistant (MCR), (Clinical and Laboratory Standards Institute, 2020)).

### **AMR index scheme**

Each herd was given an AMR index score based on the average of the AMR index of each *E. coli* isolate from that herd. The AMR index is a summary measure of the public health significance of resistance present in an isolate. Herds resistance to CIAs were given a higher weighting than resistance to antimicrobials of lower concern (Table 1). The weighting of antimicrobials was based on the Australian Strategic and Technical Advisory Group’s three-scale importance rating (ASTAG, 2018), with the weightings for ciprofloxacin and meropenem increased to four because these antimicrobials are not registered for use in Australian food animals. The index score for each isolate was determined by summing each antimicrobial the product of clinical resistance status for that antimicrobial (0: susceptible, 1: resistant) and the weighting for that antimicrobial (1, 2, 3 or 4, Table 1). An isolate fully susceptible to all antimicrobials received an AMR index score of 0, while an isolate resistant to all 14 antimicrobials had an AMR index score of 30.

**Table 1.** Antimicrobials, corresponding classes and abbreviations used for susceptibility testing of commensal *E. coli* from Australian pigs. Ecological cut-off values (ECOFF, mg/L) provide the EUCAST breakpoints for wildtype interpretation. Clinical and Laboratory Standards Institute breakpoints (Clinical and Laboratory Standards Institute, 2018) or (Clinical and Laboratory Standards Institute, 2020) mg/L) provide the breakpoints for clinical resistance interpretation except where as noted. Index weight is the contribution that clinical resistance to each antimicrobial makes in the calculation of the AMR index to reflect the importance rating of each antimicrobial.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Class | Antimicrobial | Abbrev | Dilution range | ECOFF | CLSI | Index weight |
| Aminoglycosides | Apramycin | apr | 8-32 | - | >32\* | 2 |
|  | Amikacin | amk | 1-64 | >8 | >32 | 3 |
|  | Gentamicin | gen | 0.25 - 16 | >2 | >8 | 2 |
| Carbapenem | Meropenem | mer | 0.008-4 | >0.063 | >2 | 4 |
| Cephems | Cefotaxime | cta | 0.015-4 | >0.25 | >2 | 3 |
|  | Ceftazidime | ctz | 0.0625-16 | >1 | >8 | 3 |
| Fluoroquinolones | Ciprofloxacin  | cip | 0.008 - 2 | >0.063 | >0.5 | 4 |
| Folate pathway inhibitors | Trimethoprim | tri | 0.25-16 | >2 | >8 | 1 |
|  | Sulfamethoxazole | sme | 8-512 | - | >256 | 1 |
| Penicillins | Ampicillin | amp | 1 - 32 | >8 | >16 | 1 |
| Phenicols | Chloramphenicol | chl | 2 - 32 | >16 | >16 | 1 |
|  | Florfenicol | flo | 4 - 32 | >16 | >16\*\* | 1 |
| Polymyxins | Colistin | col | 0.25 - 8 | >2 | >2  | 3 |
| Tetracyclines | Tetracycline | tet | 1-32 | >8 | >8 | 1 |

## \*  Apramycin clinical breakpoint unavailable and replaced with value from NARMS (U.S. Food and Drug Administration, 2023).\*\* Florfenicol CLSI clinical breakpoint unavailable and replaced with that from Singer et al (2004).

## ***Genomic sequencing***

Twenty one isolates were selected for whole genomic sequencing (WGS). These included the only isolate resistant to third-generation cephalosporins (n=1), all isolates resistant to quinolines (n=3), a randomly selected representative isolate of the remaining phenotype with resistance to 5 or more classes of antimicrobials but not to CIAs (n=1), and a selection of isolates with meropenem MICs above the ECOFF breakpoint (n=16). DNA was extracted using the MagMAX™ Multi-Sample Kit (Thermo Fisher Scientific, USA), following the manufacturer’s instructions. Library preparation was performed using the Celero DNA-Seq Library Preparation Kit (NuGEN-Tecan), and sequencing was conducted on the Illumina NextSeq platform with a 300-cycle High Output Reagent Kit. Sequencing procedures followed a previously described protocol (O'Dea et al., 2019).

## ***Statistical analysis***

Exact binomial confidence intervals for the percent of isolates resistant to each antimicrobial were calculated using the Clopper-Pearson method in STATA v.18.5 (StataCorp, 2021) . Heatmap plotting of single resistance patterns amongst herds and dendrogram plots from an analysis of hierarchical clustering of herds were performed using Python 3.11.7 (Python Software Foundation, 2024) with the Seaborn (Waskom, 2021) and SciPy (Virtanen et al., 2020) modules, respectively.

# **Results**

## ***Overall results: bacterial isolation and national-level prevalence***

A median of 93 *E. coli* isolates were collected and appraised from each herd (range 69-94), with 2,730 isolates overall. Of these, 122 (4.47%) were non-wildtype to all tested antimicrobials. All isolates tested were clinically susceptible to amikacin, ceftazidime, colistin and meropenem. Non-wildtype resistance was observed for ampicillin (77.36%), cefotaxime (0.18%), ceftazidime (0.18%) chloramphenicol (43.52%), ciprofloxacin (3.96%), florfenicol (17.33%), gentamicin (4.73%), meropenem (5.93%) tetracycline (78.72%) and trimethoprim (42.42%). While the percentage of isolates with clinical resistances was mostly similar to the percentage with non-wildtype resistance, none of the isolates that were non-wildtype to ceftazidime and meropenem were also clinically resistant. Survey-wide single resistance prevalence data are presented graphically in Figure 1 and numerical values are shown in Supplementary Table 1.



**Figure 1.** Summary of the percentage (+/- 95% confidence intervals) of 2,730 commensal *E. coli* from Australian pigs exceeding the clinically resistant and exceeding the ECOFF breakpoint (non-wildtype) for 14 antimicrobials when assessed in the broth-microdilution assay. Antimicrobial abbreviations and breakpoints are given in Table 1.

Data were generated to describe the concurrent expression of clinical resistance of the isolates to the nine antimicrobial classes as assessed by the broth-microdilution assay. A total of 25 phenotypic AMR profiles were identified (Table 2). Of these, a total of 1,554 (56.9%) isolates were MCR. The most common AMR profile involved concurrent resistance to beta-lactams, folate pathway inhibitors, phenicols and tetracyclines (818 isolates, 30.0 %), followed by beta-lactams and tetracyclines (386 isolates, 14.1%) and beta-lactams, folate pathway inhibitors and tetracyclines (305 isolates, 11.2%).

**Table 2**. Percentage of resistance phenotypes of *E. coli* (n=2,730) collected from Australian pigs based on clinical breakpoints (Table 1) with the broth microdilution assay. Numerical digits preceeding each phenotype indicates the number of classes to which resistance is present. Each abbreviation within each phenotype is resistance to a specific antimicrobial class: ami = aminoglycosides, bla = beta-lactams, phe = phenicols, fpi = folate pathway inhibitors, tet = tetracyclines, c3g = cephems (third-generation), and qui = quinolones.

|  |  |  |
| --- | --- | --- |
| phenotype | n | percent |
| 0: nil | 129 | 4.7253 |
| 1: bla | 171 | 6.2637 |
| 1: fpi | 10 | 0.3663 |
| 1: phe | 23 | 0.8425 |
| 1: tet | 192 | 7.0330 |
| 2: bla\_fpi | 86 | 3.1502 |
| 2: bla\_phe | 29 | 1.0623 |
| 2: bla\_tet | 386 | 14.1392 |
| 2: fpi\_phe | 9 | 0.3297 |
| 2: fpi\_tet | 137 | 5.0183 |
| 2: phe\_tet | 4 | 0.1465 |
| 3: ami\_bla\_tet | 1 | 0.0366 |
| 3: bla\_fpi\_phe | 122 | 4.4689 |
| 3: bla\_fpi\_tet | 305 | 11.1722 |
| 3: bla\_phe\_tet | 51 | 1.8681 |
| 3: fpi\_phe\_tet | 124 | 4.5421 |
| 4: ami\_bla\_fpi\_phe | 1 | 0.0366 |
| 4: ami\_bla\_fpi\_tet | 18 | 0.6593 |
| 4: ami\_fpi\_phe\_tet | 19 | 0.6960 |
| 4: bla\_c3g\_fpi\_phe | 1 | 0.0366 |
| 4: bla\_fpi\_phe\_tet | 818 | 29.9634 |
| 4: bla\_fpi\_qui\_tet | 1 | 0.0366 |
| 5: ami\_bla\_fpi\_phe\_tet | 91 | 3.3333 |
| 5: bla\_fpi\_phe\_qui\_tet | 1 | 0.0366 |
| 6: ami\_bla\_fpi\_phe\_qui\_tet | 1 | 0.0366 |

The distribution of the AMR index across all isolates in the survey is presented in Figure 2. Values ranged from 0 to 14 (mean: 3.3 , median: 3.0, interquartile range: 2.0-5.0), noting that the maximum possible value of the AMR index (sum of all the index weights) was 30. The relationship between the AMR index for isolates and the maximum weighting used in calculating the same is given in Figure 3. Because of the rarity of clinical resistance to high importance antimicrobials (cefotaxime, ceftazadine, ciprofloxacin, colistin, meropenem) in this isolate collection, only four isolates (0.15%) had a maximum index weighting greater than two.

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**Figure 2.** Distribution of the antimicrobial resistance index for 2,730 commensal *E. coli* isolates from Australian pigs. Bar colours represent the maximum index weighting applied at each value of the AMR index. Isolates to the left (green and blue bars) have a lower index resulting from having no resistance or resistance only to drugs of low importance. Isolates to the right have a higher index due to resistance to drugs of medium and high importance. Index weights applied to each antimicrobial are given in Table 1.



**Figure 3.** Relationship between the AMR index of an isolate and the maximum weighting used to generate the index for 2,730 commensal *E. coli* isolates from Australian pigs. A bubble plot displaying sample distribution, where the position of each circle represents the group, and the size of the circle indicates the number of samples in that group.

**Herd-level variation in resistance and AMR index**

Herd-based estimates of percent of isolates clinically resistant to each of the 14 antimicrobials are given in an antibiogram heatmap where herds are ranked in order of their AMR index (Figure 4). For drugs of low importance, which represent classes that have been available for use in pig production for many decades, the herd-level percent of isolates resistant to each drug had a very wide range; for example, ampicillin (11.8% to 100%), sulfamethoxazole (9.7% to 100%), trimethoprim (4.3% to 95.8%) and tetracyclines (45.7% to 100%). For most drugs that are either not registered for use in Australian food animals or have only limited use, there was little or no resistance detected and thus neglible variation between herds (e.g. for amikacin, colistin, meropenem). Importantly, resistance to ciprofloxacin was confined to single isolate detections in three herds, and resistance to cefotaxime was confined to a single isolate detection in one herd. The herd-level resistance range for the aminoglycosides apramycin (0% to 48.3%) and gentamicin (0% to 30.0%), two closely related drugs of medium importance, was much less than the range seen for resistance to the drugs of low importance mentioned above. In summary, amongst the isolates, the range with resistance todrugs of low importance was wide between herds and had high maximum herd-levels; those with resistance to medium importance drugs had an intermediate variation between herds; and those resistant to high importance drugs had almost no variation between herds and were associated with absence or virtual absence of resistance.



**Figure 4.** Heatmap antibiogram showing variation in phenotypic AMR traits for 2,730 commensal *E. coli* isolates from 30 pig herds in Australia. Data are the percent of clinical resistance to each of 14 antimicrobials measured by broth microdilution. Herds are identified on the left side y-axis by their ranking in ascending order of herd-level index (1 to 30) while the right side y-axis gives the numbers of isolates evaluated for each herd. Antimicrobial abbreviations and clinical breakpoints are given in Table 1.

An analysis of the distribution of herd AMR index and isolate AMR index within herds is shown in Figure 5. The herd AMR index summarizes the microbiological data in Figure 4, providing a simplified output that can be used for informing and influencing herd owners, prescribers and stakeholders engaged in antimicrobial stewardship. The herd AMR index for the 30 herds ranged from 1.51 to 5.76 with a median value of 3.20. A clearly visible relationship between the occurrence of low importance vs high importance resistance in individual isolates is shown in Plot B (indicated by maximum index weight for isolates) and the value of the herd AMR index is shown in Plot A. Figure 6 provides detailed scrutiny of the within herd variation in the isolate level AMR index for three example herds. As the herd AMR index increased from left to right there was a general shifting of the distribution of the isolate level AMR index to the right and there was a much greater contribution to the herd AMR index from resistance to antimicrobials of medium and high importance. Figure 7 provides a dendrogram illustrating herd similarity, generated by hierarchical cluster analysis of clinical resistance status; positions in the dendrogram correspond to the herd AMR index, with lower-ranking herds clustering on the left (yellow) and higher-ranking herds on the right (green).


**Figure 5.** Comparison of within- and between-herd variation in antimicrobial (AMR) index scores. Plot A: herd-level AMR index score for each of the 30 herds ordered from lowest to highest. Plot B: within- and between-herd variation in the proportion of isolates with each AMR index score. The size of the markers within each herd indicates the percent of isolates with each index value. The colour of the markers represents the maximum index weight applied for each index value within each herd.



**Figure 6**. Detailed representation of the distribution of the isolate level AMR index within three example herds. The figure shows the diversity of the values of the AMR index and variation in the emphasis on resistance to low, medium and high importance antimicrobials (depicted using colour coding to signify the maximum weighting at each value of the index).



**Figure 7.** Dendrogram showing the similarity amongst the 30 herds according to hierarchical cluster analysis of clinical resistance phenotypes present in each herd, with the relationship with herd AMR index shown on the horizontal axis. The latter gives the ranking of herds by AMR index followed by the value of the herd AMR index.

**Genomic characterisation of isolates resistant to CIAs**

***AMR genes***

The phenotypic resistance profiles and sequence types (STs) of the five isolates that were resistant to CIAs and were subjected to WGS are shown in Table 2.

The quinolone resistant isolates (n=3) belong to ST10, ST69 and ST744. Two isolates harboured the plasmid-mediated gene *qnrS1* (ST10 and ST69); one of these also carried the efflux pump genes *oqxA* and *oqxB* (ST69), which confer reduced susceptibility to both phenicols and quinolones. The third isolate (ST774) lacked acquired quinolone genes but possessed mutations in the Quinolone Resistance Determining Region (QRDR), specifically chromosomal substitutions in *gyrA* S83L/D87N, *parC* S80I/E84V and *parE* I529L that are associated with high-level FQ resistance (Azargun et al., 2020; Hooper and Jacoby, 2015).Although ST744 isolate did carry the extended spectrum cephalosporin resistance gene *bla*CTXM-27, the other cephalosporin resistant isolate (ST17737) did not carry any known associated cephalosporin resistance genes. The fifth isolate (ST101) was not resistant to CIAs but was MDR.

The other 16 isolates were sequenced because they had elevated meropenem MICs . They all either carried carbapenemase genes or characteristic porin/penicillin-binding-protein mutations, indicating that their non-wild-type phenotype likely reflects minor MIC drift inherent to *in-vitro* testing rather than true carbapenem resistance (Clinical and Laboratory Standards Institute, 2020).

Across the entire sequenced set, a broad array of resistance genes was detected: these included aminoglycoside genes *aac(3)-Iva*, *aadA1/2/5* and *aph(3″, 4″, 6′);* β-lactam genes *bla*TEM-1, *bla*LAP-2 and *bla*CTX-M-27; phenicol genes *cmlA1*, *floR*, *oqxA/B;* trimethoprim genes *dfrA5/12/17;* sulfonamide genes *sul1-3;* macrolide gene *mph(A);* and tetracycline genes *tetA/B/H/M*. Point mutations known to reduce susceptibility (Gross et al., 2024; Hooper and Jacoby, 2015) were also common, including *ampC C-42T* (β-lactams) and additional quinolone-target mutations (*gyrA S38L*, parC S80I/E84V, parE I529L).

**Table 3: Sequence type (ST) and genotypes of porcine-derived *E. coli* (n=5) with phenotypic resistance to the critically important antimicrobials quinolones and exttended spectrum cephalosporins.**

|  |  |  |  |
| --- | --- | --- | --- |
| Isolate ID  | ST  | Phenotype  | Associated genotype  |
| 22070107\_C01  | 10  | Beta-lactams, folate pathway inhibitors, quinolones, tetracycline  | *aadA2, acrF, blaTEM1, mdtM,qnrS1, sul3, tetA* |
| 22080092\_C02  | 69  | Aminoglycosides, beta-lactams, folate pathway inhibitors, phenicols, tetracyclines, quinolones  | *aac3IVa, aadA1, aadA2, acrF, aph3Ib, aph3IIa, aph4Ia, aph6Id, blaEC, blaTEM1, ble, bleO, cmlA1, cyaA\_S352T, dfrA12, emrD, glpT\_E448K, lnuF, lnuG, mdtM, oqxA, oqxB, qnrS1, sul1, sul3, tetA, tetH* |
| 22080154\_C01 | 744  | beta-lactams, folate pathway inhibitors, phenicols, tetracyclines, quinolones   | *aadA5, acrF, aph3Ib, aph6Id, blaEC, blaCTXM27, dfrA17, emrD, gyrA\_D87N, gyrA\_S83L, glpT\_E448K, mdtM, mphA, parC\_E84V, parC\_S80I, parE\_I529L, , ptsI\_V25I, sul1, sul2, tetA, uhpT\_E350Q* |
| 22110024\_C21 | 17737  | Beta-lactams, third, generation, cephalosporins,, folate, pathway, inhibitors, phenicols  | *aadA1, aadA2, acrF, ampC\_C42T, blaTEM1, cmlA1 dfrA5, fosA75, glpT\_E448K mdtM,  sul1, sul3, pmrB\_Y358N*  |
| 22080081\_C43  | 101  | Aminoglycosides, beta-lactams, folate pathway inhibitors, phenicols, tetracyclines  | *acrF, glpT\_E448K mdtM*   |

**Discussion**

This study utilised a high-throughput robotic platform to perform the first national, high-resolution profile of AMR in *E. coli* from healthy Australian pigs that were sampled at slaughter. By characterising phenotypic antimicrobial resistance of 2,730 isolates across each of 30 herds, we demonstrated how cluster-based sampling at high intensity could improve the detection of resistance to CIAs and define their distribution across an animal population. Strategic application of genomics to isolates selected on the basis of phenotypic expression of resistance provided added definition of the AMR landscape that was present. The visibility of resistance patterns across the population provided by this approach exceeds what is available from standard surveillance, with the advantage of yielding herd-level metrics, such as the AMR index, informing antimicrobial stewardship. Unlike using conventional laboratory methods, where attempting higher throughput carries the risk of a reduction in accuracy due to fatigue and tedium, the use of RASP gave results without compromising the quality of the measurements. This provided a scalable model for routine surveillance, involving scrutiny at the herd-level and where results were delivered to decision makers in a more timely fashion – within a week, compared to the AMR national surveillance report, which is typically available after 12-18 months.

At the national scale, resistance to first-line antimicrobials registered for use in pigs was frequently detected. Non-wild-type (NW) prevalences reached 77 % for ampicillin and 79 % for tetracycline, whereas resistance remained rare to the CIAs ciprofloxacin (0.11 %), third-generation cephalosporins (0.04 %) carbapensms (0%) and colistin (0 %). These proportions remained similar to those reported in the 2015 Australian survey (Kidsley et al., 2018), and indicated there has been only a modest upward drift in β-lactam and tetracycline resistance over the past decade, with no measurable expansion of CIA resistance. Across all isolates, 24 phenotypic resistance profiles were identified and 56.9 % of isolates were MDR, most often to the combination of β-lactams, folate-pathway inhibitors, phenicols and tetracyclines. The MDR phenotypes primarily reflect the antimicrobials that are registered for first-line treatment in Australian pigs (Australian Veterinary Association, 2018). Nevertheless, fewer than 0.2 % of isolates accrued index weightings driven by resistance to CIAs, demonstrating effectiveness of Australia’s stringent antimicrobial-stewardship policies and regulatory restrictions on critically important antimicrobials (CIAs).

These Australian data occupy a unique position among major pork-producing regions. On one hand the prevalences of ampicillin and tetracycline resistance in commensal *E. coli* from pigs are in the upper end of European estimates (EFSA/ECDC, 2024), and align more with prevalence data from North America (U.S. Food and Drug Administration, 2023). On the other hand, resistance to CIAs such as fluoroquinalones and extended-spectrum cephalosporins are notably rare in comparison, of up to 43.9% and up to 12.5% respectively in European pigs (EFSA/ECDC, 2024). The single cefotaxime-resistant *E. coli* isolate recovered here equates to a prevalence of 0.04 %, compared with ~5 % across Europe. This is consistant with previous reports on both commensal and pathogenic *E. coli* and *Salmonella spp.* from Australian pigs, poultry and beef cattle (Abraham et al., 2023; Abraham et al., 2022; Abraham et al., 2014; Abraham et al., 2015; Abraham et al., 2019; Barlow et al., 2022; Veltman et al., 2021). These contrasts reinforce the benefit of Australia’s long-standing restriction on the use of fluoroquinalones and strong label constraints limiting the use of third-generation cephalosporins in Australian livestock (Cheng et al., 2012; Mukerji et al., 2017).

Intensive herd-level subsampling (average of 93 colonies per farm) also exposed pronounced herd-to-herd variation for resistance to some antimicrobials. The herd AMR index, encapsulating resistance information from every antimicrobial in the test panel weighted by its importance rating, ranged from 1.51 to 5.76 (median 3.20). Herds at the lower end were dominated by fully susceptible isolates or isolates resistant only to low-importance drugs, whereas herds with higher index values contained increasing proportions of MDR isolates. For low-importance drugs, resistance varied substantially between herds. For example, the range of resistance for ampicillin and tetracycline was 11.8–100 % and 45.7–100 % respectively. Medium-importance agents such as apramycin (0–48.3 %) and gentamicin (0–30.0 %) showed narrower ranges, and resistance to CIAs displayed virtually no variability owing to virtual absence - single isolates were detected in just a few herds. Hierarchical clustering provided a method of ranking herds independent of the herd AMR index, and due to good agreement between the two, supports reliance on the latter as a summary of complex phenotypic resistance data.

The composite AMR index offered a concise, scalable metric of antimicrobial-resistance pressure—from individual animals to the national herd—by collapsing complex phenotypic resistance data into a single score that diverse stakeholders can use to benchmark performance and track stewardship progress. The index’s four-tier weighting assigns greatest influence to CIAs, so even small shifts in high-priority resistance are immediately apparent, while agents of lower public-health concern contribute proportionally less; this makes the index sensitive enough to prompt rapid investigation yet rewarding when CIA resistance falls. Calculated as the mean of all isolate scores on a farm, the index yields an anonymous, farm-specific “resistance rating” that can be compared across operations and over time to identify outliers, demonstrate the impact of interventions such as vaccination or reduced antimicrobial use, and aggregate seamlessly into regional or national surveillance metrics. Coded identifiers of herds managed by an independent custodian preserve confidentiality, which lowers barriers to participation and encourages producers to share data; simultaneously, it enables veterinarians to collaborate across farms, exchange best-practice insights, and coordinate targeted stewardship initiatives without exposing the identity of individual enterprises (Arnold et al., 2024; van Panhuis et al., 2014). Together, these features make the AMR index a practical metric that bridges laboratory results and on-farm decisions, underpins evidence-based policy, and provides a transparent means to demonstrate tangible reductions in resistance.

To explore the molecular basis of resistance to critically important antimicrobials and multi-class resistance (≥5 classes), 21 isolates were sequenced. All three quinolone-resistant isolates were included: two carried plasmid-mediated *qnrS1* and one of these possessed *oqxA/oqxB;* the third displayed canonical chromosomal mutations in *gyrA, parC* and *parE.* The single cefotaxime-resistant isolate harboured no recognised β-lactamase genes, suggesting either a novel mechanism or a false-positive phenotype. Sixteen meropenem NW isolates contained no carbapenemase determinants, indicating that their elevated MICs likely reflected assay drift (Clinical and Laboratory Standards Institute, 2020) rather than genuine carbapenem resistance. Although limited in scope, these genomic data illustrate that the rare CIA-resistant events detected were globally disseminated fluroquinlone -resistant clones such as ST744 (Abraham et al., 2015; Boehmer et al., 2018; Fuga et al., 2022; Laird et al., 2022; Maciuca et al., 2019; Oteo et al., 2018) and broad host range ST10 (Fuga et al., 2022) highlighting the value of targeted sequencing for confirmation and source-tracking.

Four stewardship implications follow from this work. First, intensive subsampling provides an early-warning margin that single-colony designs cannot match and provides comprehensive herd-level monitoring of AMR. Second, resistance to first-line antimicrobials—most notably β-lactams and tetracyclines—has become the dominant selective pressure on *E. coli* and likely on other commensal and pathogenic bacteria in Australian pigs (Australian Veterinary Association, 2018). In the absence of alternative tools such as vaccines against common procine bacterial pathogens (e.g. *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, enterotoxigenic *E. coli*, *Mycoplasma hyopneumoniae* and *Brachyspira hyodysenteriae*), it is unlikely that there will be substantial reductions in selection for resistance to first-line antimicrobials. Third, sustained restriction on the use of CIAs, together with high-resolution monitoring, continue to protect the effectiveness of antimicrobials that are critical to maintaining human health. Fourth, the herd AMR index translates detailed laboratory outputs into a single value that is readily understood by producers, prescribers and regulators, facilitating benchmarking and risk-based interventions.

There are several limitations to this study. Firstly, this study offers a cross-sectional snapshot restricted to finisher pigs and therefore cannot resolve potential seasonal or age-related trends. Secondly, commensal *E. coli* in this work were isolated with the standard, *E. coli* selective agar commonly included in national and international guidelines on AMR surveillance. This approach neither measures bacterial concentration nor does it target early emergence of resistance to CIAs. Consequently, CIA-resistant strains present at low concentrations in faeces can escape detection. For example, in an Australian poultry survey, for example, fluoroquinolone-resistant *E. coli* occurring at only 10^2–10^4 CFU g⁻¹ were found only after selective media were applied (Truswell et al., 2023), demonstrating how similar low-level carriage in pigs could remain hidden. Integrating CIA-selective plates and quantitative enumeration into routine monitoring would make surveillance more sensitive and allow emerging resistance to be recognised before it becomes entrenched (World Health Organization, 2022). Despite these constraints, the combination of large numbers of isolates, detailed resistance phenotyping and herd-level analyses has yielded the most comprehensive picture to date of the Australian porcine *E. coli* resistome.

In conclusion, pig production in Australia is frequently challenged by the occurrence of AMR to first-line antimicrobials registered for use in pigs, yet resistance to critically important antimicrobials remains particularly low. Protecting this favourable balance rests on two pillars: continued exclusion of CIAs from food-animal use and the use of a surveillance approach sensitive enough to detect early incursions of rare but clinically significant phenotypes. The robotic, high-density approach showcased here—and its potential extension to quantitative screening—meets these requirements and should be integrated into routine monitoring programmes to safeguard both animal health and public health worldwide.

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**Supplementary material.**

**Table S1**: Distribution of minimum inhibitory concentrations for *Escherichia coli* (n=2,730) isolated from Australian pigs. Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, solid vertical bars between cells show the position of the microbiological breakpoint and the shaded areas indicate the range of dilutions evaluated. Blank cells within the shaded area indicate that no isolates tested had an MIC at that concentration. Numbers outside the shaded area indicate the percent of isolates that had growth at all concentrations tested and the MIC is above the tested range. “.” Indicates the breakpoint was not available and the confidence interval was not calculated.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| drug | n | 0.008 | 0.016 | 0.031 | 0.063 | 0.13 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2048 | nw(nw\_ci) | cr(cr\_ci) |
| Amikacin | 2730 |  |  |  |  |  |  |  | 66.52 | 27.58 | 5.24 | .66 |  |  |  |  |  |  |  |  | 0(0,.14) | 0.00(0,.14) |
| Ampicillin | 2730 |  |  |  |  |  |  |  | 1.54 | 6.92 | 10 | 4.18 | 1.06 | .18 | 76.12 |  |  |  |  |  | 77.36(75.75,78.92) | 76.30(74.66,77.88) |
| Apramycin | 2730 |  |  |  |  |  |  |  |  |  |  | 93.77 | 2.6 | .04 | 3.59 |  |  |  |  |  | . | . | 3.63(2.96,4.4) |
| Cefotaxime | 2730 |  | 3.7 | 30.66 |  | 11.17 | .04 |  | .07 | .07 | .04 |  |  |  |  |  |  |  |  |  | .18(.06,.43) | 0.04(0,.2) |
| Ceftazidime | 2730 |  |  |  |  | 50 | 35.57 | 1.76 |  | .07 | .07 | .04 |  |  |  |  |  |  |  |  | .18(.06,.43) | 0.00(0,.14) |
| Chloramphenicol | 2730 |  |  |  |  |  |  |  |  | 2.56 | 23.3 | 25.97 | 4.65 | 8.64 | 34.87 |  |  |  |  |  | 43.52(41.65,45.4) | 43.52(41.65,45.4) |
| Ciprofloxacin | 2730 |  | 13.92 | 2.16 |  | 1.54 | 2.16 | .15 | .07 |  | .04 |  |  |  |  |  |  |  |  |  | 3.96(3.26,4.76) | 0.11(.02,.32) |
| Colistin | 2730 |  |  |  |  |  | 95.35 | 4.29 | .33 | .04 |  |  |  |  |  |  |  |  |  |  | 0(0,.14) | 0.00(0,.14) |
| Florfenicol | 2730 |  |  |  |  |  |  |  |  |  | 21.68 | 38.1 | 22.89 | 5.82 | 11.5 |  |  |  |  |  | 17.33(15.92,18.8) | 17.33(15.92,18.8) |
| Gentamicin | 2730 |  |  |  |  |  | 50.15 | 38.35 | 6.08 | .7 | .62 | 2.16 | .92 | 1.03 |  |  |  |  |  |  | 4.73(3.96,5.59) | 1.94(1.46,2.53) |
| Meropenem | 2730 |  | 16.56 | 53.44 |  | 5.93 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 5.93(5.08,6.89) | 0.00(0,.14) |
| Sulfamethoxazole | 2730 |  |  |  |  |  |  |  |  |  |  | 10.84 | 2.12 | 20.15 | 4.69 | 1.21 | .04 | 3.04 | 57.91 |  | . | . | 60.95(59.09,62.79) |
| Tetracycline | 2730 |  |  |  |  |  |  |  | 15.09 | 5.09 | .44 | .66 | .22 | 1.1 | 77.4 |  |  |  |  |  | 78.72(77.13,80.24) | 78.72(77.13,80.24) |
| Trimethoprim | 2730 |  |  |  |  |  | 31.72 | 21.1 | 3.48 | 1.28 | .18 | .04 | .11 | 42.09 |  |  |  |  |  |  | 42.42(40.55,44.3) | 42.20(40.34,44.08) |

**References:**

Abraham, R., Allison, H.S., Lee, T., Pavic, A., Chia, R., Hewson, K., Lee, Z.Z., Hampson, D.J., Jordan, D., Abraham, S. 2023. A national study confirms that Escherichia coli from Australian commercial layer hens remain susceptible to critically important antimicrobials. PLoS ONE 18, e0281848. <https://doi.org/10.1371/journal.pone.0281848>.

Abraham, R., Sahibzada, S., Jordan, D., O'Dea, M., Hampson, D.J., McMillan, K., Duffy, L., Mellor, G., Barlow, R., Abraham, S. 2022. Antimicrobial resistance and genomic relationships of Salmonella enterica from Australian cattle. Int. J. Food Microbiol. 371, 109672. <https://doi.org/10.1016/j.ijfoodmicro.2022.109672>.

Abraham, S., Groves, M.D., Trott, D.J., Chapman, T.A., Turner, B., Hornitzky, M., Jordan, D. 2014. Salmonella enterica isolated from infections in Australian livestock remain susceptible to critical antimicrobials. Int. J. Antimicrob. Agents 43, 126-130. <https://doi.org/10.1016/j.ijantimicag.2013.10.014>.

Abraham, S., Jordan, D., Wong, H.S., Johnson, J.R., Toleman, M.A., Wakeham, D.L., Gordon, D.M., Turnidge, J.D., Mollinger, J.L., Gibson, J.S., Trott, D.J. 2015. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals. Journal of Global Antimicrobial Resistance 3, 273-277. <https://doi.org/10.1016/j.jgar.2015.08.002>.

Abraham, S., O'Dea, M., Sahibzada, S., Hewson, K., Pavic, A., Veltman, T., Abraham, R., Harris, T., Trott, D.J., Jordan, D. 2019. Escherichia coli and Salmonella spp. isolated from Australian meat chickens remain susceptible to critically important antimicrobial agents. PLoS ONE 14, e0224281. <https://doi.org/10.1371/journal.pone.0224281>.

Arnold, K.E., Laing, G., McMahon, B.J., Fanning, S., Stekel, D.J., Pahl, O., Coyne, L., Latham, S.M., McIntyre, K.M. 2024. The need for One Health systems-thinking approaches to understand multiscale dissemination of antimicrobial resistance. The Lancet Planetary Health 8, e124-e133. [https://doi.org/10.1016/S2542-5196(23)00278-4](https://doi.org/10.1016/S2542-5196%2823%2900278-4).

ASTAG, 2018. Importance ratings of antimicrobials in human medicine. Canberra: Health, A.G.D.o. <https://www.amr.gov.au/sites/default/files/2022-10/importance-ratings-and-summary-of-antibacterial-uses-in-human-and-animal-health-in-australia.pdf>.

Australian Pork Limited, 2020. National Farm Biosecurity Manual for Pork Producers. Limited, A.P.

Australian Veterinary Association, 2018. Antimicrobial Prescribing Guidelines for Pigs. Sydney, Australia: <https://www.ava.com.au/siteassets/advocacy-resources/antimicrobial-prescribing-guidelines-for-pigs.pdf>.

Azargun, R., Sadeghi, V., Leylabadlo, H.E., Alizadeh, N., Ghotaslou, R. 2020. Molecular mechanisms of fluoroquinolone resistance in Enterobacteriaceae clinical isolates in Azerbaijan, Iran. Gene Reports 21, 100924. [https://doi.org/https://doi.org/10.1016/j.genrep.2020.100924](https://doi.org/https%3A//doi.org/10.1016/j.genrep.2020.100924).

Barlow, R., McMillan, K., Mellor, G., Duffy, L., Jordan, D., Abraham, R., O'Dea, M., Sahibzada, S., Abraham, S. 2022. Phenotypic and Genotypic Assessment of Antimicrobial Resistance in Escherichia coli from Australian Cattle Populations at Slaughter. J. Food Prot. 85, 563-570. <https://doi.org/10.4315/jfp-21-430>.

Boehmer, T., Vogler, A.J., Thomas, A., Sauer, S., Hergenroether, M., Straubinger, R.K., Birdsell, D., Keim, P., Sahl, J.W., Williamson, C.H. 2018. Phenotypic characterization and whole genome analysis of extended-spectrum beta-lactamase-producing bacteria isolated from dogs in Germany. PLoS ONE 13, e0206252.

Cheng, A.C., Turnidge, J., Collignon, P., Looke, D., Barton, M., Gottlieb, T. 2012. Control of Fluoroquinolone Resistance through Successful Regulation, Australia. Emerging Infect. Dis. 18, 1453-1460. <https://doi.org/10.3201/eid1809.111515>.

Clinical and Laboratory Standards Institute, 2018. VET08: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals - Fourth Edition. USA: Institute, C.a.L.S.

Clinical and Laboratory Standards Institute, 2020. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA: Institute, C.a.L.S. <https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf>.

DANMAP, 2023. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Kgs. Lyngby: Statens Serum Institut and National Food Institute, T.U.o.D. <https://www.danmap.org/reports/2023>.

EFSA/ECDC. 2024. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021/2022. EFSA J. 22. <https://doi.org/10.2903/j.efsa.2024.8583>.

Ellis, S., Coombe, J., Page, S., Gomboso, J., Iglesias, R., Schipp, M. 2024. Response to "Global trends in antimicrobial use in food-producing animals: 2020 to 2030 ". Plos Glob Publ Hlth 4. <https://doi.org/ARTN> e0003133

10.1371/journal.pgph.0003133.

EUCAST, 2023. MIC and zone diameter distributions and ECOFFs. <https://www.eucast.org/mic_and_zone_distributions_and_ecoffs>.

Fuga, B., Sellera Fábio, P., Cerdeira, L., Esposito, F., Cardoso, B., Fontana, H., Moura, Q., Cardenas-Arias, A., Sano, E., Ribas Rosineide, M., Carvalho Albalúcia, C., Tognim Maria Cristina, B., de Morais Marcia Maria, C., Quaresma Ana Judith, P.G., Santana Ângela, P., Reis Joice, N., Pilonetto, M., Vespero Eliana, C., Bonelli Raquel, R., Cerqueira Aloysio, M.F., Sincero Thaís, C.M., Lincopan, N. 2022. WHO Critical Priority Escherichia coli as One Health Challenge for a Post-Pandemic Scenario: Genomic Surveillance and Analysis of Current Trends in Brazil. Microbiol. Spectr. 10, e01256-01221. <https://doi.org/10.1128/spectrum.01256-21>.

Government of Canada, 2023. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2023. Ottawa, Canada: Canada, P.H.A.o. <https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars.html>.

Gross, R., Yelin, I., Viktória, L., Datta, M.S., Kishony, R. 2024. Beta-lactamase dependent and independent evolutionary paths to high-level ampicillin resistance. Nature Communications 15, 5383. <https://doi.org/10.1038/s41467-024-49621-2>.

Hooper, D.C., Jacoby, G.A. 2015. Mechanisms of drug resistance: quinolone resistance. Ann. N. Y. Acad. Sci. 1354, 12-31. <https://doi.org/10.1111/nyas.12830>.

Kidsley, A.K., Abraham, S., Bell, J.M., O'Dea, M., Laird, T.J., Jordan, D., Mitchell, P., McDevitt, C.A., Trott, D.J. 2018. Antimicrobial Susceptibility of Escherichia coli and Salmonella spp. Isolates From Healthy Pigs in Australia: Results of a Pilot National Survey. Front Microbiol 9, 1207. <https://doi.org/10.3389/fmicb.2018.01207>.

Laird, T.J., Jordan, D., Lee, Z.Z., O'Dea, M., Stegger, M., Truswell, A., Sahibzada, S., Abraham, R., Abraham, S. 2022. Diversity detected in commensals at host and farm level reveals implications for national antimicrobial resistance surveillance programmes. J. Antimicrob. Chemother. 77, 400-408. <https://doi.org/10.1093/jac/dkab403>.

Landers, T.F., Cohen, B., Wittum, T.E., Larson, E.L. 2012. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Rep. 127, 4-22. <https://doi.org/Doi> 10.1177/003335491212700103.

Maciuca, I.E., Cummins, M.L., Cozma, A.P., Rimbu, C.M., Guguianu, E., Panzaru, C., Licker, M., Szekely, E., Flonta, M., Djordjevic, S.P. 2019. Genetic features of mcr-1 mediated colistin resistance in CMY-2-producing Escherichia coli from Romanian poultry. Front. Microbiol. 10, 2267.

Marshall, B.M., Levy, S.B. 2011. Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev. 24, 718-733. <https://doi.org/10.1128/cmr.00002-11>.

Mukerji, S., O'Dea, M., Barton, M., Kirkwood, R., Lee, T., Abraham, S. 2017. Development and transmission of antimicrobial resistance among Gram-negative bacteria in animals and their public health impact. Essays Biochem 61, 23-35. <https://doi.org/10.1042/Ebc20160055>.

Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S.C., Browne, A.J., Chipeta, M.G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B.H.K., Kumaran, E.A.P., McManigal, B., Agarwal, R., Akech, S., Albertson, S., Amuasi, J., Andrews, J., Aravkin, A., Ashley, E., Bailey, F., Baker, S., Basnyat, B., Bekker, A., Bender, R., Bethou, A., Bielicki, J., Boonkasidecha, S., Bukosia, J., Carvalheiro, C., Castañeda-Orjuela, C., Chansamouth, V., Chaurasia, S., Chiurchiù, S., Chowdhury, F., Cook, A.J., Cooper, B., Cressey, T.R., Criollo-Mora, E., Cunningham, M., Darboe, S., Day, N.P.J., De Luca, M., Dokova, K., Dramowski, A., Dunachie, S.J., Eckmanns, T., Eibach, D., Emami, A., Feasey, N., Fisher-Pearson, N., Forrest, K., Garrett, D., Gastmeier, P., Giref, A.Z., Greer, R.C., Gupta, V., Haller, S., Haselbeck, A., Hay, S., Holm, M., Hopkins, S., Iregbu, K.C., Jacobs, J., Jarovsky, D., Javanmardi, F., Khorana, M., Kissoon, N., Kobeissi, E., Kostyanev, T., Krapp, F., Krumkamp, R., Kumar, A., Kyu, H.H., Lim, C., Limmathurotsakul, D., Loftus, M.J., Lunn, M., Ma, J., Mturi, N., Munera-Huertas, T., Musicha, P., Mussi-Pinhata, M.M., Nakamura, T., Nanavati, R., Nangia, S., Newton, P., Ngoun, C., Novotney, A., Nwakanma, D., Obiero, C.W., Olivas-Martinez, A., Olliaro, P., Ooko, E., Ortiz-Brizuela, E., Peleg, A.Y., Perrone, C., Plakkal, N., Ponce-de-Leon, A., Raad, M., Ramdin, T., Riddell, A., Roberts, T., VictoriaRobotham, J., Roca, A., Rudd, K.E., Russell, N., Schnall, J., Scott, J.A.G., Shivamallappa, M., Sifuentes-Osornio, J., Steenkeste, N., Stewardson, A.J., Stoeva, T., Tasak, N., Thaiprakong, A., Thwaites, G., Turner, C., Turner, P., van Doorn, H.R., Velaphi, S., Vongpradith, A., Vu, H., Walsh, T., Waner, S., Wangrangsimakul, T., Wozniak, T., Zheng, P., Sartorius, B., Lopez, A.D., Stergachis, A., Moore, C., Dolecek, C., Naghavi, M., Collabora, A.R. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399, 629-655. [https://doi.org/10.1016/S0140-6736(21)02724-0](https://doi.org/10.1016/S0140-6736%2821%2902724-0).

O'Dea, M., Sahibzada, S., Jordan, D., Laird, T., Lee, T., Hewson, K., Pang, S., Abraham, R., Coombs, G.W., Harris, T., Pavic, A., Abraham, S. 2019. Genomic, Antimicrobial Resistance, and Public Health Insights into *Enterococcus* spp. from Australian Chickens. J. Clin. Microbiol. 57, e00319-19. <https://doi.org/10.1128/JCM.00319-19>.

Oteo, J., Mencia, A., Bautista, V., Pastor, N., Lara, N., Gonzalez-Gonzalez, F., García-Peña, F.J., Campos, J. 2018. Colonization with Enterobacteriaceae-producing ESBLs, AmpCs, and OXA-48 in wild avian species, Spain 2015–2016. Microb. Drug Resist. 24, 932-938.

Python Software Foundation, 2024. Python Language Reference, version 3.11.7 [software]. <https://www.python.org/>.

Singer, R.S., Patterson, S.K., Meier, A.E., Gibson, J.K., Lee, H.L., Maddox, C.W. 2004. Relationship between Phenotypic and Genotypic Florfenicol Resistance in Escherichia coli. Antimicrobial Agents and Chemotherapy 48, 4047-4049. <https://doi.org/10.1128/aac.48.10.4047-4049.2004>.

StataCorp, 2021. Stata Statistical Software: Release 16.1 (16.1) [software]. StataCorp LLC. <https://www.stata.com>.

Truswell, A., Abraham, R., O’Dea, M., Lee, Z.Z., Lee, T., Laird, T., Blinco, J., Kaplan, S., Turnidge, J., Trott, D.J., Jordan, D., Abraham, S. 2021. Robotic Antimicrobial Susceptibility Platform (RASP): a next-generation approach to One Health surveillance of antimicrobial resistance. J. Antimicrob. Chemother. 76, 1800-1807. <https://doi.org/10.1093/jac/dkab107>.

Truswell, A., Lee, Z.Z., Stegger, M., Blinco, J., Abraham, R., Jordan, D., Milotic, M., Hewson, K., Pang, S., Abraham, S. 2023. Augmented surveillance of antimicrobial resistance with high-throughput robotics detects transnational flow of fluoroquinolone-resistant *Escherichia coli* strain into poultry. J. Antimicrob. Chemother. 78, 2878–2885. [https://doi.org/https://doi.org/10.1093/jac/dkad323](https://doi.org/https%3A//doi.org/10.1093/jac/dkad323).

U.S. Food and Drug Administration, 2010. National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS): 2007 Executive Report. Administration, U.S.F.a.D.

U.S. Food and Drug Administration, 2023. About NARMS 2023. <https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/about-narms>.

van den Bogaard, A.E., Stobberingh, E.E. 2000. Epidemiology of resistance to antibiotics - Links between animals and humans. Int. J. Antimicrob. Agents 14, 327-335. <https://doi.org/Doi> 10.1016/S0924-8579(00)00145-X.

van Panhuis, W.G., Paul, P., Emerson, C., Grefenstette, J., Wilder, R., Herbst, A.J., Heymann, D., Burke, D.S. 2014. A systematic review of barriers to data sharing in public health. BMC Public Health 14, 1144. <https://doi.org/10.1186/1471-2458-14-1144>.

Veltman, T., Jordan, D., McDevitt, C.A., Bell, J., Howden, B.P., Valcanis, M., O'Dea, M., Abraham, S., Scott, P., Kovac, J.H., Chia, R., Combs, B., Chousalkar, K., Wilson, T., Trott, D.J. 2021. Absence of high priority critically important antimicrobial resistance in Salmonella sp. isolated from Australian commercial egg layer environments. Int. J. Food Microbiol. 340, 109042. <https://doi.org/10.1016/j.ijfoodmicro.2021.109042>.

Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S.J., Brett, M., Wilson, J., Millman, K.J., Mayorov, N., Nelson, A.R.J., Jones, E., Kern, R., Larson, E., Carey, C.J., Polat, İ., Feng, Y., Moore, E.W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E.A., Harris, C.R., Archibald, A.M., Ribeiro, A.H., Pedregosa, F., van Mulbregt, P., Vijaykumar, A., Bardelli, A.P., Rothberg, A., Hilboll, A., Kloeckner, A., Scopatz, A., Lee, A., Rokem, A., Woods, C.N., Fulton, C., Masson, C., Häggström, C., Fitzgerald, C., Nicholson, D.A., Hagen, D.R., Pasechnik, D.V., Olivetti, E., Martin, E., Wieser, E., Silva, F., Lenders, F., Wilhelm, F., Young, G., Price, G.A., Ingold, G.-L., Allen, G.E., Lee, G.R., Audren, H., Probst, I., Dietrich, J.P., Silterra, J., Webber, J.T., Slavič, J., Nothman, J., Buchner, J., Kulick, J., Schönberger, J.L., de Miranda Cardoso, J.V., Reimer, J., Harrington, J., Rodríguez, J.L.C., Nunez-Iglesias, J., Kuczynski, J., Tritz, K., Thoma, M., Newville, M., Kümmerer, M., Bolingbroke, M., Tartre, M., Pak, M., Smith, N.J., Nowaczyk, N., Shebanov, N., Pavlyk, O., Brodtkorb, P.A., Lee, P., McGibbon, R.T., Feldbauer, R., Lewis, S., Tygier, S., Sievert, S., Vigna, S., Peterson, S., More, S., Pudlik, T., Oshima, T., Pingel, T.J., Robitaille, T.P., Spura, T., Jones, T.R., Cera, T., Leslie, T., Zito, T., Krauss, T., Upadhyay, U., Halchenko, Y.O., Vázquez-Baeza, Y., SciPy, C. 2020. SciPy 1.0: fundamental algorithms for scientific computing in Python. Nat. Methods 17, 261-272. <https://doi.org/10.1038/s41592-019-0686-2>.

Waskom, M.L. 2021. seaborn: statistical data visualization. Journal of Open Source Software 6, 3021. <https://doi.org/10.21105/joss.03021>.

World Health Organization, 2017. WHO guidelines on use of medically important antimicrobials in food-producing animals. Geneva: Organization, W.H. <https://www.who.int/publications/i/item/9789241550130>.

World Health Organization, 2022. Global antimicrobial resistance and use surveillance system (GLASS) report: 2022. Geneva: Organization, W.H. <https://www.who.int/publications/i/item/9789240062702>.

World Health Organization, 2024a. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: Organization, W.H. <https://www.who.int/publications/i/item/9789240093461>.

World Health Organization, 2024b. WHO’s List of Medically Important Antimicrobials: A risk management tool for mitigating antimicrobial resistance due to non-human use. Geneva: Organization, W.H. <https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf>.

World Health Organization; Food and Agriculture Organization of the United Nations; United Nations Environment Programme; World Organisation for Animal Health, 2023. A one health priority research agenda for antimicrobial resistance. Geneva: Organization, W.H. <https://www.who.int/publications/i/item/9789240075924>.