Surveillance for antimicrobial resistance in enteric commensals and pathogens in Australian meat chickens 2022

ACKNOWLEDGEMENTS

The Australian Chicken Meat Federation would like to thank The Department of Agriculture, Fisheries and Forestry (DAFF; formerly Department of Agriculture, Water and the Environment) and the AgriFutures Australia Chicken Meat Program for the funding to complete this survey, Dr. Kylie Hewson for project management, Birling Laboratories for exceptional coordination of sample collection and bacterial isolation and Dr. Sam Abraham and his research team for their dedication to precision, transparency and accuracy with the AMR analyses and reporting. Completion of this survey would not have been possible without the commitment of those within all participating chicken meat processing companies who were able to collect the samples under extraordinary circumstances.

ABBREVIATIONS

ACMF	Australian Chicken Meat Federation Inc
AMR	Antimicrobial resistance
AMS	Antimicrobial stewardship
APVMA	Australian Pesticides and Veterinary Medicines Authority
AS	Australian Standard
ASTAG	Australian Strategic and Technical Advisory Group on AMR
CLSI	Clinical and Laboratory Standards Institute
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
DANMAP	Danish Programme for surveillance of antimicrobial consumption and resistance
ECOFF	Epidemiological Cut-off Values
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MALDI-TOF	Mass spectrometry, matrix-assisted laser desorption/ionization
MCR	Multi-class resistance
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence type
NARMS	National Antimicrobial Resistance Monitoring System
NATA	National Accreditation of Testing Authorities, Australia
PBS	Phosphate-buffered saline
PUBCRIS	Public Chemical Registration Information System
QRDR	Quinolone Resistance Determining Region
RASP	Robotic Antimicrobial Susceptibility Platform
SBA	Sheep blood agar
WOAH	World Organisation for Animal Health

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EXECUTIVE SUMMARY

Background

Antimicrobial resistance (AMR) is a serious threat to public health globally. The cornerstone of national and international efforts to address AMR is antimicrobial stewardship – programs and activities designed to halt the emergence and spread of resistance in animal and human populations. Surveillance for AMR can help identify emerging AMR and provide valuable feedback on how to ensure stewardship programs are effective. The purpose of this study was to conduct a repeat of the 2016 survey (i.e. 5 years between the surveys) using the recommended surveillance model for the Australian chicken meat industry to re-assess the AMR status of the Australian meat chicken. The results of the 2016 study showed that the Australian chicken meat industry is in an enviable position globally, with low levels of AMR in commensal bacteria from meat chickens and, importantly, low levels of resistance to antimicrobials that are priorities for use in human health.

This project reassessed the prevalence of resistance against specified antimicrobials amongst key indicator and foodborne pathogens (*Escherichia (E) coli*, *Salmonella* spp., *Enterococcus* spp. and *Campylobacter* spp.) isolated from caecal contents of Australian meat chickens at slaughter.

Approach

This project used internationally recognised methods in AMR surveillance along with cutting edge robotics, mass spectrometry and genomics platforms. Sampling was designed to be comparable to international surveillance programs in terms of sample size and processing. Samples were collected at slaughter from 20 processing plants which provide the majority of chicken meat in Australia (>90%) in proportion to the number of birds processed at each facility. Samples were collected between October 2021 and May 2022.

Each sample consisted of 5 caeca. *Salmonella* and *Campylobacter* spp. were isolated using traditional methods and a single isolate of *Salmonella* and *C. coli* and *C. jejuni* (where both species were present) per sample was collected. *E. coli* and *Enterococci* spp. were isolated using selective agar on the Robotic Antimicrobial Susceptibility Platform (RASP). Each individual caeca within a sample was swabbed and the swabs pooled in phosphate-buffered saline (PBS) before robotic processing. Between 13-16 *E. coli* isolates were selected per sample and a single *E. faecium* and *E. faecalis*, when present, were also collected. The identity of all isolates was confirmed by MALDI-TOF before AMR profiling. Antimicrobial susceptibility testing was performed using the RASP which conforms to Clinical Laboratory Sciences Institute (CLSI) and ISO 20776-1:2019 guidelines.

Minor adjustments were made to the antimicrobial panels, in collaboration with representative experts from industry and government departments, to improve resource use and alignment with international guidance without compromising the ability to compare resistance to antimicrobial classes from previous surveys. All *Salmonella* and *Campylobacter* isolates and any *E. coli* or Enterococci displaying phenotypic resistance of importance were selected for whole genome sequencing.

Key results

A total of 190 samples were accepted into the study over the course of the collection period. A total of 3,308 bacterial isolates were collected – 171 *Enterococcus*, 2950 *E. coli*, 9 *Salmonella* and 178 *Campylobacter*.

Overall, antimicrobial resistance was found to be low and consistent with the results from the 2016 survey.

Enterococcus

No resistance to the critically important antimicrobials vancomycin and linezolid was detected and only one isolate (0.58%; an *E. faecium*) was multi-class resistant (MCR); defined as being clinically resistant to three or more classes of antimicrobial).

The majority of *E. faecium* (64.6%) were susceptible to all tested antimicrobials. Resistance to erythromycin in *E. faecium* continued to decrease, a trend observed in the 2016 study, to a low of 5.44%. This could reflect the reduction in use of macrolides in the industry since the introduction of the *Mycoplasma* vaccines in the 1990s. The frequency of resistance to quinupristin-dalfopristin in *E. faecium* decreased to 6.1% (from 54.5%) in this study compared to the previous study, which might be reflective of the removal of virginiamycin use in chickens between the 2016 and 2022 surveys (resistance to virginiamycin also causes resistance to quinopristin-dalfopristin). Tetracycline resistance was the most prevalent (30.6 %) but was lower than the 2016 study (40.3 %).

The majority of *E. faecalis* isolates were resistant to tetracycline (87.5%) and a large proportion resistant to erythromycin (41.67%). However, no conclusions can be drawn due to the low number of isolates obtained in this study (n=24). All isolates were susceptible to the other antimicrobials tested.

E. coli

A total of 2950 *E. coli* isolates were collected in this study using the RASP, 14 times more than in the 2016 study. The majority of isolates were susceptible to all tested antimicrobials (56.8%). No clinical resistance to third generation cephalosporins was detected in this study. Ninety-six isolates (3.25%) were considered microbiologically resistant to ciprofloxacin while 32 (1.2%) were clinically resistant. Due to the fact that fluoroquinolones are not used in the commercial Australian chicken meat industry, these isolates were investigated further. Mutations known to confer quinolone resistance were identified in 25 of the sequenced clinically resistant isolates. The most common multi-locus sequence typing (MLST) amongst sequenced isolates was ST354 (n=16) and all of these were phenotypically resistant to ciprofloxacin with associated genetic mutations. These quinolone resistant isolates were heterogenous in AMR phenotype and genotype. Quinolone inducing resistance mutations were also found in isolates from STs 2083, 10130, 1078, 2398 and 2985. Only 2.92% of *E. coli* isolates were classified as MCR.

Salmonella

Despite using the same isolation protocols, laboratories and operators as for the 2016 survey, the number of *Salmonella* isolated from this study was very low (n=9). No resistance to the antimicrobials tested was observed.

Campylobacter

The majority of *Campylobacter* isolates were susceptible to all antimicrobials tested (C. jejuni 68.7%; C. coli 88.9 %). No MCR profiles were identified in the isolates in this study. All *Campylobacter* isolates were microbiologically susceptibile to azithromycin, chloramphenicol, clindamycin, erythromycin, florfenicol and gentamicin. Resistance to tetracycline (18.26% *C. jejuni*; 1.59% *C. coli*), nalidixic acid (21.74% *C. jejuni*; 4.76% *C. coli*) or ciprofloxacin (24.35% *C. jejuni*; 3.17% *C. coli*) were the most commonly detected resistance. Despite there being no use of fluoroquinolones in the commercial chicken industry in Australia, all the ciprofloxacin resistant isolates had mutations in the region known to confer resistance to quinolones.

Similar to the 2016 survey, no resistance to macrolides among campylobacter isolates was detected in this study, one of the key antimicrobials used for treating human campylobacteriosis.

Conclusion

Overall, there was a decrease in the prevalence of AMR in this study compared with the previous study in 2016 [1]. AMR carriage was generally low, if present at all, in comparison to other countries. The RASP processing with *E. coli* demonstrated the resolution achievable using high throughput robotics. Fluoroquinolone resistance was detected in all sequenced *E. coli* ST354 and ST773 isolates as well as the majority of ST752 isolates. These sequence types are globally disseminated multi-host strains. Considering the global prevalence of these fluoroquinolone resistant strains and the fact that fluoroquinolones are not used in the chicken meat industry it is likely the strains have been introduced through an external source.

These results suggest the Australian chicken meat industry’s efforts through its AMS program to reduce, refine and replace the use of antibiotics used in human medicine in the chicken industry are having a positive impact on the occurrence of AMR in chicken meat. The results also provide opportunities to continue improving antimicrobial stewardship efforts and make further progress in minimising industry’s impacts on the levels of AMR in animals, the environment and the community.

INTRODUCTION

Background

Antimicrobial resistance (AMR) is a serious threat to public health globally. The cornerstone of national and international efforts to deal with AMR is antimicrobial stewardship (AMS) – programs and activities designed to minimise the emergence of resistance and its spread in animal and human populations. Whilst the development of AMR impacting public health is foremost a consequence of antimicrobial use in human medicine, the use of antimicrobials in food-producing animals and companion animals can also potentially contribute. Therefore, the application of AMS across both human and animal populations offers the community the greatest protection from the harmful consequences of AMR.

Surveillance for AMR can help identify new risks and opportunities, and also provide valuable feedback on how AMS programs should be conducted. Globally, European and North American countries stand out as having well established surveillance systems that incorporate data from food animals on an ongoing basis. These include, for example, DANMAP (Denmark) [2], CIPARS (Canada) [3], and NARMS (USA) [4]. In Australia, a pilot program in food-producing animals was commissioned by the Australian Department of Agriculture, Fisheries and Forestry (DAFF) in 2003/2004 [5] followed by a national study in 2016 that utilised a recommended surveillance model for use in Australian livestock sectors based on the World Organisation for Animal Health (WOAH; formerly OIE) Chapter 6.8 'Harmonisation of national antimicrobial resistance surveillance and monitoring programmes' (previously Chapter 6.7) [1].

The results of the 2016 study showed that the Australian chicken meat industry is in an enviable position globally, with low levels of AMR in commensal bacteria from meat chickens and, importantly, low levels of resistance to antimicrobials that are priorities for use in human health [1]. The chicken meat industry in Australia is highly vertically integrated with chicken farmers predominantly contracted by processing companies who determine the farming practises employed [1]. This dynamic means that the processing companies are responsible for the inputs to the farm that relate directly to the chickens. Flock health is always managed by at least one registered veterinarian specialising in poultry, often directly employed by the processing company, who oversee and manage disease surveillance, diagnosis and treatment. There are few antimicrobials approved for use in Australian food production systems, with less approved for use in poultry (Table 1). There are further voluntary restrictions on antimicrobial use adopted by the companies that produce >90% of Australian chicken meat, including no use for growth promotion, and highly important antibiotics (as rated by the Australian Strategic and Technical Advisory Group on AMR; ASTAG) only used as a last resort [6, 7].

Table 1. Antibiotics that are permitted for use in the Australian meat chicken industry

Antimicrobial class	Antimicrobial	Route of administration	Registered use
Aminocyclitol, Lincosamide	Spectinomycin + Lincomycin	Water, Injection	treatment or prevention
Aminoglycoside	Apramycin	Water	treatment or prevention
	Neomycin	Feed, water	treatment or prevention
Arsenical	Roxarsone	Feed	growth promotion [a]
Glycophospholipid	Flavophospholipol	Feed	growth promotion [b]
Ionophore	Lasalocid	Feed	treatment or prevention
	Maduramicin	Feed	treatment or prevention
	Monensin	Feed	treatment or prevention
	Narasin	Feed	treatment or prevention
	Salinomycin	Feed	treatment or prevention
	Semduramicin	Feed	treatment or prevention
Macrolide	Erythromycin	Water	treatment or prevention
	Tylosin	Feed, water	treatment or prevention
Orthosomycin	Avilamycin	Feed	treatment or prevention + growth promotion [c]
Pleuromutilin	Tiamulin	Feed, water	treatment or prevention
Polypeptide	Bacitracin	Feed	treatment or prevention
Streptogramin	Virginiamycin	Feed	treatment or prevention
Sulfonamide, Diaminopyrimidine	Sulfadiazine + Trimethoprim	Water	treatment or prevention
	Sulfadimidine + Trimethoprim	Water	treatment or prevention
Sulfonamide	Sulfadimidine	Water	treatment or prevention
	Sulfaquinoxaline	Water	treatment or prevention
Tetracycline	Chlortetracycline	Feed, water	treatment or prevention
	Oxytetracycline	Feed, water	treatment or prevention
Moderate spectrum penicillin	Amoxicillin	Water	treatment or prevention

[a] Product registration discontinued in 2018; [b] Used off-label as a therapeutic treatment for necrotic enteritis or enteritis when other medications are inappropriate.; [c] Although the avilamycin formulation having a growth promotion claim is approved for use there are presently no such products available for sale in Australia. (Source: APVMA PUBCRIS database https://portal.apvma.gov.au/pubcris)

Objective

Repeat the recommended surveillance model for use in the Australian chicken meat industry based on the WOAH Chapter 6.8 within 5 years of the 2016 study to re-assess the AMR status of the Australian meat chicken. This includes surveillance of important indicator and potentially pathogenic species *E. coli*, *Salmonella, Enterococcus* and *Campylobacter* isolated from the caeca contents of Australian meat chickens at slaughter.

Roles and responsibilities

Successful completion of this work required collaboration amongst several individuals and institutions. A number of people contributed their time and expertise to assist this collaboration, and their contributions are gratefully acknowledged.

- Australian Chicken Meat Federation (ACMF), Dr. Kylie Hewson – project management, sample collection coordinator and industry engagement; overall coordination of the project and sample collection, first contact point for stakeholders; establishing and providing protocols for sample collection; primary responsibility for the project. kylie.hewson@chicken.org.au

- Company Coordinator for each company involved in the study – coordinated collection of samples in each plant associated with that company and training, as needed, for those collecting the samples; trained quality assurance staff or poultry veterinarians at the participating chicken processing plants; responsibility for ensuring samples were collected and shipped as per the protocol.

- Antimicrobial Resistance and Infectious Diseases Laboratory (AMRID), Murdoch University (Prof. Sam Abraham, Dr. Rebecca Abraham, Dr. Hui San Allison, Mr. John Blinco, Mr. Joe Lee, Ms. Nikki Asuming-Bediako and Ms. Michaela Burton), AMR testing laboratories – specialist ability at isolating bacterial species (*E. coli* and Enterococci) and phenotypic AMR testing on bacterial isolates using the Robotic Antimicrobial Susceptibility Platform (RASP) platform; responsible for providing scientific and technical advice to the project and assist the project coordinator in analysis and interpretation of results and compilation of the report. s.abraham@murdoch.edu.au

- Birling Laboratories, Dr. Sue Sharpe, Taha Harris and Dr Tony Pavic; Primary laboratory – NATA accreditation, broad expertise in veterinary microbiology with capacity and infrastructure for collation of caecal samples; isolation and identification of *Campylobacter* and *Salmonella*; storage of isolates and collation of data sent to the AMR laboratories in coordination with the project coordinator; responsible for ensuring only one sample from each farm collected at processing was submitted, isolation protocol was followed, and isolates were characterised, stored and shipped appropriately; maintains a copy of all isolates for reference. Sue_Sharpe@baiada.com.au; Tony_Pavic@baiada.com.au

- Dr Leigh Nind and Dr. David Jordan (NSW DPI); Management group – general oversight of the project; responsible for making final decisions on protocols and reporting. Leigh.nind@agriculture.gov.au; david.jordan@dpi.nsw.gov.au.

MATERIALS AND METHODS

The methods followed for this study are in line with recommendations from the WOAH Chapter 6.8 "*Harmonisation of national antimicrobial resistance surveillance and monitoring programmes*", which also aligns with the approaches undertaken for other DAFF-funded AMR surveillance projects in Australian livestock. The study also follows recommendations from the review *'Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia'* (the AMRIA report), which identified one of the major components of surveillance being the assessment of AMR in commensal bacteria and pathogens present in the gut of food animals at slaughter [8].

Animal population under study

The work focused on AMR in bacteria from the gut of meat chickens at slaughter from meat chicken processing plants around Australia. Companies that produce the bulk (> 90%) of Australian chicken meat were included in this study, to ensure the surveillance undertaken considered the key risk of volume of product/extent of human exposure.

There was a Company Coordinator for each of the six companies involved in the study (one company had closed since the 2016 survey), and in some cases, took the samples themselves, or coordinated other trained personnel to take the samples as per the below protocol. The Project Coordinator was the intermediary between the Company Coordinators and Birling Laboratories to enable an additional level of anonymity and scrutiny. Smaller processors were regarded as out of scope of this study.

Sampling of caecal contents from chickens at processing for AMR surveillance

Sample collection

Samples were collected as outlined in the previous report [1]. Briefly, each sample consisted of 5 pooled caecal pairs as per the NARMS protocol [9]. A maximum of 200 were planned to be collected from the 20 processing plants operated by the six major chicken meat companies between September 2021 and May 2022.

The number of samples to be collected at each plant was proportionally distributed based on the approximate number of chickens processed by each plant in each category each week and the most accurate estimate of the total number of chickens processed in Australia in 2020 (estimated at 12,756,000/week) [1, 10]. This is the method used for calculating sampling requirements for the National Residue Survey as actual number of chickens processed by each plant is commercially sensitive data and was therefore not available to the ACMF or the project team.

Only one sample from any single farm being processed on each day of sampling was collected, with duplicate collections from the same farm to be avoided. The exception was for situations were sample numbers required from a processing plant exceeded the number of farms supplying that plant during the study period. In these cases, an additional sample was collected from the farm but from a different batch of chickens. Samples were shipped overnight to Birling Laboratories for sample verification. Upon receival of the samples, the time and temperature inside the shipping container was recorded. Any samples that arrived more than 24hrs after collection or at a temperature >8°C were deemed unacceptable and

discarded. In these instances, the collection staff at the processing plant were notified and sent additional sampling kits to collect replacement samples.

Each caecal pair was cut into individual caeca. Five of the individual caeca were processed by Birling Laboratories to isolate *Salmonella* and *Campylobacter* while the other 5 caeca were processed by Murdoch University to isolate *Enterococci* and *E. coli*.

Sample processing and bacterial isolation – *Salmonella* and *Campylobacter* (Birling Laboratories)

The caeca for each sample were placed into individual stomacher bags and stomached to homogenise for 60 seconds as per the Australian Standard AS 5013.20-2004 (12.2) and left at room temperature for 5 minutes for gravity settling of large particles. For isolation of *Salmonella* spp., 25g of the homogenised sample was combined with 225 ml of sterile buffered peptone water and mixed well then processed as outlined below. For isolation of *Campylobacter* spp. 10g of homogenised sample was combined with 90ml of Bolton broth and mixed well then processed as outlined below.

Salmonella isolation

Salmonella was isolated using the Australian Standard (AS) 5013.10-2009 method (ISO 6579:2002) for *Salmonella* spp. using RV and MK media with two different selective and differential plates (XLD as the primary and Hektone as a secondary selective). A portion of remaining homogenate was mixed well and incubated at 37°C for 24 hours. A post incubation screen using Atlas PCR (validated to AS 5013. 10-2009 and NATA approved) was conducted to screen for *Salmonella* in addition to the AS method. Samples positive for both methods were confirmed using the AS reference method stated above with the following validated and NATA approved modification. A *Salmonella* specific chromogenic media (SMID2, BioMereriux) was used in place of biochemical testing by subculturing any suspect colonies onto nutrient agar for serological confirmation. From a pure subculture from the original colony, bacteria were harvested for storage at -20°C on cryo-beads in two separate, identical containers labelled with the sample code and the laboratory reference number. Low-dose positive controls were used to confirm isolation success (30 CFU of *S*. Alford; Bioball, Biomerieux).

Campylobacter isolation

Campylobacter was isolated as per the AS 5013.6-2015 method using *Campylobacter* selective Bolton broth. A portion of the remaining homogenate was shaken to suspend the particles and for samples that were <12 hours post-sampling, 100µL was streaked direct from Bolton broth/homogenate onto CSK (Skirrow, BioMerieux) and CFA (Campy food Agar, BioMerieux) agar and incubated at 42°C for 48 hours. For samples that were >12 hours post-sampling, the direct streaking method was performed along with a preliminary incubation of the Bolton broth/homogenate sample at 42°C for 48 hrs under microaerophilic conditions, prior to streaking onto CSK and CFA agar. The *Campylobacter* was speciated using Vitek 2 (BioMerieux) mass spectrometry. From a pure subculture from the original colony, bacteria were harvested for storage at -20°C on cryo-beads, using a proprietal suspension media which prevents damage to the

bacteria from freezing, in two separate, identical containers labelled with the sample code and the laboratory reference number.

Dispatch to AMR laboratories

One vial of cryo-beads for each isolate of *Campylobacter* spp., and *Salmonella* spp., recovered by Birling Laboratories was shipped to Murdoch University for species identification/confirmation using MALDI-TOF MS (Microflex, Bruker, MA, USA) and antimicrobial susceptibility testing.

Sample processing and bacterial isolation – *E. coli* and *Enterococci* (Murdoch University AMRID Laboratory)

Upon receival of the samples at Murdoch University, the time and temperature inside the shipping container was recorded. Any samples that arrived more than 24 hours after collection or at a temperature > 8°C were deemed unacceptable and discarded. In these instances, bacteria isolated at Birling Laboratories related to those samples were also discarded, the collection staff at the processing plant were notified and sent additional sampling kits to collect replacement samples. Each caecum from the sample container was laid out on sterile surgery cloth, cut with sterile scissors and a sterile swab inserted into the caeca. Each swab was placed in a 50ml tube containing 25ml of 1x PBS (phosphate buffered saline). The tube was vortexed and transferred onto the RASP for the isolation of *Enterococcus* and *E. coli* as previously described by Truswell [11].

Enterococcus faecium and Enterococcus faecalis isolation

For the isolation of *E. faecium* and *E. faecalis*, serial dilutions of pooled swabs were plated onto Slantez and Bartley agar (ThermoFisher) using the RASP Platform and incubated at 42°C for 48 hours. Four presumptive *Enterococcus* colonies on Slantez and Bartley were picked manually and streaked onto sheep blood agar (SBA, Edwards) and incubated at 37°C overnight. A pure sub-culture was picked manually for MADLI-TOF (Bruker) identification, and if identified as *E. faecalis* or *E. faecium*, re-streaked onto SBA and incubated at 37°C overnight. A pure sub-culture was picked for MADLI-TOF (Bruker) identification, and if identified as *E. faecalis* or *E. faecium*, frozen down in a tube containing 1mL brain heart infusion broth with 20% glycerol and stored at -80 °C. All samples were speciated using MALDI-TOF (Bruker).

Escherichia coli isolation

For the isolation of *E. coli* using the RASP Platform [11], serial dilutions of pooled swabs were plated onto CHROMagar ECC (Edwards) and incubated at 37°C overnight. Using the RASP Platform, 13 - 16 colonies were picked, inoculated into Luria Bertani (LB) broth and incubated at 37°C overnight. Equal volumes of LB broth and 40% glycerol was added to each well and stored at -80 °C. All samples were speciated using MALDI-TOF (Bruker).

Antimicrobial susceptibility testing (Murdoch University AMRID Laboratory)

Recovery of isolates for Antimicrobial Susceptibility Testing

For *E. coli*, frozen-down isolates were picked on the RASP into fresh cation-adjusted Mueller-Hinton broth (CAMHB, BD) and incubated overnight at 37°C. The overnight culture was used for susceptibility testing. For *Salmonella* and *Enterococcus*, from each frozen-down vial, a loop-full of broth was streaked onto SBA for pure colonies and incubated at 37°C for 24 hours. The overnight SBA were examined for purity and one colony was inoculated into CAMHB and incubated overnight at 37°C. The overnight culture was used for susceptibility testing. For *Campylobacter*, from each frozen-down vial, a loop-full of broth was streaked onto SBA and incubated in microaerophilic conditions at 37°C for 48 hours. A single colony was streaked on to another SBA and incubated at 37°C for 48 hours before performing antimicrobial susceptibility testing.

Susceptibility testing

For *E. coli* and *Salmonella* spp., the antimicrobials tested were: amikacin, ampicillin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, meropenem, trimethoprim, tetracycline, sulfamethoxazole (*E. coli* only) and azithromycin (*Salmonella* only). For *Enterococcus*, the antimicrobials tested were: ampicillin, daptomycin, erythromycin, gentamicin, lincomycin, linezolid, quinupristin/dalfopristin, teicoplanin, tetracycline, vancomycin and virginiamycin. For *Campylobacter* spp., the antimicrobials tested were: azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, tetracycline, chloramphenicol and streptomycin.

Antimicrobial susceptibility for the isolates was determined using the RASP platform by the broth microdilution method based on in-house panels prepared according to Clinical and Laboratory Standards Institute (CLSI) standards using custom Tecan robotics [12]. The required antimicrobials were diluted and dispersed onto test plates. The test plates were made and used on the same day. The complete list of antimicrobials along with the concentration ranges that were tested are listed according to their antimicrobial classes in Tables 2, 3 and 4 for *Enterococcus* spp., *E. coli* / *Salmonella* spp. and *Campylobacter* spp., respectively. Briefly, the method for antimicrobial susceptibility testing on the RASP platform started with the dilution of bacteria equivalent to a 0.5 McFarland standard. The suspension was further diluted to achieve a final dilution in the testing plate of ~5 x10^5 CFU/ml. The CFU in the final testing plate was performed on the first and 49th isolate of each run. Quality control was performed on control strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Campylobacter jejuni* ATCC 33560 throughout the study period.

Interpretation

The minimum inhibitory concentration (MIC) for each drug/bug combination was determined according to CLSI guidelines. Resistance breakpoints from the CLSI or M100 document [13] as well as Epidemiological Cut-off Values (ECOFF)[14] were used to interpret the data and classify each isolate as resistant or susceptible to tested antimicrobials. In this study, 'clinical resistance' to an antimicrobial refers to isolates that, in a clinical setting, would not be successfully removed by use of that antimicrobial and is determined using CLSI breakpoints. Whilst clinical breakpoints are useful in a clinical setting to guide therapy, they have

a limited role in surveillance. Microbiological resistance is characterised according to ECOFF breakpoints. 'Microbiologically resistant' refers to isolates that may show signs of emerging resistance and are more useful in surveillance situations. ECOFF breakpoints are used in the interpretation of surveillance data in international systems such as DANMAP.

Multi-class resistant (MCR) isolates were those that were clinically resistant to three or more classes of antimicrobial.

Table 2. Breakpoints used for susceptibility testing of *Enterococcus faecalis* and *E Enterococcus faecium* species

Class	Agent	Species	Range (mg/L)	Microbiological Breakpoint [a]	Clinical breakpoint [b]
Aminoglycosides	Gentamicin	*E. faecium*	4 - 1024	>32	-
		E. faecalis	4 - 1024	>64	-
Glycopeptides	Vancomycin	Both	0.25 - 128	>4	>16
	Teicoplanin	Both	0.25 - 128	>2	>16
Lincosamide	Lincomycin	Both	1 - 8	-	-
Lipopeptides	Daptomycin	*E. faecium*	0.25 - 16	>8	>4
		E. faecalis	0.25 - 16	>4	>4
Macrolides	Erythromycin	Both	0.25 - 16	>4	>4
Oxazolidinones	Linezolid	*E. faecium*	0.5 - 16	>4	>4
		E. faecalis	0.5 - 16	-	>4
Penicillins	Ampicillin	*E. faecium*	0.5 - 32	>8	>8
		E. faecalis	0.5- 32	>4	>8
Streptogramins	Quinupristin-Dalfopristin	*E. faecium*	0.5 - 32	-	>2
		E. faecalis	0.5 - 32	-	-
	Virginiamycin	*E. faecium*	0.25 - 64	>4	-
		E. faecalis	0.25 - 64	>32	-
Tetracyclines	Tetracycline	Both	0.25 - 128	>4	>8

[a] EUCAST epidemiological cut-off values (ECOFFS) (mg/L)

[b] CLSI M100S [15] breakpoints (mg/L)

- Not defined

Table 3. Breakpoints used for susceptibility testing of *Escherichia coli* and *Salmonella* species

Class	Agent	Range (mg/L)	Microbiological Breakpoint [a]		Clinical Breakpoint [b]
			E. coli	***Salmonella spp.***	**Enterobacterales**
Aminoglycosides	Gentamicin	0.25 - 16	>2	>2	>8
	Amikacin	1 - 64	>8	>4	>32
Cephems	Cefotaxime	0.015 - 4	>0.25	>0.5	2
	Ceftazidime	0.0625 - 16	>0.5	>2	>8
Quinolones	Ciprofloxacin	0.008 - 2	>0.06	-	>0.5
Carbapenem	Meropenem	0.008 - 4	>0.06	>0.06	>2
Folate pathway inhibitors	Sulfamethoxazole (*E. coli* only)	8 - 512	-	-	>256
	Trimethoprim	0.25 - 16	>2	>2	>8
Macrolides	Azithromycin (*Salmonella only*)	1 - 64	-	>16	>16
Penicillins	Ampicillin	1 - 32	>8	>4	>16
Phenicols	Chloramphenicol	2 - 32	>16	>16	>16
	Florfenicol	1 - 64	>16	>16	-
Polymyxins	Colistin	0.25 - 8	>2	-	>2
Tetracyclines	Tetracycline	1 - 32	>8	>8	>8

[a] EUCAST epidemiological cut-off values (ECOFFs) (mg/L)

[b] CLSI M100S [15] (CLSI 2021) breakpoints (mg/L)

- Not defined

Table 4. Breakpoints used for susceptibility testing of *Campylobacter* species

Class	Agent	Species	Range (mg/L)	Microbiological Breakpoint [a]	Clinical Breakpoint [b]
Aminoglycosides	Gentamicin	All	0.12 - 16	>2	-
	Streptomycin	All	0.5 - 16	>4	-
Ketolides	Telithromycin	*C. jejuni*	0.5 - 8	>4	>4
Lincosamide	Clindamycin	*C. coli*	0.03125 - 32	>1	-
		C. jejuni	0.03125 - 32	>0.5	-
Macrolides	Azithromycin	*C. coli*	0.03125 - 2	>0.5	>0.5
		C. jejuni	0.03125 - 2	>0.25	>0.25
	Erythromycin	*C. coli*	0.0625 - 128	>8	>16
		C. jejuni	0.0625 - 128	>4	>16
Phenicols	Florfenicol	All	0.03125 - 32	>4	-
	Chloramphenicol	All	2-32	>16	-
Quinolones	Ciprofloxacin	All	0.008 - 16	>0.5	>2
	Nalidixic acid	All	1 - 64	>16	-
Tetracyclines	Tetracycline	*C. coli*	0.125 - 64	>2	>8
		C. jejuni	0.125 - 64	>1	>8

[a] EUCAST epidemiological cut-off values (ECOFFs) (mg/L)
[b] CLSI M100S[15] (CLSI 2021) breakpoints (mg/L)
- Not defined

Genetic analysis (Murdoch University AMRID Laboratory)

All *Salmonella* and *Campylobacter* isolates were sequenced for typing. All *E. coli* isolates clinically resistant to ciprofloxacin were also sequenced. Enterococci were only sequenced if they demonstrated resistance to the highly important antimicrobials linezolid and vancomycin.

DNA extraction and library preparation

DNA extraction was performed on all isolates using the MagMAX Multi-sample extraction kit (Thermofisher Scientific, USA) as per the manufacturer's instructions. DNA library preparation was conducted using an Illumina Nextera XT Library Preparation kit, with variation from the manufacturer's instructions for an increased time for tagmentation to 7 mins. Library preparations were sequenced via Illumina Nextseq platform with a high output 2x150 kit.

DNA sequencing and analysis

The genomic data was de novo assembled using SPAdes. All isolates were analysed using the Centre for Genomic Epidemiology and the nullabor pipeline for the screening of multi-locus sequence type, AMR genes, virulence genes and plasmids. The presence of various known mutations was detected using the Centre for Genomic Epidemiology website (https://www.genomicepidemiology.org/) [16, 17]. Samples were included in analysis if they had a read depth of >40x and the major species identified was *E. coli.*

Statistical analysis

Confidence intervals of proportions were calculated using exact binomial confidence intervals derived by the Clopper-Pearson method in Stata version 14.2 (StataCorp LLC, College Station, Texas USA, www.stata.com).

RESULTS

Reporting of the results is in-line with recommendations in WOAH chapter 6.8 which states that "*For surveillance and monitoring purposes, use of the microbiological breakpoint (also referred to as epidemiological cut-off point), which is based on the distribution of MICs or inhibition zone diameters of the specific bacterial species tested, is preferred*.". The results using clinical resistance breakpoints are reported but the purpose of this project was on AMR surveillance, meaning interrogation of results based on microbiological breakpoints was the priority. Therefore, where possible, genetic analysis was used to better understand the results captured using the microbiological breakpoints.

Sample collection

A total of 202 samples were received at Murdoch University. One sample was removed due to an error in robotic processing and 11 submissions were omitted from the study due to the incorrect number of caeca being collected (less than 4). Efforts were made to recollect these samples; however, the re-collection efforts were further unable to meet study design therefore these submissions were not included in this study. In total, 190 samples were included in the study.

Bacterial isolation

A total of 3308 bacterial isolates were recovered from 190 submissions (Table 5). Using the RASP platform, 13-16 *E. coli* were isolated from each sample which resulted in in a total of 2950 *E. coli* that could be used for susceptibility testing. For isolation of Enterococci, *E. faecium* (86%) was more commonly isolated than *E. faecalis (*14%). Only 9 *Salmonella* were isolated in total. For *Campylobacter*, there was an isolation rate of approximately 94% from the 190 samples collected, with *C. jejuni* (64.6%) more commonly isolated than *C. coli* (35.4%).

Table 5. Isolates recovered

Genus	Species	Number (% of genus)
Escherichia	*coli*	2950
Enterococcus	*E. faecium*	147 (86)
	E. faecalis	24 (14)
Salmonella	various	9
Campylobacter	*C. coli*	63 (35.4)
	C. jejuni	115 (64.6)

Phenotypic analysis

Enterococcus species

All *E. faecalis* and *E. faecium* isolates were clincially susceptible to the antimicrobials vancomycin and linezolid, which are highly important (as rated by ASTAG) [7]. All isolates were microbiologically susceptible to ampicillin. Among the *Enterococcus* spp*.,* microbiological resistance to tetracycline was common (30.61 - 87.5%). The majority of *E. faecium* isolates (64.6%) were susceptible to all antimicrobials tested. A small proportion of *E. faecium* isolates were microbiologically resistant to quinupristin-dalfopristin (6.1%), and 41.67% of *E. faecalis* were microbiologically resistant to erythromycin. No *Enterococcus* isolates demonstrated clinical resistance to the aminoglycosides class. Refer to Figures 1-2 and Tables 6 - 7 for the complete description of results. Note that the small sample size (*E. faecium*, n = 147; *E. faecalis*, n= 24) may confound interpretation of the results. *Enterococcus* spp. are intrinsically resistant to lincosamides and low-level aminoglycosides. In addition, *E. faecalis* is intrinsically resistant to the streptogramin class (virginiamycin and quinupristin-dalfopristin) [16].

A total of 7 unique microbiological resistance profiles were identified among the 171 *Enterococci* isolates of which 0.58% (n=1) was MCR (defined as being clinically resistant to three or more classes of antimicrobial) with a single profile of resistance to macrolides, streptogramins and tetracyclines in *E. faecium*. The AMR profiles for *Enterococcus* spp. are shown in Tables 8 - 9, and any isolates classified as MCR have been highlighted.

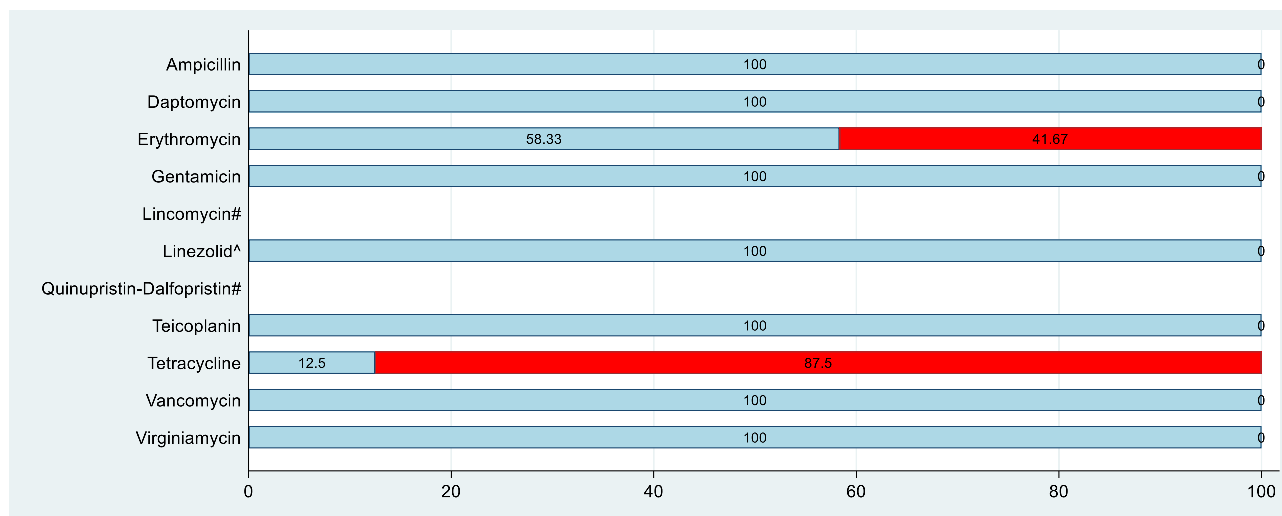

Figure 1. Antimicrobial resistance patterns for *Enterococcus faecalis* (n=24) based on microbiological (ECOFF) break points. The proportion of susceptible is shown in blue and the proportion resistant in red. ^Denotes use of clinical breakpoints where no microbiological breakpoints are available. #No data available due to lack of both ECOFF and clinical breakpoints.

Table 6. Distribution of minimum inhibitory concentrations for *Enterococcus faecalis* (n=24) isolated from Australian meat chickens

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)															Microbiological Resistance		Clinical Resistance	
drug	n	0.06	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	nw	nw_ci	cr	cr_ci
Ampicillin	24			12.5	83.33	4.17											0	0,14.2	0.0	0,14.2
Daptomycin	24		25	37.5	37.5												0	0,14.2	0.0	0,14.2
Erythromycin	24		8.33	20.83	25		4.17	8.33		33.33							41.67	22.1,63.4	41.7	22.1,63.4
Gentamicin	24						91.67	8.33									0	0,14.2	.	.
Lincomycin	24							4.17	95.83								.	.	.	.
Linezolid	24			8.33	62.5	29.17											.	.	0.0	0,14.2
Quinupristin-Dalfopristin	24						83.33	16.67									.	.	.	.
Teicoplanin	24		75	25													0	0,14.2	0.0	0,14.2
Tetracycline	24			12.5					8.33	37.5	4.17	37.5					87.5	67.6,97.3	87.5	67.6,97.3
Vancomycin	24			25	41.67	33.33											0	0,14.2	0.0	0,14.2
Virginiamycin	24					8.33	75	16.67									0	0,14.2	.	.

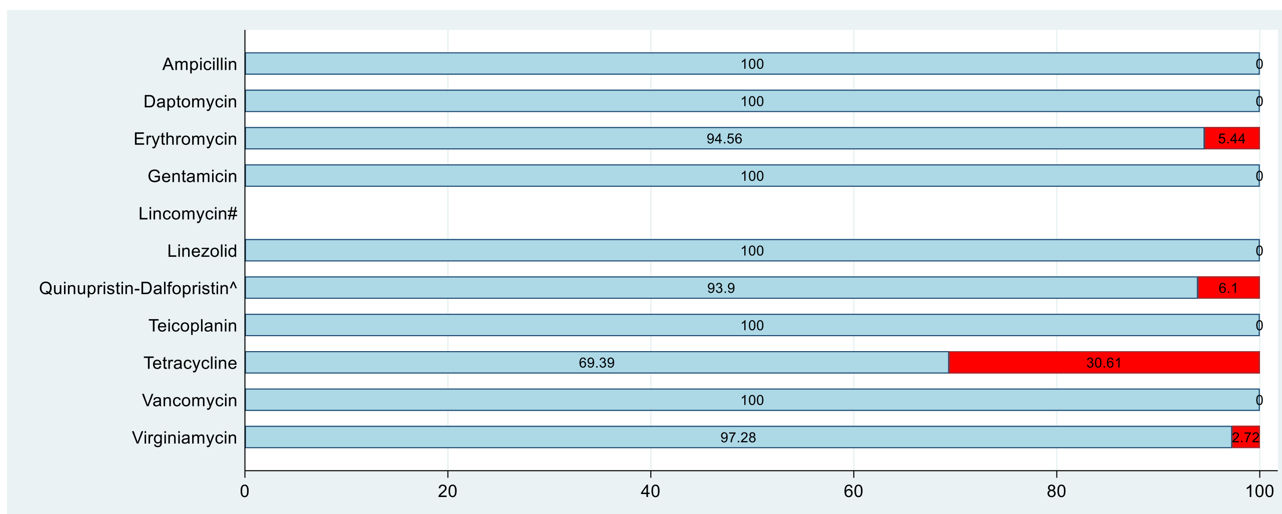

Figure 2. Antimicrobial resistance patterns for *Enterococcus faecium* (n=147) based on microbiological (ECOFF) break points. The proportion of susceptible is shown in blue and the proportion resistant in red. ^Denotes use of clinical breakpoints where no microbiological breakpoints are available. # No data available due to lack of ECOFF and clinical breakpoints.

Table 7. Distribution of minimum inhibitory concentrations for *Enterococcus faecium* (n=147) isolated from Australian meat chickens.

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)															Microbiological Resistance		Clinical Resistance	
drug	n	0.06	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	nw	nw_ci	cr	cr_ci
Ampicillin	147			81.63	16.33	1.36	.68										0	0,2.5	0.0	0,2.5
Daptomycin	147		33.33	38.1	26.53	2.04											0	0,2.5	0.0	0,2.5
Erythromycin	147		82.31	2.04	3.4	4.76	2.04	1.36	2.04	2.04							5.44	2.4,10.4	5.4	2.4,10.4
Gentamicin	147						96.6	3.4									0	0,2.5	.	.
Lincomycin	147				41.5	5.44	8.16	2.72	42.18								.	.	.	.
Linezolid	147			57.82	12.24	29.93											0	0,2.5	0.0	0,2.5
Quinupristin-Dalfopristin	147			65.31	23.81	4.76	4.76	1.36									.	.	6.1	2.8,11.3
Teicoplanin	147		92.52	7.48													0	0,2.5	0.0	0,2.5
Tetracycline	147		61.22	3.4	1.36	2.72	.68		.68	3.4	9.52	15.65	1.36				30.61	23.3,38.7	30.6	23.3,38.7
Vancomycin	147		46.94	46.94	4.08	2.04											0	0,2.5	0.0	0,2.5
Virginiamycin	147		59.18	27.21	5.44	4.08	1.36	2.04	.68								2.72	.7,6.8	.	.

Table 8. Antimicrobial resistance profiles of *Enterococcus faecalis* isolates (n=24)

No. of Resistances	Resistance*	No. of isolates	% of total
0	nil	3	12.5
1	tet	11	45.8
2	mac|tet	10	41.7

* mac= macrolides, tet= tetracyclines

Table 9. Antimicrobial resistance profiles of *Enterococcus faecium* isolates (n=147)

No. of Resistances	Resistance*	No. of isolates	% of total
0	nil	95	64.6
1	mac	2	1.4
1	str	3	2.0
1	tet	38	25.9
2	mac|str	2	1.4
2	mac|tet	3	2.0
2	str|tet	3	2.0
3	mac|str|tet	1	0.7

* mac= macrolides str= streptogramins, tet= tetracyclines

E. coli

Of the 2950 *E. coli* isolates, 56.8% were susceptible to all of the antibiotics tested. All *E. coli* isolates tested were microbiologically susceptibile to amikacin and colistin. Microbiological resistance was observed for ampicillin (11.66%), cefotaxime (0.24%), ceftazidime (0.20%), chloramphenicol (0.14%), ciprofloxacin (3.25%), florfenicol (0.24%), gentamicin (1.05%), tetracycline (23.25%), meropenem (0.20%) and trimethoprim (5.39%). Clinical resistance was detected to sulphamethoxazole (13.9%). Ninety six isolates (3.25%) demonstrated microbiological resistance to the fluroquinolone class (ciprofloxacin MICs from 0.13 to 4 mg/L). However, only 32 of these isolates (1.08% of the total ciprofloxacin microbiologically resistant) were also classified as resistant based on the clinical breakpoint. The AMR prevalence for *E. coli* based on microbiological break points is shown in Figure 3. Comprehensive distribution of MIC concentrations for *E. coli* including frequency of clinical resistance is shown in Table 10.

A total of 33 microbiological resistance profiles were identified among the 2950 isolates. Only 2.92% of isolates were classified as MCR. The most common multi-class resistance profile (1.40%) was beta-lactams, folate pathway inhibitors and tetracyclines (bla/fpi/tet). Three isolates (0.10%) were resistant to aminoglycosides, beta-lactams, folate pathway inhibitors, quinolones and tetracyclines (ami/bla/fpi/qui/tet). A single isolate (0.03%) was resistant to third generation cephalosporins, carbapenems, folate pathway inhibitors, quinolones and tetracyclines (c3g/car/fpi/qui/tet). The MCR profiles for *E. coli* are shown in Table 11, and isolates classified as MCR have been highlighted.

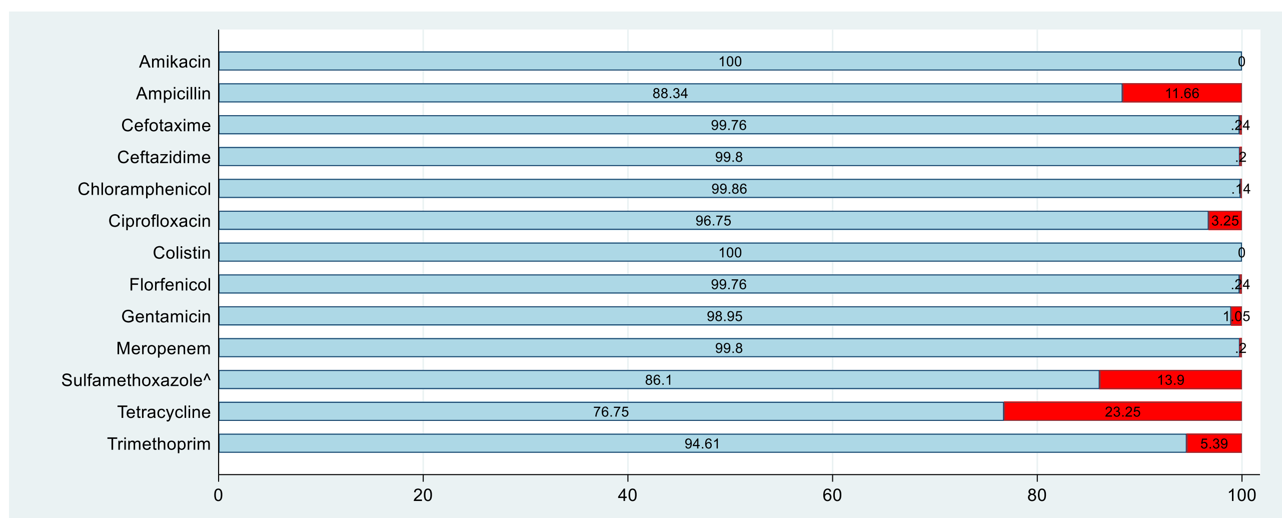

Figure 3. Antimicrobial resistance patterns for *Escherichia coli* (n=2950) based on microbiological (ECOFF) break points. Clinical break points are used when microbiological break point is unavailable. The proportion of susceptible is shown in blue and the proportion resistant in red. ^ Denotes no microbiological breakpoints available, therefore clinical breakpoints were used. # No data available due to lack of ECOFF and clinical breakpoints.

Table 10. Distribution of minimum inhibitory concentrations for *Escherichia coli* (n=2950) isolated from Australian meat chickens.

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)																		Microbiological Resistance		Clinical Resistance	
drug	n	0.008	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	nw	nw_ci	cr	cr_ci
Amikacin	2950								69.63	24.88	5.15	.34								0	0,.1	0.0	0,.1
Ampicillin	2950								3.39	38.17	42.34	4.44	.14	.07	11.46					11.66	10.5,12.9	11.5	10.4,12.7
Cefotaxime	2950		1.69	14.2	61.39	20.88	1.59	.07		.17										.24	.1,.5	0.0	0,.1
Ceftazidime	2950				10.58	45.39	41.49	2.34		.07	.07	.07								.2	.1,.4	0.0	0,.1
Chloramphenicol	2950									2.54	46.37	44.58	6.37	.1	.03					.14	0,.3	0.1	0,.3
Ciprofloxacin	2950		6.17	.03	1.02	1.97	.07		.03	.2	.98									3.25	2.6,4	1.2	.9,1.7
Colistin	2950						98.75	.88	.31	.07										0	0,.1	0.0	0,.1
Florfenicol	2950								.07	.78	36.31	58.68	3.93	.24						.24	.1,.5	.	.
Gentamicin	2950						53.02	38.31	7.25	.37	.03	.2	.41	.41						1.05	.7,1.5	0.8	.5,1.2
Meropenem	2950		47.15	49.93	.24	.2														.2	.1,.4	0.0	0,.1
Sulfamethoxazole	2950											15.32	5.63	50.24	14.41	.47	.03	.1	13.8	.	.	13.9	12.7,15.2
Tetracycline	2950								40.2	35.12	1.15	.27	.47	1.08	21.69					23.25	21.7,24.8	23.3	21.7,24.8
Trimethoprim	2950						75.39	18.44	.68	.1	.03	.14	.17	5.05						5.39	4.6,6.3	5.2	4.4,6.1

Table 11. Antimicrobial resistance profiles of *Escherichia coli* isolates (n=2950)

No. of Resistances	Resistance*	No. of isolates	% of total
0	nil	1675	56.8
1	bla	165	5.6
1	car	1	<0.1
1	fpi	288	9.8
1	phe	5	0.2
1	qui	44	1.5
1	tet	468	15.9
2	ami_fpi	5	0.2
2	bla_c3g	2	0.1
2	bla_fpi	30	1.0
2	bla_phe	2	0.1
2	bla_qui	18	0.6
2	bla_tet	73	2.5
2	fpi_qui	2	0.1
2	fpi_tet	60	2.0
2	phe_tet	1	<0.1
2	qui_tet	8	0.3
3	ami_fpi_qui	6	0.2
3	ami_fpi_tet	11	0.4
3	bla_fpi_phe	1	<0.1
3	bla_fpi_tet	41	1.4
3	bla_qui_tet	2	0.1
3	c3g_car_fpi	1	<0.1
3	c3g_car_qui	1	<0.1
3	car_fpi_tet	1	<0.1
3	fpi_qui_tet	4	0.1

4	ami_bla_fpi_qui	1	<0.1
4	ami_bla_fpi_tet	2	0.1
4	ami_fpi_qui_tet	3	0.1
4	bla_fpi_phe_tet	1	<0.1
4	bla_fpi_qui_tet	3	0.1
4	c3g_car_fpi_tet	1	<0.1
5	ami_bla_fpi_qui_tet	3	0.1
5	c3g_car_fpi_qui_tet	1	<0.1

* ami= aminoglycosides, bla= beta lactams, phe= phenicols, fpi= folate pathway inhibitors, tet=tetracycline, c3g = cephems – third generation, qui = quinolones. ^ numbers are rounded to 1 decimal place, <0.1 indicates the percentage would round to zero (0.04 or less).

Salmonella species

All *Salmonella* isolates tested were microbiologically and clinically susceptible to all the antimicrobials tested in this study. Note that the small sample size may confound interpretation of the results (Figure 4, Table 12).

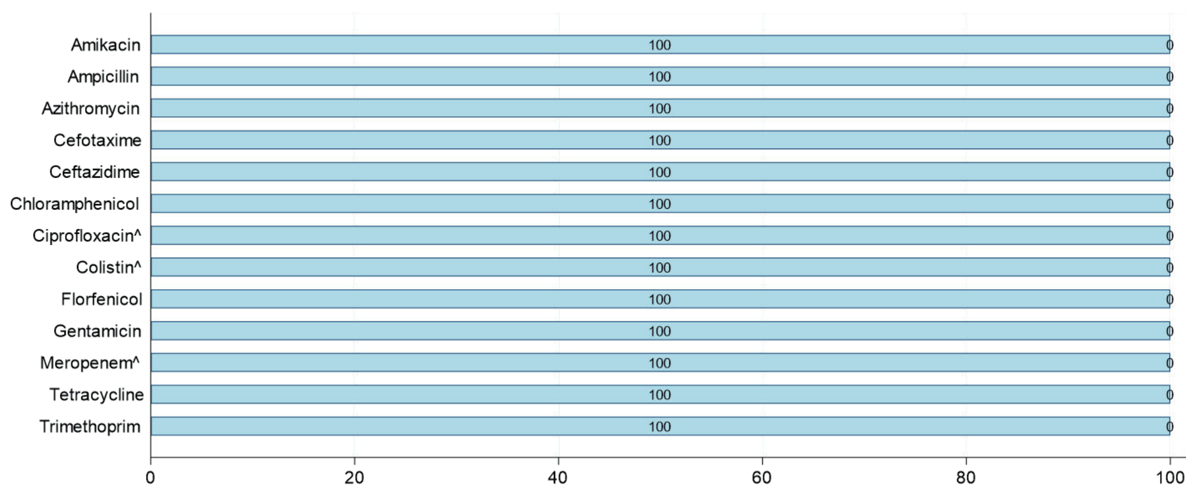

Figure 4. Antimicrobial resistance patterns for *Salmonella* spp. (n=9) based on microbiological (ECOFF) break points. The proportion of susceptible is shown in blue and the proportion resistant in red. ^ Denotes use of clinical breakpoints where no microbiological breakpoints are available.

Table 12. Distribution of minimum inhibitory concentrations for *Salmonella* spp. (n=9) isolated from Australian meat chickens.

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)															Microbiological Resistance		Clinical Resistance	
drug	n	0.008	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	nw	nw_ci	cr	cr_ci
Amikacin	9								100								0	0,33.6	0.0	0,33.6
Ampicillin	9								88.9	11.1							0	0,33.6	0.0	0,33.6
Azithromycin	9								11.1	66.7	22.2						0	0,33.6	0.0	0,33.6
Cefotaxime	9				66.7	33.3											0	0,33.6	0.0	0,33.6
Ceftazidime	9					11.1	88.9										0	0,33.6	0.0	0,33.6
Chloramphenicol	9										22.2	77.8					0	0,33.6	0.0	0,33.6
Ciprofloxacin	9		88.9														.	.	0.0	0,33.6
Colistin	9						55.6	33.3		11.1							.	.	0.0	0,33.6
Florfenicol	9										55.6	44.4					0	0,33.6	.	.
Gentamicin	9						66.7	33.3									0	0,33.6	0.0	0,33.6
Meropenem	9			11.1	88.9												0	0,33.6	0.0	0,33.6
Tetracycline	9								33.3	66.7							0	0,33.6	0.0	0,33.6
Trimethoprim	9						44.4	55.6									0	0,33.6	0.0	0,33.6

Campylobacter species

All *Campylobacter* isolates tested were microbiologically susceptibile to azithromycin, erythromycin, chloramphenicol, clindamycin, gentamicin, telithromycin, florfenicol and gentamicin. No resistance was detected in 68.7% of *C. jejuni* and 88.9% of *C. coli* isolates. Microbiological and clincial resistance to ciprofloxacin was detected in 24.35% and 22.6% of *C. jejuni* isolates respectively. Microbiological resistance to ciprofloxacin was detected in 3.17% of *C. coli* isolates. Microbiological resistance to tetracycline in *C. jejuni* and *C. coli* isolates was 18.26 % and 1.59% respectively. The AMR prevalence for *Campylobacter* spp. based on microbiological break points is shown in Figures 5 and 6. Comprehensive distribution of MIC concentrations for *Campylobacter* spp. including frequency of clinical resistance is shown in Tables 13 and 14. Eight isolates were unable to grow under the MIC test conditions and no resistance profiles were identified for these.

A total of four unique microbiological resistance profiles were identified among the 178 *Campylobacter* isolates for which an MIC could be determined, with no MCR profiles identified. The AMR profiles for *Campylobacter* spp. is shown in Tables 15 and 16.

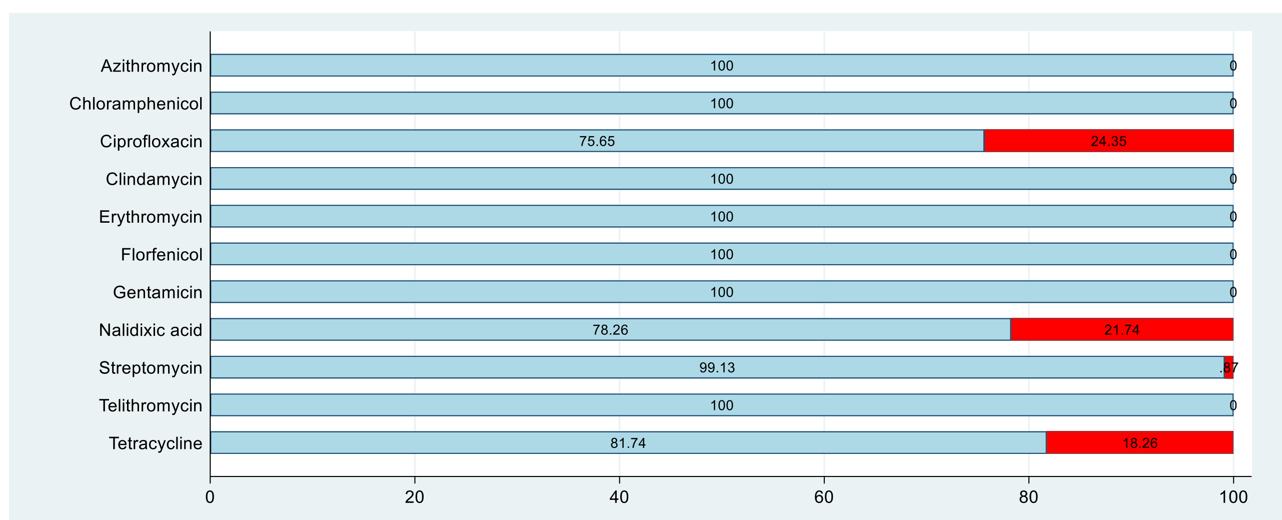

Figure 5. Microbiological resistance patterns for *Campylobacter jejuni* (n=115) based on microbiological (ECOFF) break points. The proportion of susceptible is shown in blue and the proportion resistant in red.

Table 13. Distribution of minimum inhibitory concentrations for *Campylobacter jejuni* (n=115) isolated from Australian meat chickens.

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)																Microbiological Resistance		Clinical Resistance	
drug	n	0.008	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	nw	nw_ci	cr	cr_ci
Azithromycin	115			93.04	6.09	.87												0	0,3.2	.	.
Chloramphenicol	115									96.52	2.61		.87					0	0,3.2	.	.
Ciprofloxacin	115			6.09	35.65	17.39	9.57	.87	.87	.87	9.57	5.22	7.83					24.35	16.8,33.2	22.6	15.3,31.3
Clindamycin	115			53.91	33.91	9.57	2.61											0	0,3.2	.	.
Erythromycin	115				19.13	14.78	40	24.35	.87	.87								0	0,3.2	0.0	0,3.2
Florfenicol	115			6.96		3.48	8.7	39.13	38.26	3.48								0	0,3.2	.	.
Gentamicin	115					55.65	33.04	11.3										0	0,3.2	.	.
Nalidixic acid	115								6.96	3.48	33.91	20.87	13.04	9.57	7.83	4.35		21.74	14.6,30.4	.	.
Streptomycin	115							66.96	28.7	3.48		.87						.87	0,4.7	.	.
Telithromycin	115							80	11.3	7.83	.87							0	0,3.2	.	.
Tetracycline	115					78.26	3.48						4.35	1.74	9.57	2.61		18.26	11.7,26.5	18.3	11.7,26.5

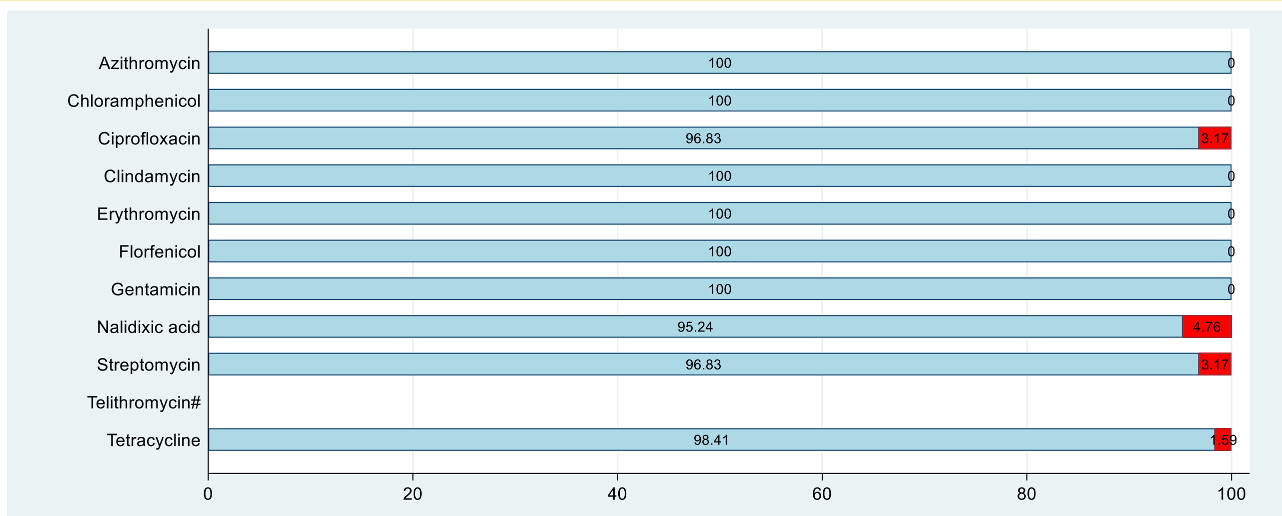

Figure 6. Microbiological resistance patterns for *Campylobacter coli* (n=63) based on microbiological (ECOFF) break points. The proportion of susceptible is shown in blue and the proportion resistant in red. # No data available due to lack of ECOFF and clinical breakpoints.

Table 14. Distribution of minimum inhibitory concentrations for *Campylobacter coli* (n=63) isolated from Australian meat chickens.

Percentage of isolates classified as microbiologically resistant (nw) and clinically resistant (cr) with corresponding 95% confidence intervals (ci). For each drug, vertical bars show position of the microbiological breakpoint and 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.

		Minimum Inhibitory Concentration (mg/L)																Microbiological Resistance		Clinical Resistance	
drug	n	0.008	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	nw	nw_ci	cr	cr_ci
Azithromycin	63			41.27	20.63	23.81	14.29											0	0,5.7	.	.
Chloramphenicol	63									74.6	11.11	14.29						0	0,5.7	.	.
Ciprofloxacin	63		4.76	14.29	36.51	11.11	23.81	4.76	1.59	1.59								3.17	.4,11	0.0	0,5.7
Clindamycin	63			7.94	23.81	26.98	22.22	12.7	6.35									0	0,5.7	.	.
Erythromycin	63				7.94	6.35	14.29	17.46	19.05	19.05	14.29	1.59						0	0,5.7	0.0	0,5.7
Florfenicol	63			1.59		1.59	3.17	22.22	36.51	17.46	17.46							0	0,5.7	.	.
Gentamicin	63					15.87	42.86	38.1	3.17									0	0,5.7	.	.
Nalidixic acid	63								3.17	4.76	34.92	31.75	20.63	3.17	1.59			4.76	1,13.3	.	.
Streptomycin	63							12.7	46.03	30.16	7.94			3.17				3.17	.4,11	.	.
Telithromycin	63							42.86	19.05	3.17	12.7	7.94	14.29					.	.	.	.
Tetracycline	63					76.19	20.63	1.59								1.59		1.59	0,8.5	1.6	0,8.5

Table 15. Antimicrobial resistance profiles of *Campylobacter jejuni* isolates (n=115)

No. of Resistances	Resistance*	No. of isolates	% of total
0	nil	79	68.7
1	ami	1	0.9
1	qui	14	12.2
1	tet	7	6.1
2	qui_tet	14	12.2

* ami= aminoglycosides, qui= quinolones, tet= Tetracycline

Table 16. Antimicrobial resistance profiles of *Campylobacter coli* isolates (n=63)

No. of Resistances	Resistance*	No. of isolates	% of total
0	nil	56	88.9
1	ami	2	3.2
1	qui	4	6.3
1	tet	1	1.6

* ami= aminoglycosides, qui= quinolones, tet= tetracycline

GENETIC ANALYSIS

All *Campylobacter* and *Salmonella* were subjected to whole genome sequencing due to their importance as zoonotic bacterial pathogens. All *E. coli* isolates demonstrating clinical resistance to fluroquinolones (ciprofloxacin MIC>0.5) were also subjected to whole genome sequencing (Table 17). No *Enterococci* isolates were sequenced as none were resistant to the critically important antimicrobials linezolid and vancomycin.

Table 17. Isolates selected for genetic analysis

Species	Isolates (n)
E. coli	32
Enterococcus	0
Salmonella	9
Campylobacter	186
Total	227

Escherichia coli

All *E. coli* isolates (n=32) that displayed phenotypic clinical resistance to ciprofloxacin (MIC >0.5 mg/L) were selected for whole genome sequencing. Of the isolates sequenced, only 25 were identified as having mutations known to confer fluroquinolone resistance in the quinolone resistance-determining region (QRDR) region (Table 18). The remaining seven isolates had no known quinolone resistance mutations and did not carry the plasmid mediated quinolone resistance gene and require further investigation.

Table 18. MLST and profile of resistance genes in *E. coli* isolates (n=2950)

MLST	number of isolates	Resistance profile*	QRDR mutation^
-	1	*mac_tet*	yes
-	1	*ami_tet*	
10	1	*bla_mac*	
354	1	*bla_mac*	yes
354	7	*bla_mac_tet*	yes
354	4	*mac*	yes
354	2	*ami_bla_fpiT_fpiS_mac_tet*	yes
354	2	*ami_fpiT_mac_tet*	yes
665	1	*mac_tet*	
752	7	*ami_fpiS_mac*	yes
752	1	*ami_mac*	
752	1	*ami_mac_tet*	
773	1	*mac_tet*	yes
949	1	*mac*	
4980	1	*mac*	

*mac=macrolide, tet=tetracyclines, bla = beta-lactamases, fpiT=folate pathway inhibitor trimethoprim, fpiS = folate pathway inhibitor sulfamethoxazole, ami=aminoglycosides. ^ QRDR = known fluroquinolone mutation occurs in this genetic region

Antimicrobial resistance genes for aminoglycosides, beta-lactams, folate pathway inhibitors and tetracycline were identifed. Of the 32 isolates sequenced, 16 were phenotypically resistant to beta-lactams with 11 having a resistant genotype (*blaTEM*-1B). Eleven of the sequenced isolates had phenotypic tetracycline resistance with 9 having a resistant genotype also (*tet(A)* n=4, *tet(B)* n=5). Another 7 isolates had a variant *tet(A)* gene, however, none of these were phenotypically resistant to tetracycline. All isolates with phenotypic resistance to trimethoprim (n=4) had an associated known resistance gene (*dfrA17*) present.

There were 7 known sequence types identified amongst the isolates sequenced. The most prevalent ST was ST354 (n=16) and ST752 (n=9).

Of the ST354 isolates, there were 5 unique genotypes and 4 unique phenotypes (Table 19). Of the ST752 isolates there were 5 unique phenotypes and 3 unique genotypes (Table 20).

Table 19: Phenotypic and genotypic profiles of *E. coli* ST354 isolates

Phenotype	Genotype	Number of isolates
qui	mac_qui	4
bla_qui	bla_mac_qui	1
bla_qui	bla_mac_tet_qui	7
ami_fpi_qui_tet	ami_fpiT_mac_tet_qui	2
ami_bla_fpi_qui_tet	ami_bla_fpiT_fpiS_mac_tet_qui	2

Table 20: Phenotypic and genotypic profiles of *E. coli* ST752 isolates

Phenotype	Genotype	Number of isolates
bla_qui	ami_mac	1
qui_tet	ami_mac_tet	1
ami_fpi_qui	ami_fpiS_mac_qui	5
ami_fpi_qui_tet	ami_fpiS_mac_qui	1
ami_bla_fpi_qui_tet	ami_fpiS_mac_qui	1

Salmonella species

All nine *Salmonella enterica* isolates were subjected to whole genome sequencing to determine sequence type and serovar. Five of the isolates were ST19, serovar Typhimurium, two were ST32 serovar Infantis, one ST768 serovar Abortusovis and one ST64 serovar Anatum. There were no known AMR genes detected among these isolates. Although Abortusovis is considered species-specific to ovine hosts, it has previously been detected in chicken from Australia [18].

Campylobacter species

Successful sequencing was achieved for 176 of the 186 Campylobacter isolates (120 *C. jejuni*; 56 *C. coli*).

Campylobacter jejuni

The 120 *C. jejuni* isolates sequenced belonged to 29 known sequence types, with the most prominent being ST10143 (n=16), 2083 (n=11), 48 (n=9), 46 (n=6) and 583 (n=6) (Table 21).

24.4% of *C. jejuni* isolates demonstrated microbiological resistance to fluoroquinolones. The fluoroquinolone-resistant *C. jejuni* belonged to sequence types ST10130 (n=4), ST2083 (n=11) ST2398 (n=3), ST2895 (n=4), ST7323 (n=4) and ST1078 (n=2). All the fluoroquinolone-resistant isolates had a single mutation, T-86-I, in the QRDR of DNA gyrase A subunit (*GyrA*), known to decrease the sensitivity of *Campylobacter* spp. to quinolones. Tetracycline resistant isolates had an associated resistant gene, *tet(O*).

Table 21. MLST and resistance profile of *Campylobacter jejuni* isolates (n=120)

MLST*	Number of Isolates	Resistance genotype	QRDR#	Ciprofloxacin (S/R)
-	4	^		S
-	1	tet(O)		S
21	2			S
42	1			S
45	1			S
46	5			S
46	1	tet(O)		S
48	11			S
48	1	tet(O)		S
50	8			S
51	3			S
52	2			S
128	1			S
137	4			S
161	1			S
190	2			S
520	2			S
525	1	tet(O)		S
528	2			S
538	2			S
538	1	tet(O)		S
567	1			S
583	4			S
583	1	tet(O)		S
699	1			S
825	2			S
1078	2		yes	R
1301	1	tet(O)		S
1911	1			S
1911	1	tet(O)		S
2083	10		yes	R
2083	1	tet(O)	yes	R
2347	1			S
2349	2			S
2398	1	tet(O)		S
2398	3	tet(O)	yes	R
2895	4	tet(O)	yes	R
4187	1			S
4896	2			S
6775	1			S
7323	4		yes	R
10130	4		yes	R
10143	16			S

^Gaps represent no presence of known resistance genes. #QRDR; quinolone resistance-determining region. All isolates with QRDR mutations were also microbiologically resistant to ciprofloxacin. S = sensitive, R = resistant. - Sequence type not found

Campylobacter coli

From all 63 *C. coli* isolates, 56 passed quality control for sequencing. The predominant sequence types present were ST827 (n=14) and ST825 (n=14) with a further 10 known sequence types (Table 22). ST825 has previously been isolated from Australian livestock. ST825 has been reported to cause gastroenteritis in humans [19].

Mechanisms for quinolone resistance were detected in four *C. coli* isolates. Two *C. coli* isolates were resistant to ciprofloxacin, and had a known mutation in the *gyrA_2* gene known to confer quinolone resistance (T-86-I). ST860 and 894 were the sequence type identified among the fluoroquinolone-resistant *C. coli,* with ST860 sequence type previously reported in chickens and humans from the United Kingdom and Germany [19].

Table 22. MLST and resistance profile of *Campylobacter coli* (n=56)

MLST*	Number of Isolates	Resistance genotype	QRDR mutation#	Ciprofloxacin (S/R)
-	7	^		S
-	1	lnu(C)		S
583	1			S
825	12			S
827	14			S
828	1			S
829	1			S
860	1			S
860	1		Yes	S
860	1	lnu(C)		R
894	1		Yes	R
1017	3			S
1181	1			S
1243	1			S
4175	1			S
6775	4			S
9419	5			S

^Gaps represent no presence of known resistance genes. #QRDR; quinolone resistance-determining region. All isolates with QRDR mutations were also microbiologically resistant to ciprofloxacin. S = sensitive, R = resistant. - Sequence type not found

DISCUSSION

This study collected caeca samples from meat chickens at slaughter from processing plants that produce >90% of Australian chicken meat to estimate the current prevalence of antimicrobial resistance and to compare to previous studies. The key indicator organisms and potential zoonotic pathogens, *Enterococcus* spp. (171 isolates), *E. coli* (2950 isolates), *Salmonella* spp. (9 isolates), and *Campylobacter* spp. (186 isolates; with 178 able to be used for analysis) were isolated from 190 samples collected from the caeca of meat chickens at slaughter for AMR profiling.

The antimicrobial panel used for the susceptibility testing was modified slightly from the 2016 survey. For *Enterococci* the aminoglycosides kanamycin and streptomycin were removed as neither antimicrobial is routinely used in human or animal medicine and resistance to aminoglycosides can be captured with the inclusion of gentamicin in the panel. Benzylpenicillin was also removed as ampicillin is able to adequately capture resistance to penicillins. The removal of these antimicrobials was important to increase the concentration range of the remaining antimicrobials to cover the microbiological and clinical breakpoints.

For the gram-negative panels (*E. coli* and *Salmonella*) the aminoglycoside streptomycin was replaced with amikacin and the cephems (cefoxitin, ceftiofur and ceftriaxone) were replaced with cefotaxime and ceftazidime. Trimethoprim and sulfamethoxazole were tested individually rather than in combination, and meropenem, a carbapenem, was added to the panel. All of these changes were made to align with the European Union guidelines for antimicrobial testing in surveillance [20]. Amoxicillin-clavulanate was removed due to instability of the compound for testing. Chloramphenicol and Streptomycin were added to the *Campylobacter* panel in this study to reflect EU *Campylobacter* panels [20].

The project had a strict methodological design to minimise the variability in sample collection and shipping, while also ensuring minimal impacting on the practicality of the study. This enables refinements of future studies to be made that allow ongoing monitoring of how AMR profiles of various microbial isolates from meat chickens change over time.

There were several challenges experienced conducting this survey during the COVID-19 crisis, which particularly impacted the Australian chicken meat sector over the 2021/22 summer (the time of sample collection). This included severe staff shortages in processing plants and laboratories due to illness, remaining staff either not trained in required processes for the survey or with no capacity to prioritise the survey activities over daily burden of tasks when covering for other staff on leave. Further, these issues were compounded by transport and logistics issues due to shipping delays which meant a substantial number of samples had to be recollected, which placed further strain on those contributing their time and significantly increased the cost of undertaking the AMR survey. Future surveys will need to take into consideration ways to reduce the burden and cost of collection and shipping as the current approach is likely unsustainable (particularly if progressed solely with industry funding). Despite these challenges, however, the survey from start of collection to finalisation of results was substantially shorter than for the 2016 survey, due in large part to the adoption of the use of the RASP system.

Enterococcus spp.

None of the 171 *Enterococcus* isolates were resistant to vancomycin or linezolid. Resistance to tetracycline was common among *Enterococcus spp.*, likely reflecting past historical use in the industry. Although there appears to be an increase in the prevalence of tetracycline resistance for *E. faecalis* since the 2016 survey, no conclusions can be drawn due to the low number of isolates (Figure 7). The frequency of resistance to quinupristin-dalfopristin in *E. faecium* decreased to 6.1% (from 54.5%) in this study compared to the previous study (Table 7; [16]). This might be reflective of the removal of virginiamycin use in chickens between the 2016 and 2022 surveys [1], as resistance to virginiamycin also causes resistance to quinopristin-dalfopristin.

The prevalence of erythromycin resistance continued to decrease, with only 5.44% resistant *E. faecium* isolated in this study (Figure 8). While there appeared to be an increase in erythromycin resistance in *E. faecalis* since the 2016 survey (from 26.8% to 41.67%)[1], it is difficult to draw conclusions as there were almost half the number of *E. faecalis* isolated in this current survey (n=24). Among the *Enterococci* isolates, 0.7% of isolates were classified as MCR, much lower than the previous 2016 study which found 17.5% of isolates to be MCR (Figure 7). However, this could potentially be due to a difference in the number of isolates and the antimicrobials tested between the two studies.

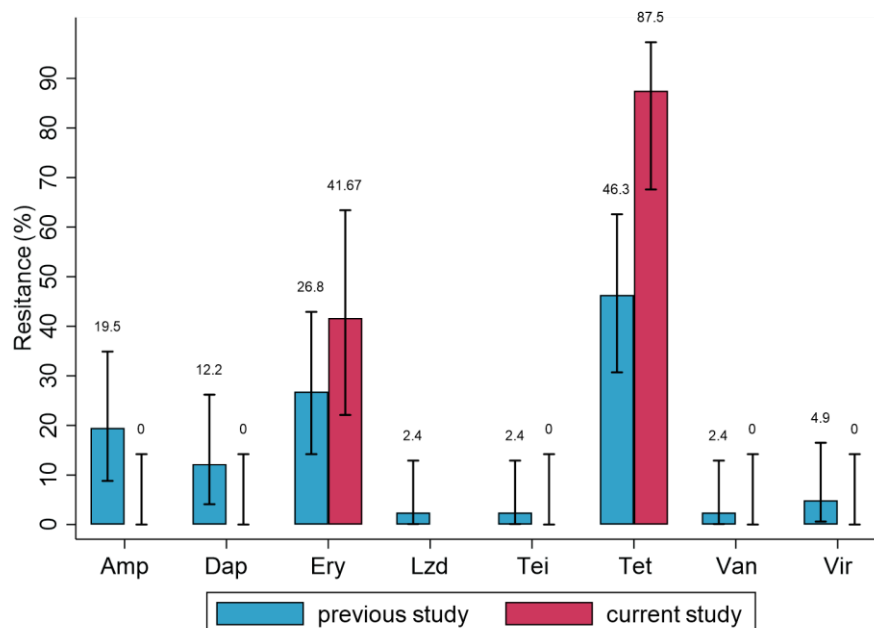

Figure 7: Comparison of resistance to select antimicrobials in *E. faecalis* isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 41, current study n = 24. Amp = ampicillin; Dap = daptomycin; Ery = erythromycin; Lzd = linezolid; Tei = teicoplanin; Tet = tetracycline; Van = vancomycin; Vir = virginiamycin.

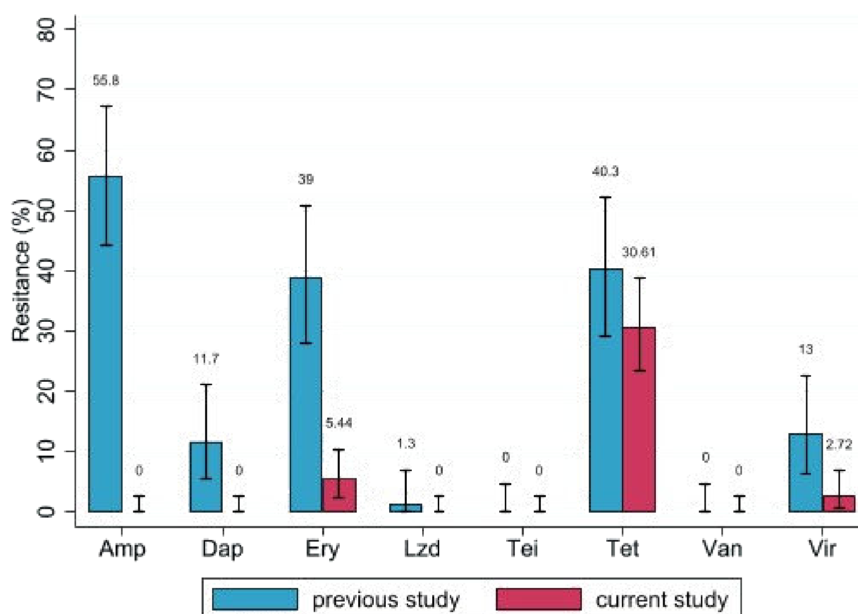

Figure 8: Comparison of resistance to select antimicrobials in *E. faecium* isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 77, current study n = 147. Amp = ampicillin; Dap = daptomycin; Ery = erythromycin; Lzd = linezolid; Tei = teicoplanin; Tet = tetracycline; Van = vancomycin; Vir = virginiamycin.

E. coli

One of the major advancements in this study, compared to the previous 2016 study [1], is the significant increase in the number of *E. coli* isolated; 209 in 2016 compared to 2950 in this study. This was achievable due to the use of advanced robotics to select isolates and perform susceptibility testing. Despite the 14-fold increase in the number of isolates collected and tested, the overall resistance profile remained consistent and within the confidence interval range for the 2016 study (Figure 9). Resistance to the fluoroquinolone antimicrobial, ciprofloxacin, appeared to increase slightly from 1% to 3.25% from 2016. Considering quinolones (including ciprofloxacin) have never been approved for use in chickens in Australia, it is highly likely these clones have been introduced into the industry and not arisen due to selection through use in the industry. The diversity in the genotype and phenotype of isolates from the same ST (e.g. ST354) and the fact that they are globally disseminated multi-host strains also indicates that the clones were likely introduced rather than selected for within the host [21]. It is also possible that these clones have been present in the population for an unknown period of time but have gone largely unnoticed due to the constraints in the number of isolates that can be collected using manual isolation methods. A small number of isolates were microbiologically resistant to the carbapenem, meropenem. This is likely due to the phenomenon known as MIC drift, as the MIC is just above the ECOFF breakpoint for meropenem. Sequencing of these isolates would be required to confirm the presence or absence of genes associated with carbapenem resistance.

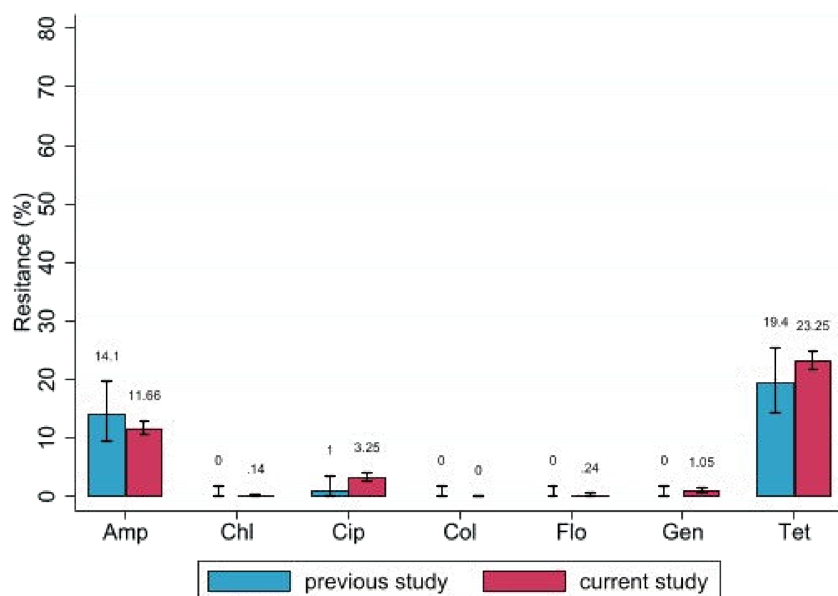

Figure 9: Comparison of resistance to select antimicrobials in *E. coli* isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 206, current study n = 2950. Amp = ampicillin; Chl = chloramphenicol; Cip = ciprofloxacin; Col = colistin; Flo = florfenicol; Gen = gentamycin; Tet = tetracycline.

Salmonella sp.

The recovery of *Salmonella* spp. was very low compared to 2016, and there were considered to be no issues with isolation techniques or processing followed (all low-dose positive controls were reisolated and the operators and procedure were the same as for the 2016 survey). All isolates demonstrated susceptibility to all antimicrobials tested (Figure 10), which is unsurprising given the low resistance levels detected in other surveys [1, 22].

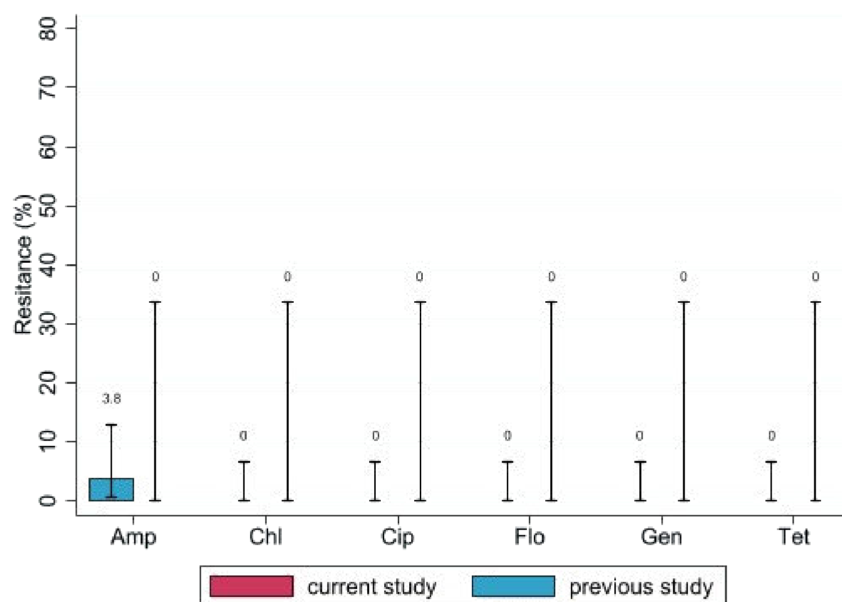

Figure 10: Comparison of resistance to select antimicrobials in *Salmonella* spp. isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 53, current study n = 9. Amp = ampicillin; Chl = chloramphenicol; Cip = ciprofloxacin; Flo = florfenicol; Gen = gentamycin; Tet = tetracycline.

Campylobacter spp.

No antimicrobial resistance was detected to any of the antibiotics tested in 68.7% of *C. jejuni* isolates and 88.9% *C. coli* isolates. All *Campylobacter* isolates tested were microbiologically susceptibile to florfenicol, erythromycin, azithromycin, clindamycin and chloramphenicol and gentamicin. A reduction in the prevalence of erythromycin resistance has been observed since 2004 and this reduction has continued to a point where erythromycin resistance was not detected in this study [1, 5].

Resistance to tetracycline (18.26% *C. jejuni*; 1.59% *C. coli*), nalidixic acid (21.74% *C. jejuni*; 4.76% *C. coli*) or ciprofloxacin (24.35% *C. jejuni*; 3.17% *C. coli*) were the most commonly detected forms of resistance. The observed resistance to ciprofloxacin in *C. jejuni* is higher than in the 2016 study, however it is still within range of the confidence intervals for the results from the 2016 survey so may not be significant (Figure 11, 12). The prevalence of ciprofloxacin resistance continues to be unexpected since fluoroquinolones have not ever been approved for use in the Australian chicken meat industry, and have been confirmed as not being used in commercial chicken meat production in Australia [23]. Half of the *C. jejuni* ciprofloxacin resistant isolates (n=14) were only resistant to quinolones, suggesting they are likely to be evolved from a situation where fluoroquinolone were used as a first-line therapy. The remaining ciprofloxacin resistant *C. jejuni* were also resistant to tetracycline. Recent reports from New Zealand (which also do not approve use of fluoroquinolones in livestock) demonstrated that fluoroquinolone resistance in poultry was attributed to the emergence of a new clone of *C. jejuni* (ST6964) that was resistant to both ciprofloxacin and tetracycline [24, 25]. The dual resistant *C. jejuni* isolates in this study were not ST6964 (detected in New Zealand). Theses isolates belonged to a range of STs; 10130, 1078, 2083, 2398, 2895. Some of these STs were

detected in the previous study, while some were only detected in this study [16]. The levels of resistance to fluoroquinolones is similar to that detected in meat chickens in other countries that also do not approve the use fluoroquinolones in chickens [26].

In the absence of fluoroquinolone use in the Australian chicken industry, the fluoroquinolone resistant isolates are unlikely to have evolved as a result of local selection pressure. It is likely that these isolates may have been introduced by anthropozoonosis i.e. human-chicken transmission. However, further longitudinal and genomic studies are required to fully validate this hypothesis as there may be 'bridge' species that transfer resistant bacteria directly to the chickens or to chickens via humans [27].

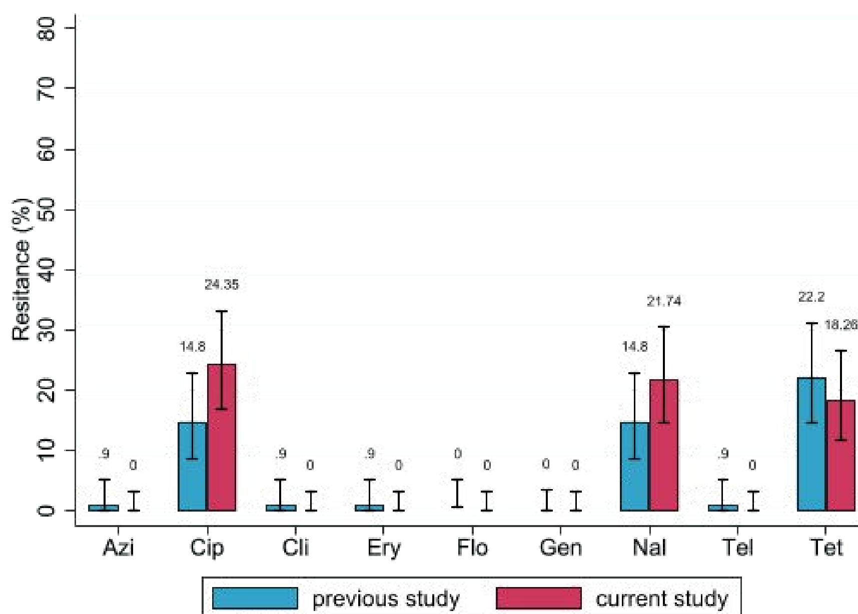

Figure 11: Comparison of resistance to select antimicrobials in *C. jejuni* isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 108, current study n = 115.

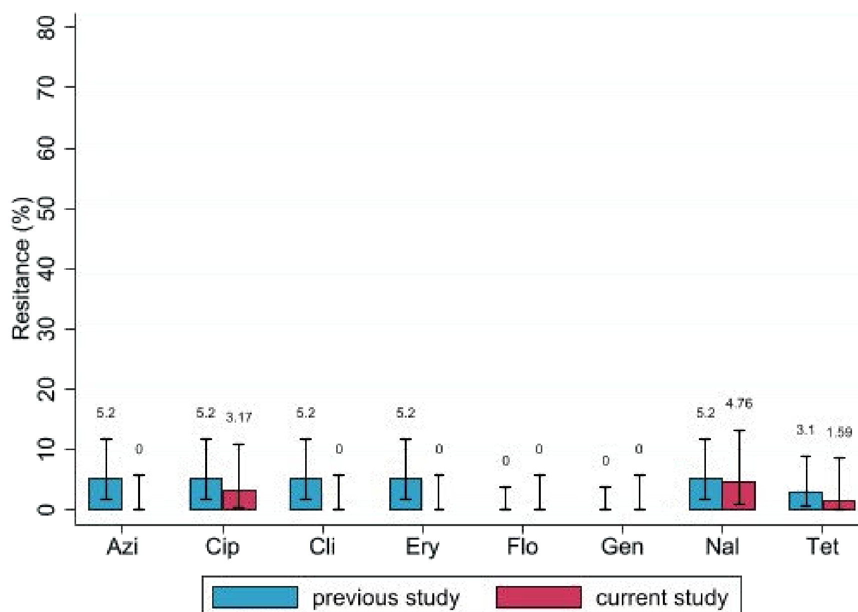

Figure 12: Comparison of resistance to select antimicrobials in *C. coli* isolated from Australian chickens. The percent resistance refers to results using microbiological breakpoints. Only antimicrobials used in both studies with microbiological breakpoints available were used. Error bars refer to 95% confidence intervals. Previous study (2016) n = 96, current study n = 63.

General conclusion

Overall, resistance to antimicrobials that are of critical importance to human health remains considerably low in commensal bacteria from Australian meat chickens. Ciprofloxacin resistance in *E. coli* and *Campylobacter* spp. remains stable and has been detected despite fluoroquinolones never being used in the commercial chicken meat industry in Australia, suggesting alternative pathways exist for entry of AMR bacteria into Australian chicken flocks. The low detection of resistance supports the antimicrobial stewardship efforts in place in the Australian chicken industry and provides a basis for areas of future improvement.

Recommendations Arising from this AMR survey

1. Sampling protocols and logistics of collection within plants should be reviewed to reduce the cost of the survey and the burden on those in industry who donate their time and expertise to conducting the survey. The current protocols are intended to be comparable with major AMR surveillance systems abroad and improve the veracity of the surveillance system, meaning they cannot be replaced entirely.

2. Transportation of caecal samples to the primary processing lab within 12-24h was also challenging. Alternative sampling strategies for caecal collection require investigation before undertaking any future AMR surveys in the chicken meat industry. In the current survey, *E. coli* and Enterococci were isolated using pooled or individual samples directly sampling the caecal contents using a swab followed by processing swabs on the RASP platform. Preliminary validation studies (not reported here) have indicated that swabs from caeca could be used and may also be suitable for isolating *Campylobacter* spp. Caecal swabs appear viable if appropriate transport media is used, and this may provide some additional flexibility on transportation time. Investigation and validation of the potential replacement of collection of whole caeca with swabs of caecal contents may improve sample collection, transportation and cost-efficiency without compromising the integrity of the study. However, where *Salmonella* is specifically targeted whole caeca may still be necessary.

3. The low number of *Salmonella* (n=9) recovered in this survey suggest a change in approach is required to obtain a sufficient number of *Salmonella* isolates, if it is to remain as a regular component of these surveys in the future. Any future AMR survey that intends to specifically target *Salmonella* should consider different specimen volumes and isolation methods.

4. Consider options for including in future studies bacteria and antimicrobial combinations that may be of animal health concern. If preventative or first line recommended treatments using antimicrobials to minimise animal health issues (despite high standards of biosecurity and husbandry having been implemented) fail, the alternative may be to use antimicrobials that are of higher importance to human health. Therefore, for the industry to contribute to a One Health approach, it is imperative that the sector considers AMR that may be of concern to animal health, not just human health.

5. Determine relevant risks for incursion of AMR bacteria into Australian chicken flocks to identify approaches that may reduce the level of these bacteria in chicken flocks (in the absence of environmental pressure due to use).

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