Journal of Food Protection 87 (2024) 100245

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Antimicrobial Resistance in Selected Bacteria from Food Animals in New Zealand 2018–2022

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ARTICLE INFO

Keywords:
Calves
Cattle
Food safety
Pigs
Poultry
Susceptibility

ABSTRACT

Antimicrobial resistance (AMR) presents a significant threat to human health worldwide. One important source of antimicrobial-resistant infections in humans is exposure to animals or animal products. In a phased survey, we investigated AMR in 300 *Escherichia coli* isolates and 300 enterococci (*Enterococcus faecalis* and *E. faecium*) isolates each from the carcasses of poultry, pigs, very young calves, and dairy cattle (food animals); all *Salmonella* isolates from poultry, very young calves, and dairy cattle; and 300 *Campylobacter* (*Campylobacter jejuni* and *C. coli*) isolates from poultry. The highest resistance levels in *E. coli* were found for sulfamethoxazole, tetracycline, and streptomycin, for all food animals. Cefotaxime-resistant *E. coli* were not found and low resistance to ciprofloxacin, colistin, and gentamicin was observed. The majority of enterococci isolates from all food animals were bacitracin-resistant. Erythromycin- and/or tetracycline-resistant enterococci isolates were found in varying proportions from all food animals. Ampicillin- or vancomycin-resistant enterococci isolates were not identified, and ciprofloxacin-resistant *E. faecalis* were not found. *Salmonella* isolates were only recovered from very young calves and all eight isolates were susceptible to all tested antimicrobials. Most *Campylobacter* isolates were susceptible to all tested antimicrobials, although 16.6% of *C. jejuni* were resistant to quinolones and tetracycline. Results suggest that AMR in *E. coli*, enterococci, *Salmonella,* and *Campylobacter* isolates from food animals in New Zealand is low, and currently, AMR in food animals poses a limited public health risk. Despite the low prevalence of AMR in this survey, ongoing monitoring of antimicrobial susceptibility in bacteria from food animals is recommended, to ensure timely detection of AMR with potential impacts on animal and human health.

Antimicrobials are widely used in human and veterinary medicine to prevent disease, treat infections, and permit life-preserving surgery and cancer chemotherapy (The Review on Antimicrobial Resistance, 2016). A consequence of antimicrobial use is the potential for microorganisms to develop antimicrobial resistance (AMR), which makes infections harder to treat resulting in an increased number of deaths, health complications, and health expenditure (The Review on Antimicrobial Resistance, 2016). It has been estimated that by 2050, 10 million human lives a year will be at risk from AMR if proactive solutions are not found to slow the rise of resistance (The Review on Antimicrobial Resistance, 2016). In 2016, the United Nations General Assembly recognized the inappropriate use of antimicrobials in multiple sectors, including animals, as a leading cause of rising AMR (United Nations, 2016).

Many antimicrobial-resistant infections in humans result from exposure to contaminated animals or animal products (Van Boeckel et al., 2017). Infections can result from direct or indirect contact with animals, including eating or handling food derived from animals infected with pathogens that have AMR – particularly bacteria (World Health Organization, 2014). Recent data suggest that antimicrobial use in animals may lead to AMR in commensal bacteria such as *Escherichia coli* and enterococci (Makarov et al., 2022; Nhung et al., 2022). Evidence linking the transfer of bacteria containing AMR between animals and humans is also particularly strong for some common foodborne pathogens; for example, *Campylobacter* and *Salmonella* isolates resistant to quinolones (Rukambile et al., 2019). Awareness and understanding of AMR in bacteria from carcasses from animals raised for human consumption (herein referred to as 'food animals') is therefore vital for slowing the rise of AMR.

A lack of surveillance data on AMR in bacteria from food animals worldwide is a major gap in the fight against AMR (World Health Organization, 2014). In 2005, the New Zealand Food Safety Authority

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https://doi.org/10.1016/j.jfp.2024.100245
Received 23 July 2023; Accepted 13 February 2024
Available online 20 February 2024

(NZFSA) Expert Panel on Antibiotic Resistance recommended that a program of surveillance and monitoring of AMR in bacteria from New Zealand (NZ) food animals be implemented (Heffernan et al., 2011). Consequently, a survey was conducted in 2009–2010 to describe AMR among *E. coli,* enterococci, *Campylobacter,* and *Salmonella* isolates obtained from carcasses of poultry, pigs, and very young calves. The food animals considered for inclusion were poultry, pigs, very young calves, and dairy cattle as these species represent the industries that use the greatest quantities of antimicrobials in NZ agriculture (very young calves are a by-product of the dairy industry) (Agricultural Compounds and Veterinary Medicines team, 2023), although at substantially lower levels than often used in comparator countries (Hillerton et al., 2021; Hillerton et al., 2017). Dairy cattle were not included, because at the time, dairy and beef cattle were not distinguished in the NMD programme.

The 2009–2010 study showed resistance to most tested antimicrobials was lower in NZ food animals than observed in similar studies in Denmark and the United States of America (Heffernan et al., 2011). In the years following this survey, only limited data were published on AMR in bacteria isolated from NZ food animals.

A follow-up to the 2009–2010 survey, recommended by the NZFSA Expert Panel on Antibiotic Resistance, was funded in 2017 to provide more up-to-date data on AMR in priority food animals, specifically poultry, pigs, very young calves (<14 days of age), and dairy cattle. Each food animal species was to be sampled over a 12-month period, with the first sample collection period beginning in 2018 (poultry), and the last ending in 2022 (dairy cattle). The purpose of this survey is to increase the amount of data available on AMR in NZ food animals. More specifically, to estimate the prevalence of isolates resistant to important and commonly used antimicrobials among representative collections of indicator commensal bacteria (*E. coli* and enterococci) and pathogenic bacteria (*Salmonella* and *Campylobacter*) isolated from freshly dressed carcasses of poultry, pigs, very young calves, and dairy cattle. There are many parallels between this follow-up survey and the initial 2009–2010 survey. However, several changes were made to the antimicrobials and concentrations tested to ensure they reflected the range of results expected in contemporary samples. Here, we present findings from the follow-up survey of bacterial isolates collected between 2018 and 2022, comparing them to 2009–2010 findings.

Materials and methods

Sample Collection. A phased survey of carcasses from selected food animals in NZ was performed. Samples collected as part of the regulatory National Microbiological Database (NMD) monitoring program (Kinsella, 2021) were utilized for this survey, where possible. Samples were collected from all eight standard-throughput (>1,000,000 birds per annum) poultry processors from August 2018 to July 2019 and tested for *E. coli*, enterococci (*Enterococcus faecalis* and *E. faecium*), *Salmonella,* and *Campylobacter* (*Campylobacter jejuni* and *C. coli*). Samples were collected from five of the six pig processors from November 2019 to October 2020 and tested for *E. coli* and enterococci. Testing for *Salmonella* is no-longer part of NMD testing for pigs due to consistent low prevalence. Samples were collected from 17 of the 26 very young calf processors during 2020, with processing occurring from March to November 2020. Samples from very young calves were tested for *E. coli*, enterococci, and *Salmonella*. Samples were collected from 21 of the 30 dairy cattle processors from April 2021 to March 2022 and tested for *E. coli*, enterococci, and *Salmonella*. Participation by processors was voluntary. They were selected to ensure a good geographical distribution and be representative of the total numbers of carcasses processed in NZ. Carcass rinsates (for poultry) and carcass swab suspensions (for pigs, very young calves, and dairy cattle) were prepared as described in the Animal Products Notice: Specifications for National Microbiological Database Programme (Kinsella, 2021). Three whole poultry carcasses were sampled at random times across each processing day from the line after primary chilling at the last readily accessible point prior to the carcass entering any packing bins, being bagged, cut up, or sent for further processing (Kinsella, 2021). For pigs, very young calves, and dairy cattle, one sampling day and five sampling times were selected on a random or rotational basis to ensure different shifts, food animal classes, runs, times, and chains/boning rooms were included (Kinsella, 2021). At the selected day and time, one carcass was sampled within 30 min of the post-mortem examination before any physical procedure or chemical intervention had occurred (Kinsella, 2021). The three carcass sites swabbed for pigs were outside the hindleg, lower flap, and outside shoulder with 5 cm^2 swabbed per site using one wet and one dry swab and using 10 mL of 0.1% Peptone Diluent (0.1% Peptone, 0.85% NaCl) (Kinsella, 2021). The three carcass sites swabbed for very young calves were fore rump, flank, and foreleg with 25 cm^2 swabbed per site using two wet and two dry swabs and using 15 mL of 0.1% Peptone Diluent (Kinsella, 2021). The three carcass sites swabbed for dairy cattle were rump, flank, and brisket with 100 cm^2 swabbed per site using three wet and three dry swabs and using 15 mL of 0.1% Peptone Diluent (Kinsella, 2021).

***E. coli* Isolation and Confirmation.** *E. coli* were isolated from carcass swab suspensions for pigs, very young calves, and dairy cattle as described in the NMD documentation (Kinsella, 2021). *E. coli* testing is not prescribed for poultry, but the same NMD method was applied to poultry carcass rinsates for this survey. Petrifilms (3 M Saint Paul, Minnesota) showing blue colonies were held at 0–10 °C while awaiting further testing. Purity plates were prepared from well-isolated presumptive *E. coli* colonies on Tryptic Soy Agar (Fort Richard, Auckland, NZ) and incubated at 35 °C for 24 ± 4 h. *E. coli* were confirmed by a positive indole test. Isolates were grown in Tryptone Broth (Condalab, Madrid, Spain), incubated at 35 °C for 20 ± 2 h, with a pink color change when Kovacs reagent (Merck, Kenilworth, New Jersey) was added indicating a positive result. In addition, isolates were streaked on L-EMB agar (Merck) and incubated at 35 °C for 20 ± 2 h. *E. coli* produced characteristic purple colonies with a metallic green sheen. Confirmed *E. coli* isolates were stored in Brain Heart Infusion (Difco, Franklin Lakes, New Jersey) with 15% glycerol (Thermo Fisher Scientific, Waltham, Massachusetts) at −80 °C.

Enterococci Isolation and Confirmation. Carcass rinsates (for poultry) and carcass swab suspensions (for pigs, very young calves, and dairy cattle) were held at 0–10 °C while awaiting testing. Enrichments were prepared by thoroughly mixing rinsates or swab suspensions, transferring 2 mL to 18 mL of Azide Dextrose Broth (Fort Richard) and incubating at 37 °C for 20 ± 2 h. For very young calves, the three enrichments for the three sites from the same carcass were composited (2 mL from each carcass swab suspension to 54 mL of Azide Dextrose Broth). A 1 μL loop was used to streak the incubated enrichment onto Stanetz and Bartley Medium (Thermo Fisher Scientific). These plates were incubated at 44 °C for 44 ± 2 h and light pink to dark red colonies were identified as possible *Enterococcus* isolates. Purity streak plates were prepared on Columbia Sheep Blood Agar plates (Fort Richard) and incubated at 35 °C for 24 ± 2 h. Well-isolated colonies were used to inoculate one Brain Heart Infusion (Fort Richard) tube which was incubated at 45 °C for 24 ± 2 h and a second Brain Heart Infusion with 6.5% NaCl (Fort Richard) which was incubated at 35 °C for 24 ± 2 h. DNA was extracted from isolates that grew at both 45 °C and in 6.5% NaCl by resuspending one colony in 500 μL of 2% Chelex 100 sodium form 50–100 mesh (Sigma-Aldrich, St. Louis, Missouri) in sterile water, heating at 95 °C for 10 min, holding at room temperature for 1 min, centrifuging at 16,000 x *g* for 5 min, transferring 250 μL of the supernatant to a sterile 1.5 mL tube and storing at 4 °C. To identify *E. faecalis* and *E. faecium* cultures, each DNA extract was tested using a 25 μL multiplex PCR that included Platinum Multiplex PCR Master Mix (Thermo Fisher Scientific), 5 pmol of each of the species-specific ddl *E. faecalis* and ddl *E. faecium* primers

(Dutka-Malen et al., 1995) and 2.5 μL of DNA. The thermal profile was initial denaturation of 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 45 s and then a final extension of 72 °C for 10 min. The PCR products were detected on the MCE-202 MultiNA microchip electrophoresis system (Shimadzu Corporation, Kyoto, Japan) using standard operating procedures for on-chip mixing with the DNA 1000 reagent kit (Shimadzu). Isolates identified as *E. faecalis* (941 bp PCR product) or *E. faecium* (550 bp PCR product) were stored as described for *E. coli*.

***Salmonella* Isolation and Confirmation.** *Salmonella* were isolated from poultry, very young calves, and dairy cattle, as described in the NMD documentation (Kinsella, 2021). Testing for *Salmonella* is no-longer part of NMD testing for pigs due to consistent low prevalence. These specifications require *Salmonella* testing from one carcass per processing day for poultry and one composite containing swabs from three sites on each of five carcasses on one processing day per week each year (October to September for dairy cattle, January to December for very young calves) until six consecutive weeks are negative. All isolates were tested using conventional phenotypic laboratory methods to confirm they belong to the *Salmonella* genus. *Salmonella* serotyping was performed on all confirmed Salmonellae using commercial antisera and in-house agglutination methods (slide for O [somatic] antigens and tube for H [flagella] antigens) with reference to the WHO Collaborating Centre for Reference and Research on Salmonella: Antigenic formulae of the *Salmonella* serovars (Grimont & Weill, 2007).

***Campylobacter* Isolation and Confirmation.** *Campylobacter* (*C. jejuni* and *C. coli*) were isolated from poultry carcasses as described in the NMD documentation (Kinsella, 2021). When time permitted, one well-isolated colony from the modified charcoal cefoperazone-deoxycholoate (mCCDA) plate (Fort Richard) was streaked on a Columbia Sheep Blood Agar (CBA) plate (Fort Richard) and incubated at 37 °C for 48 h in a microaerophilic atmosphere. Growth from either the mCCDA or CBA plate was transferred to a charcoal swab (Thermo Fisher Scientific) for storage prior to further testing. Swabs were streaked on mCCDA plates and used to inoculate a vial containing 25 mL of Bolton Broth (Fort Richard). The mCCDA plates and Bolton Broth cultures were incubated for 4–6 h at 37 °C and then transferred to 42 °C for the remainder of the 48 ± 2 h incubation. The mCCDA plates were incubated in a microaerophilic environment. If gray colonies were observed on the mCCDA plates, the Bolton Broths were discarded. If no gray colonies were observed, a 10 μL loopful of the Bolton Broth was streaked onto mCCDA and incubated as above. Purity plates were prepared on Columbia Sheep Blood Agar plates (Fort Richard) for one well-isolated gray colony per sample and incubated at 42 °C for 48 ± 2 h in a microaerophilic atmosphere. DNA was extracted from isolates that were oxidase-positive, Gram-negative rods as described for enterococci. Each DNA extract was tested using a 50 μL multiplex PCR that included 1.25 units FIREPol DNA Polymerase (Solis Biodyne, Tartu, Estonia), 1 X Reaction Buffer BD (Solis Biodyne), 8 μL 25 mM $MgCl_2$ (Solis Biodyne), 10 mg Bovine Serum Albumin (Thermo Fisher Scientific), 250 μM each dNTP (Thermo Fisher Scientific), 5 pmol of each of the Therm and LpxA primers and 20 pmol of each of the CeuE primers (Wong et al., 2004), and 5 μL of DNA. The thermal profile was initial denaturation of 94 °C for 3 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 74 °C for 1 min and then a final extension of 74 °C for 8 min. The PCR products were detected as described for enterococci. Samples with the Therm (246 bp) and LpxA (99 bp) PCR products were positive for *C. jejuni,* and samples with the Therm and CeuE (695 bp) PCR products were positive for *C. coli*. Isolates identified as *C. jejuni* or *C. coli* were stored as described for *E. coli*.

Selection of Isolates for Antimicrobial Susceptibility Testing (AST). A maximum of 300 isolates of each bacterial species, from each food animal, underwent AST. When excess bacterial isolates were recovered for a given food animal, isolates were subsampled using the following criteria. Where possible, the number of isolates selected for AST from each processor was proportional to the number of processing weeks for that processor during the study period. For poultry and pigs, where processing occurred almost every week of the year for all processors, the aim was to have an equal number of isolates from each processor in each season. Where there were not enough isolates from a particular processor, additional isolates were selected from processors with an excess of isolates. For poultry, the preference was to select these additional isolates from processors with higher throughput, using information supplied by the Poultry Industry Association of New Zealand. There were fewer excess isolates available to choose from for pigs, very young calves, and dairy cattle so throughput data were not used to aid selection for these food animals. The number of *E. faecalis* and *E. faecium* isolates selected for AST reflected the proportions of each species available from each processor.

Antimicrobial Susceptibility Testing (AST). All susceptibility testing was performed by broth microdilution using Sensititre plates (Thermo Fisher Scientific). For the 2018–2022 study, three plate types were used: one for *E. coli* and *Salmonella* (code NZL2ESR), one for enterococci (code NZLESRP), and one for *Campylobacter* (code EUCAMP2). Plates for *E. coli/Salmonella* and enterococci were customized for this survey whereas plates for *Campylobacter* testing were European Union surveillance plates. *E. coli* and *Salmonella* were tested for resistance to Apramycin (APR), Gentamicin (GEN), Streptomycin (STR), Cefotaxime (CTX), Ceftazidime (CAZ), Cefoxitin (FOX), Ciprofloxacin (CIP), Ampicillin (AMP), Amoxicillin/clavulanic acid, 2:1 ratio (AMC), Colistin (COL), Sulfamethoxazole (SMX), Trimethoprim (TMP), and Tetracycline (TET); enterococci for GEN, STR, CIP, Vancomycin (VAN), Erythromycin (ERY), Quinupristin/dalfopristin (QDA, *E. faecium* only), Tylosin tartrate (TYL), Ampicillin (AMP), Bacitracin (BAC), and TET; and *Campylobacter* for GEN, STR, CIP, ERY, Nalidixic acid (NAL), and TET (Table S1). The Sensititre plates used in the 2009–2010 study differed from those used in the 2018–2022 study and are described elsewhere (Heffernan et al., 2011).

Susceptibility testing was performed according to the Clinical and Laboratory Standard Institute's (CLSI's) microbroth dilution method (Clinical and Laboratory Standards Institute, 2018). All susceptibility testing was fully controlled according to the CLSI and Sensititre protocols with the quality control strains: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *C. jejuni* ATCC 33560, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. *E. coli* NCTC 13846 was also included to control for colistin testing. The interpretive standards and breakpoints used are summarized Table S2. If available, the minimum inhibitory concentrations (MICs) were interpreted using the 2022 EUCAST breakpoint tables (available at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf). Where no EUCAST breakpoints were available, CLSI standards (Clinical and Laboratory Standards Institute, 2022), EUCAST epidemiological cut-off (ECOFF) values (as of October 2022) or the ECOFFs used by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Statens Serum Institut et al., 2013) were applied instead (Table S2). ECOFFs separate bacterial populations into those with acquired and/or mutational resistance mechanisms (referred to as nonwild type, NWT) and those without such mechanisms (referred to as wild type, WT). For simplicity, nonwild type bacteria are grouped with, and referred to as, resistant bacteria. Isolates resistant to at least one antimicrobial tested in three or more antimicrobial classes, in addition to antimicrobials to which each bacterial species is intrinsically resistant, are classified as multidrug-resistant (MDR) (Magiorakos et al., 2012). When comparisons were made with other studies, resistance was classified as: 'rare': < 0.1%, 'very low': 0.1%–1.0%, 'low': > 1%–10.0%, 'moderate': > 10.0%–20.0%, 'high': > 20.0%–50.0%, 'very high': > 50.0%–70.0%, or 'extremely high': > 70.0% (EFSA (European Food Safety Authority) et al., 2023).

Phenotypic confirmatory tests and identification of specific resistance genes. *E. coli* isolates that screened positive for extended-spectrum β-lactamases (ESBLs) by growing at 1 mg/L cefotaxime and/or 1 mg/L ceftazidime were tested by the CLSI ESBL disc confirmatory method to confirm the presence of an ESBL (Clinical and Laboratory Standards Institute, 2022). Cefoxitin-resistant *E. coli* were screened for AmpC β-lactamase with a boronic acid double-disc synergy test (Yagi et al., 2005). Any screen-positive isolates were tested by PCR for plasmid-mediated AmpC β-lactamase (Pérez-Pérez & Hanson, 2002). Vancomycin-resistant enterococci were tested by PCR to determine the presence of the *van*A or *van*B genes (Clark et al., 1993).

Isolates resistant to antimicrobials of last resort (specifically colistin and vancomycin) were considered for characterization using whole genome sequencing to detect acquired resistance genes. Genomic DNA was extracted using the Roche High Pure PCR template preparation kit, the DNA library was created using the Nextera XT DNA preparation kit (Illumina, San Diego, California), and sequencing was performed on a NextSeq 500 (Illumina). The resistance genes present were identified using an in-house pipeline linking together open-source established packages and in-house scripts, which enables the acquired resistome and the multilocus sequence type to be determined. Open-source packages used included the Nullarbor2 (available at https://github.com/tseemann/nullarbor), SKESA (Souvorov et al., 2018), MLST (available at https://github.com/tseemann/mlst), and ABRicate (available at https://github.com/tseemann/abricate) using the ResFinder (Bortolaia et al., 2020) database.

Comparison of 2009–2010 and 2018–2022 surveys. Where results were included from the 2009 to 2010 survey period in these analyses, MICs were harmonized using the same breakpoints or ECOFFs used to analyze results from the 2018 to 2022 survey period. When classifying isolates from 2009 to 2010 as MDR, only antimicrobials tested for each bacterial species in both 2009–2010 and 2018–2022 survey periods were included, excluding those without breakpoints (tylosin) and those used to screen for ESBLs (cefotaxime) and AmpC β-lactamase (cefoxitin).

Data Analysis. Data cleaning, MIC interpretation, and statistical tests were performed in R version 4.1.0 (R Core Team, 2021) using the following open-source packages: tidyverse (Wickham et al., 2019), AMR (Berends et al., 2022), and janitor (https://github.com/sfirke/janitor). Statistical differences were tested using Fisher's exact test when comparing two proportions, or chi-square test when comparing three or more proportions. Differences were considered statistically significant if the associated P value ≤ 0.05 and highly statistically significant if the associated P value ≤ 0.001. Where calculated, 95% confidence intervals for proportions were based on the binomial distribution, whereas those for counts were based on the Poisson distribution.

Results

Samples selected for AST. The number of samples received for isolation and/or confirmation for each food animal, the number of isolates for each taxon confirmed and stored, and the number of isolates selected for AST are summarized in Table 1. *Salmonella* isolates were not obtained from poultry or dairy cattle during the study periods, and only eight *Salmonella* isolates were isolated from very young calves. The full MIC distributions for each bacterial species are shown in Supplementary Materials (Table S3).

***E. coli* AST**. The AST of *E. coli* isolates from each of poultry, pigs, very young calves, and dairy cattle is summarized in Figure 1. None of the *E. coli* isolates from any food animal showed resistance to apramycin or cefotaxime (Fig. 1) and none of the isolates produced an ESBL or AmpC β-lactamase. Resistance to ciprofloxacin, colistin, and gentamicin was rare or very low (≤1%) and resistance to amoxicillin-clavulanic acid was low (<4.0%) for all food animals (Fig. 1). The highest resistance levels in *E. coli* for all antimicrobials, except ciprofloxacin and gentamicin, were observed in pigs and very young calves. The highest resistance levels in *E. coli* isolates were observed for tetracycline, streptomycin, and sulfamethoxazole (Fig. 1). Whole genome sequencing of the one colistin-resistant *E. coli* isolate from a very young calf and the one dairy cattle isolate with a colistin MIC of 2 mg/L (Table S3) did not identify any mobile colistin resistance genes.

Enterococci AST. The AST of enterococci isolates from each of poultry, pigs, very young calves, and dairy cattle is summarized in Figure 2. Resistance to ampicillin or vancomycin was not observed among enterococci isolates from any food animal (Fig. 2). Similarly, ciprofloxacin-resistant *E. faecalis* and gentamicin-resistant *E. faecium* isolates were not observed. Gentamicin resistance in *E. faecalis* isolates was either not detected or detected at very low prevalence (Fig. 2A). The highest resistance levels in *E. faecalis,* for all food animals, were found for bacitracin, tetracycline, and erythromycin (Fig. 2A). The highest resistance levels in *E. faecium* isolates, for all food animals, were found for bacitracin, tetracycline, and erythromycin, with ciprofloxacin resistance also found in pig and dairy cattle isolates (Fig. 2B). Please note that EUCAST guidelines indicate that enterococci are intrinsically resistant to macrolides (such as erythromycin) (European Committee on Antimicrobial Susceptibility Testing, 2022), however, the CLSI guidelines (Clinical and Laboratory Standards Institute, 2022) do not, hence, the erythromycin resistance reported here should be interpreted judiciously. There were no interpretative standards for tylosin and enterococci. Tylosin resistance in enterococci had a bimodal distribution, with MIC results in two distinct clusters. This appears to separate isolates into a wild type population and a second, nonwild type population that may contain tylosin resistance mechanisms. In the absence of accepted international interpretive standards for tylosin in enterococci, *E. faecalis* isolates with a tylosin MIC ≥16 mg/L and *E. faecium* isolates with a tylosin MIC ≥8 mg/L were considered 'nonwild type'. For *E. faecalis,* 39.3% of isolates from pigs, 37.6% of isolates from poultry, 15.4% of isolates from very young calves, and 6.3% of isolates from dairy cattle were nonwild type for tylosin (Table S3). For *E. faecium*, 43.6% of isolates from pigs, 27.1% of isolates from dairy cattle, 21.7% of isolates from poultry, and 13.2% of isolates from very young calves were nonwild type for tylosin (Table S3).

E. faecalis isolates from poultry had statistically significant higher resistance levels to erythromycin and tetracycline than *E. faecium* isolates (Table 2). Similarly, *E. faecalis* isolates from very young calves had statistically significant higher resistance levels to bacitracin, streptomycin, tetracycline, and MDR than *E. faecium* isolates (Table 2). In contrast, *E. faecium* isolates from pigs had statistically significant higher resistance levels to ciprofloxacin and tetracycline than *E. faecalis* isolates, and *E. faecium* isolates from dairy cattle had statistically significant higher resistance levels to bacitracin and ciprofloxacin than *E. faecalis* isolates (Table 2).

***Campylobacter* AST**. Figure 3 summarizes AST of the *Campylobacter* isolates from poultry. Most *C. jejuni* isolates were susceptible to all antimicrobials tested (81.9%) although 16.6% of isolates were resistant to the quinolones, ciprofloxacin, and nalidixic acid, as well as tetracycline. None of the *C. jejuni* isolates were resistant to erythromycin, gentamicin, or streptomycin (Fig. 3). Most *C. coli* isolates were susceptible to all antimicrobials tested, but some isolates were resistant to a single antimicrobial, namely streptomycin or tetracycline. *C. jejuni* isolates had statistically significant higher resistance levels to ciprofloxacin, nalidixic acid, and tetracycline than *C. coli* isolates (Table 3). In contrast, *C. coli* isolates had statistically significant higher resistance levels to streptomycin than *C. jejuni* isolates (Fig. 3).

***Salmonella* AST**. The eight *Salmonella* isolates from very young calves were susceptible to all the tested antimicrobials. The small num-

Table 1
Number of samples received, recovery of *E. coli*, enterococci, *Campylobacter,* and *Salmonella* and the number of isolates selected for antimicrobial susceptibility testing (AST)

	E. coli	Enterococci	*Campylobacter*	*Salmonella*
Poultry				
Number of samples	875	1,264	810	NA[a]
Number of isolates stored	409	445	561	NA
Number selected for AST	300	300	300	0
Pigs				
Number of samples	915	3,246	NA	NA
Number of isolates stored	601	543	NA	NA
Number selected for AST	300	300	NA	NA
Very young calves				
Number of samples	1,434	1,069[b]	NA	NA
Number of isolates stored	584	496[c]	NA	NA
Number selected for AST	300	300	NA	8
Dairy cattle				
Number of samples	445	4,893	NA	NA
Number of isolates stored	550[c]	1,133[c]	NA	NA
Number selected for AST	300	300	NA	0

[a] Not applicable. Only poultry were tested for *Campylobacter*, only poultry, very young calves, and dairy cattle were tested for *Salmonella*. For *Salmonella*, isolates recovered as part of the National Microbiological Database were confirmed at the ESR Enteric Reference Laboratory and before being sent for AST.
[b] More than one isolate per sample was recovered and stored from some samples.
[c] Both *E. faecalis* and *E. faecium* were recovered from 30 very young calf samples.

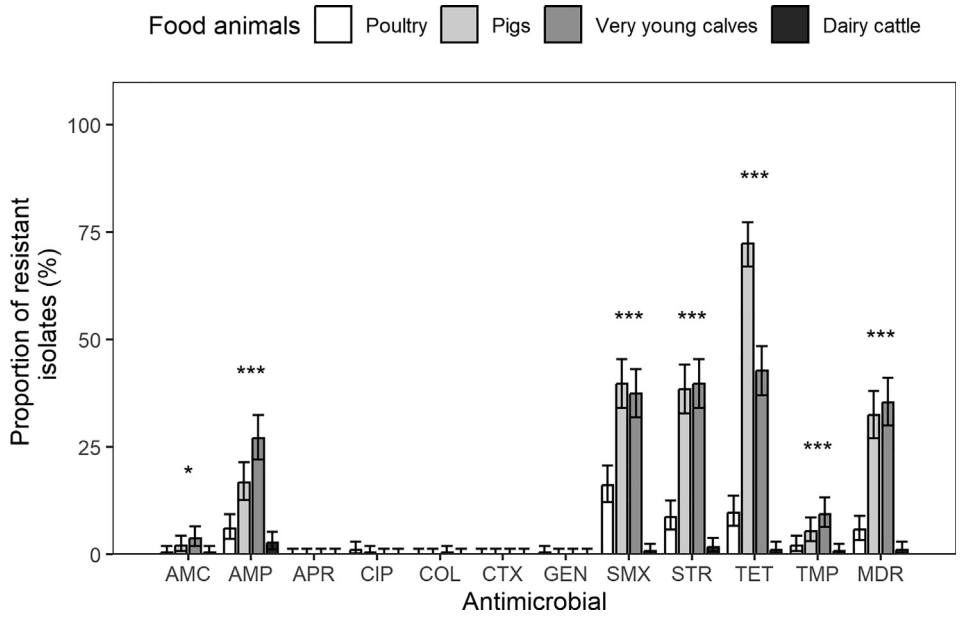

Figure 1. Antimicrobial resistance among *Escherichia coli* isolates from poultry, pigs, very young calves, and dairy cattle, 2018–2222. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; APR, apramycin; CIP, ciprofloxacin; COL, colistin; CTX, cefotaxime; GEN, gentamicin; SMX, sulfamethoxazole; STR, streptomycin; TET, tetracycline; TMP, trimethoprim; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant). Proportions are plotted with 95% confidence intervals based on the binomial distribution. *P* values were calculated using Chi-squared test to compare resistance to each antimicrobial across different food animals; * indicates $P < 0.05$ and *** indicates $P < 0.001$.

ber of *Salmonella* isolated means no meaningful comparisons were possible.

Seasonal Differences. Some significant seasonal differences in AMR were observed during this project. For *E. coli,* there was a significant ($P = 0.008$) difference in tetracycline-resistant isolates found in poultry, with higher resistance levels observed in autumn and winter. There was also a significant ($P = 0.005$) difference in sulfamethoxazole resistance in isolates from very young calves across the three seasons processing occurs, with the highest resistance levels found in spring. Erythromycin-resistant *E. faecalis* isolates from pigs were highest in autumn ($P = 0.002$) and tetracycline-resistant E. *faecalis* isolates from pigs were highest in winter ($P = 0.033$). In *E. faecium* isolates from poultry, tetracycline resistance was highest in autumn ($P = 0.007$). There was also a significant seasonal difference in bacitracin resistance among *E. faecium* isolates from pigs, with lower resistance levels found in spring ($P = 0.012$). In *E. faecium* from very young calves, only streptomycin resistance differed significantly by season, with the highest resistance levels found in spring ($P = 0.008$). For *C. jejuni*, there were significant differences by season for quinolone- and tetracycline-resistance, with the highest resistance levels found in spring, followed by winter ($P = 0.002$ and $P = 0.005$, respectively).

Multidrug resistance. Multidrug resistance (MDR), defined as resistance (or NWT) to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resis-

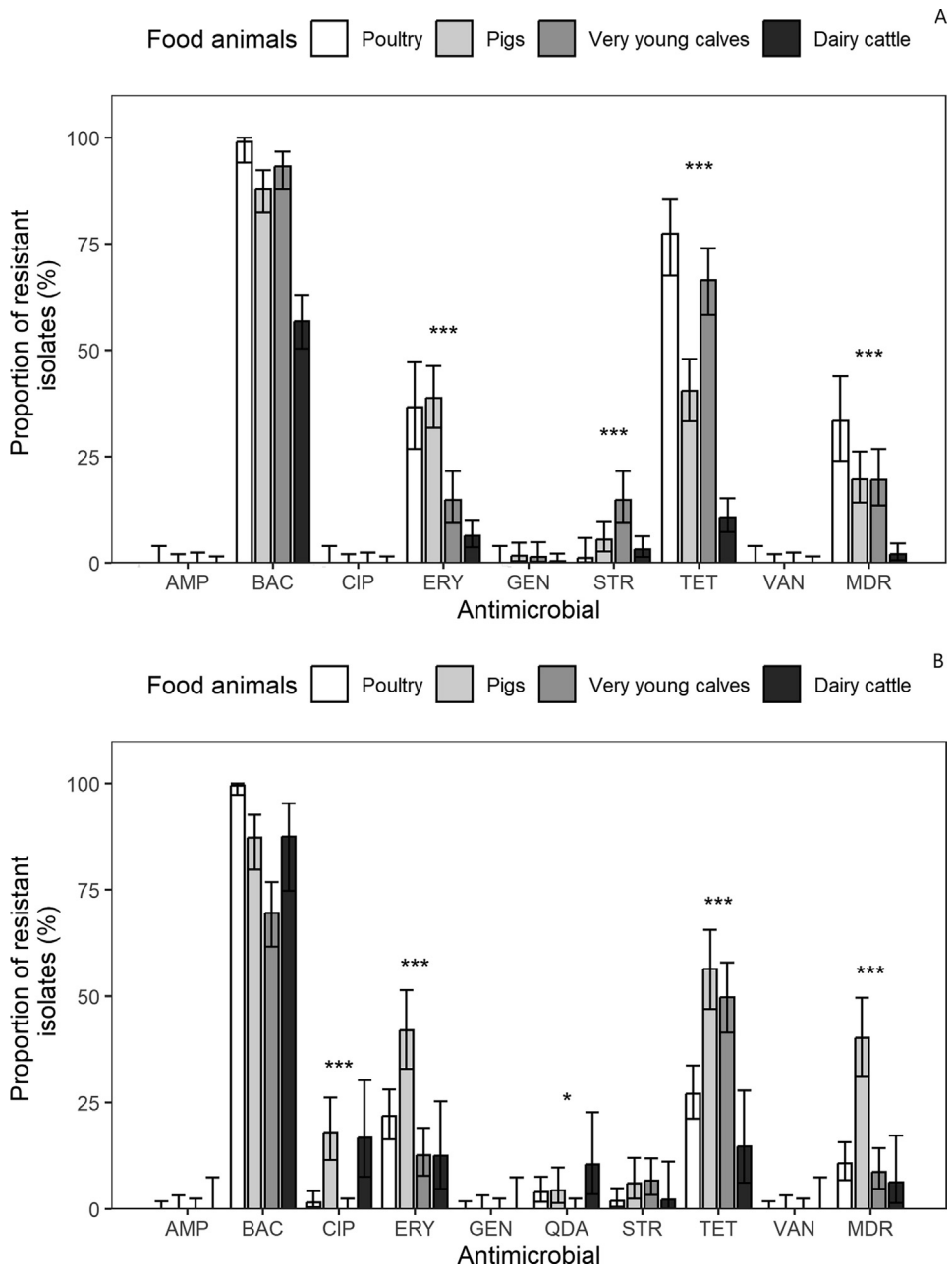

Figure 2. Antimicrobial resistance among *E. faecalis* (A) and *E. faecium* (B) from poultry, pigs, very young calves, and dairy cattle, 2018–2022. AMP, ampicillin; BAC, bacitracin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin (high level); QDA, quinupristin/dalfopristin (*E. faecium* only as *E. faecalis* are intrinsically resistant); STR, streptomycin (high level); TET, tetracycline; VAN, vancomycin; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant). Proportions are plotted with 95% confidence intervals based on the binomial distribution. *P* values were calculated using Chi-squared test to compare resistance to each antimicrobial across different food animals; * indicates $P < 0.05$ and *** indicates $P < 0.001$.

tant, (Magiorakos et al., 2012), varied across food-animal species (Figs. 1–3). MDR was not found in either *Campylobacter* or *Salmonella* isolates. For *E. coli*, 5.7% of isolates from poultry, 32.3% of isolates from pigs, 1.0% of isolates from dairy cattle, and 33.7% of isolates from very young calves were MDR. For *E. faecalis*, 33.7% of isolates from poultry, 19.7% of isolates from pigs, 19.5% of isolates from very young calves, and 2.0% of isolates from dairy cattle were MDR. For *E. faecium*, MDR isolates represented 10.6% of isolates from poultry, 40.2% of isolates from pigs, 8.7% of isolates from very young calves, and 6.3% of isolates from dairy cattle.

There were several notable resistance phenotypes among MDR isolates from poultry, pigs, and very young calves. A total of 1.0% (n = 3) of *E. coli* isolates from poultry were resistant to five antimicrobial classes: aminoglycosides, penicillins, quinolones, tetracyclines, and trimethoprim. In all, 18.7% (n = 56) of *E. coli* isolates from pigs and 19.7% (n = 59) of *E. coli* isolates from very young calves were resistant to aminoglycosides, penicillins, tetracyclines, and trimethoprim. A further 12.0% (n = 36) of *E. coli* isolates from very young calves were resistant to aminoglycosides, tetracyclines, and trimethoprim. There was a significant ($P = 0.004$) seasonal difference in MDR *E. coli* in very young calves with the higher rates in spring than in winter. For *E. faecalis*, 32.3% (n = 30) of isolates from poultry and 12.6% (n = 23) of isolates from pigs were resistant to macrolides, polypeptides, and tetracyclines, whereas 10.7% (n = 16) of *E. faecalis* isolates from very young calves were resistant to aminoglycosides, polypeptides, and tetracyclines. For *E. faecium*, 25.6% (n = 30) of iso-

Table 2
Comparison of antimicrobial resistance among *E. faecalis* and *E. faecium* from poultry, pigs, very young calves, and dairy cattle, 2018–2022

Food animal	Antimicrobial	*E. faecalis*	*E. faecium*	*P* value
Poultry	Ampicillin	0.0	0.0	–
	Bacitracin	98.9	99.5	NS
	Ciprofloxacin	0.0	1.4	NS
	Erythromycin	36.6	21.7	0.010
	Gentamicin	0.0	0.0	–
	Quinupristin/dalfopristin	–	3.9	–
	Streptomycin	1.1	1.9	NS
	Tetracycline	77.4	27.1	<0.001
	Vancomycin	0.0	0.0	–
	MDR	33.3	10.6	<0.001
Pigs	Ampicillin	0.0	0.0	–
	Bacitracin	88.0	87.2	NS
	Ciprofloxacin	0.0	17.9	<0.001
	Erythromycin	38.8	41.9	NS
	Gentamicin	1.6	0.0	NS
	Quinupristin/dalfopristin	–	4.3	–
	Streptomycin	5.5	6.0	NS
	Tetracycline	40.4	56.4	0.010
	Vancomycin	0.0	0.0	–
	MDR	19.7	40.2	<0.001
Very young calves	Ampicillin	0.0	0.0	–
	Bacitracin	93.3	69.5	<0.001
	Ciprofloxacin	0.0	0.0	–
	Erythromycin	14.8	12.6	NS
	Gentamicin	1.3	0.0	NS
	Quinupristin/dalfopristin	–	0.0	–
	Streptomycin	14.8	6.6	0.025
	Tetracycline	66.4	49.7	0.004
	Vancomycin	0.0	0.0	–
	MDR	19.5	8.6	0.008
Dairy cattle	Ampicillin	0.0	0.0	–
	Bacitracin	56.7	87.5	<0.001
	Ciprofloxacin	0.0	16.7	<0.001
	Erythromycin	6.3	12.5	NS
	Gentamicin	0.4	0.0	NS
	Quinupristin/dalfopristin	–	10.4	–
	Streptomycin	3.2	2.1	NS
	Tetracycline	10.7	14.6	NS
	Vancomycin	0.0	0.0	–
	MDR	2.0	6.3	NS

Values are proportion (%) of isolates resistant to each antimicrobial. *P* values were calculated using Fisher's exact test to compare resistance to each antimicrobial across different enterococci species. NS, not significant. MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant).

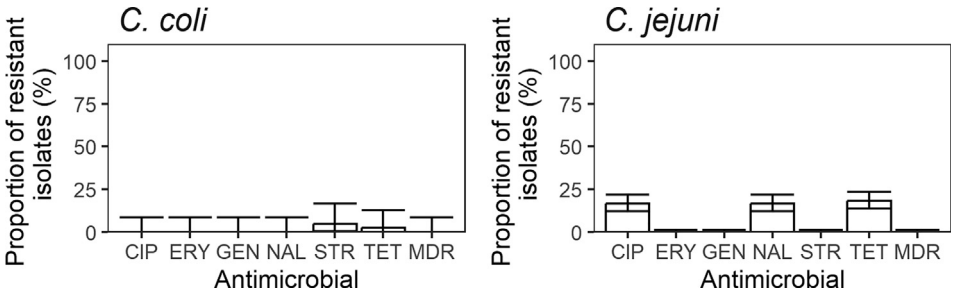

Figure 3. Antimicrobial resistance among *Campylobacter* spp. from poultry, 2018–2019. CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; TET, tetracycline; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant). Proportions are plotted with 95% confidence intervals based on the binomial distribution.

lates from pigs were resistant to macrolides, polypeptides, and tetracyclines. There was a significant ($P = 0.050$) seasonal difference in MDR *E. faecium* from poultry with the highest rate in autumn and the lowest in spring. Although none of the *Campylobacter* isolates were MDR, 16.6% of *C. jejuni* isolates, all isolated from poultry, were resistant to both quinolones and tetracyclines.

Comparison to 2009–2010 study. Data for poultry, pigs, and very young calves collected in 2018–2020 were compared with data collected in a comparable 2009–2010 survey (Heffernan et al., 2011), harmonized using contemporary AST standards (Table S2). Dairy cattle were not included in the 2009–2010 survey, so a comparison was not possible. There were some significant differences in resistance

Table 3
Comparison of antimicrobial resistance among *Campylobacter coli* and *C. coli* from poultry, 2018–2019

Antimicrobial	*C. coli*	*C. jejuni*	*P* value
Ciprofloxacin	0.0	16.6	0.002
Erythromycin	0.0	0.0	–
Gentamicin	0.0	0.0	–
Nalidixic acid	0.0	16.6	0.002
Streptomycin	4.9	0.0	0.018
Tetracycline	2.4	18.1	0.010
MDR	0.0	0.0	–

Values are proportion (%) of isolates resistant to each antimicrobial. *P* values were calculated using Fisher's exact test to compare resistance to each antimicrobial across different campylobacter species. MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant).

across survey periods, with both increasing and decreasing trends. For *E. coli* from poultry, a smaller proportion of isolates were resistant to sulfamethoxazole and trimethoprim in 2018–2019 compared to 2009–2010 (Fig. 4). For *E. coli* isolates from pigs, there was a significant increase in resistance to ampicillin and tetracycline in 2018–2022. *E. faecalis* isolates from pigs collected in 2019–2020 had higher resistance to erythromycin but significantly lower resistance to both gentamicin and streptomycin (Fig. 5A). Significantly fewer *E. faecalis* isolates from very young calves were resistant to streptomycin in 2020 compared to isolates from 2009 to 2010 (Fig. 5A). *E. faecium* isolates from poultry had lower resistance to quinupristin/dalfopristin and tetracycline in 2018–2019 (Fig. 5B). *E. faecium* isolates from pigs had higher resistance to erythromycin and tetracycline, and lower resistance to streptomycin in 2019–2020 (Fig. 5B). A smaller proportion of *E. faecium* isolates obtained from very young calves in 2020 were resistant to quinupristin/dalfopristin and tetracycline than in 2009–2010 (Fig. 5B). A greater proportion of *C. jejuni* isolates collected in 2018–2019 were resistant to ciprofloxacin and nalidixic acid and tetracycline (Fig. 6). Except for streptomycin in 2009–2010, to which 10.5% of isolates were resistant, resistance was not observed among *Salmonella* isolates in very young calves in either study period.

No further statistically significant differences were observed when comparing data between survey periods. However, there were several bacterial species/antimicrobial combinations where the proportion of resistant isolates remained high (>20.0%–50.0%), very high (>50.0%–70.0%), or extremely high (>70.0%) but without significant change between surveys. For example, the proportion of *E. coli* isolates from pigs resistant to streptomycin and the proportion of *E. coli* isolates from very young calves resistant to tetracycline remained high (Fig. 4). The proportion of *E. faecalis* isolates from poultry resistant to bacitracin and tetracycline remained extremely high (Fig. 5A). Extremely high proportions of *E. faecalis* isolates from pigs were resistant to bacitracin and high proportions were resistant to tetracycline in the 2009–2010 and 2018–2022 periods (Fig. 5A). The proportion of *E. faecalis* isolates from very young calves resistant to bacitracin remained extremely high and resistance to tetracycline remained very high (Fig. 5A). For poultry *E. faecium* resistance remained extremely high for bacitracin and resistance remained high for erythromycin (Fig. 5B).

Multidrug resistance varied between study periods for several food-animal-bacterial species combinations. A larger proportion of *E. coli* isolates from pigs were MDR in 2018–2022 compared to 2009–2010 ($P < 0.001$) (Fig. 4). A smaller proportion of *E. faecalis* from pigs in 2019 and very young calves in 2020 were MDR compared to 2009–2010 ($P = 0.029$ and $P = 0.002$, respectively) and a larger proportion of *E. faecium* from pigs in 2019 were MDR compared to 2009–2010 ($P = 0.017$) and a smaller proportion of *E. faecium* from very young calves were MDR in 2020 compared to 2009–2010 ($P = 0.028$) (Figs. 5A and 5B). No further statistically significant differences in the proportions of MDR isolates for poultry, pigs, and very young calves were observed between survey periods.

Discussion

In recent years, growing awareness of the challenge posed by AMR has led to a renewed focus on preserving the efficacy of existing antimicrobials. Measures contributing to this include conserving antimicrobials by reducing antimicrobial use and seeking alternatives to prevent and treat infections in food animals. There is also a need for improved surveillance of AMR bacteria, to quantify the threat posed by AMR and to measure the success of any changes implemented.

The need for improved surveillance of AMR in NZ has been emphasized through the Ministry for Primary Industries (MPI) Antimicrobial Resistance Direction Statement for Animals and Plants (Ministry for Primary Industries, 2016), the New Zealand Antimicrobial Resistance Action Plan (Ministry of Health & Ministry for Primary Industries, 2017), and a report issued by the Office of the Prime Minister's Chief Science Advisor (Harwood et al., 2021). The New Zealand Action Plan specifically called for "initiatives to strengthen national surveillance for antimicrobial resistance and antimicrobial consumption in animal health and agriculture production … working in partnership with livestock industries and dairy and veterinary representatives" (Ministry of Health & Ministry for Primary Industries, 2017). This survey increased knowledge of AMR in bacteria isolated from NZ food animals.

The bacterial species and antimicrobials tested were as recommended by the AMR Surveillance Advisory Group to the MPI, which considered factors such as antimicrobials of critical importance to

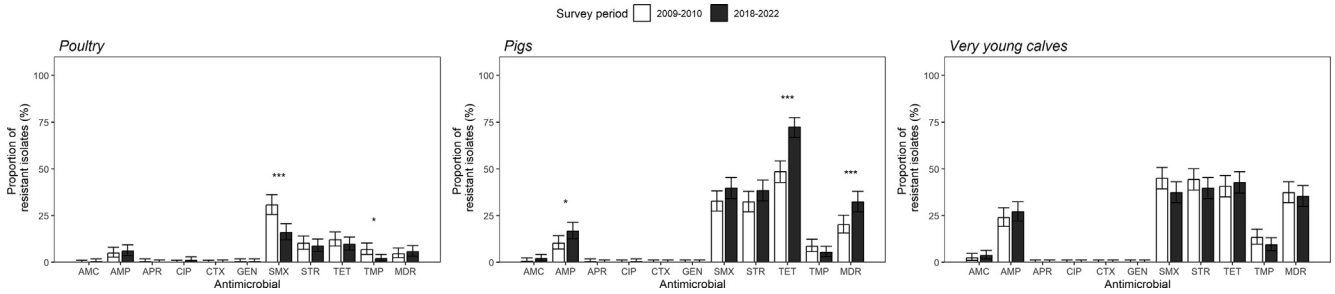

Figure 4. Comparison of resistance among *Escherichia coli* from poultry, pigs, and very young calves, in 2009–2010 versus 2018–2020. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; APR, apramycin; CIP, ciprofloxacin; CTX, cefotaxime; GEN, gentamicin; SMX, sulfamethoxazole; STR, streptomycin; TET, tetracycline; TMP, trimethoprim; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant, limited to antimicrobial agents tested in both survey periods). Proportions are plotted with 95% confidence intervals based on the binomial distribution. *P* values were calculated using Fisher's exact test to compare resistance to each antimicrobial across different food-producing animals; * indicates $P < 0.05$ and *** indicates $P < 0.001$.

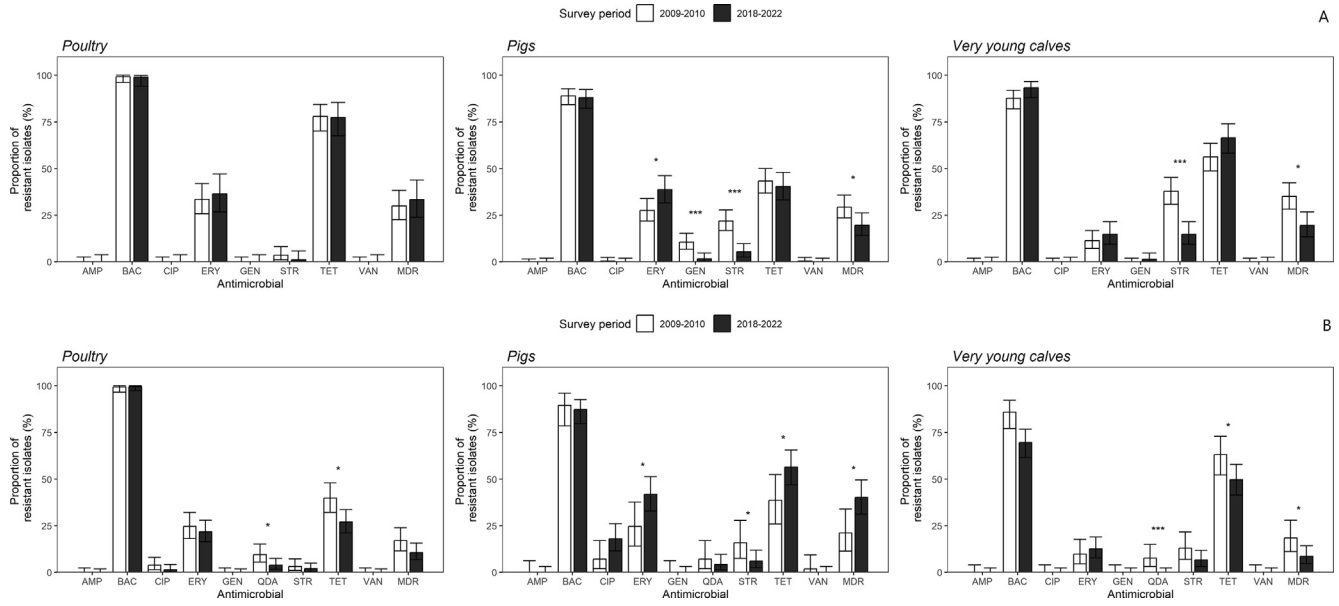

Figure 5. Comparison of resistance among *E. faecalis* (A) and *E. faecium* (B) from poultry, pigs, and very young calves in 2009–2010 versus 2018–2020. AMP, ampicillin; BAC, bacitracin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin (high level); QDA, quinupristin/dalfopristin (*E. faecium* only as *E. faecalis* are intrinsically resistant); STR, streptomycin (high level); TET, tetracycline; VAN, vancomycin; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant, limited to antimicrobial agents tested in both survey periods). Proportions are plotted with 95% confidence intervals based on the binomial distribution. *P* values were calculated using Fisher's test to compare resistance to each antimicrobial across different food-producing animals; * indicates $P < 0.05$ and *** indicates $P < 0.001$.

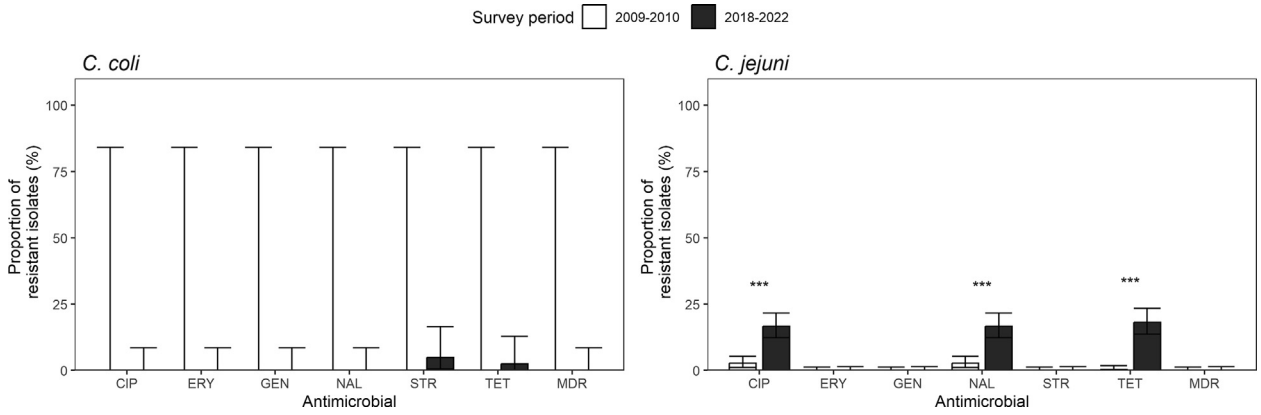

Figure 6. Comparison of resistance among *Campylobacter* spp. from poultry in 2009–2010 versus 2018–2019. CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; TET, tetracycline; MDR, multidrug-resistant (resistant/NWT to three or more antimicrobial classes in addition to antimicrobials to which each bacterial species is intrinsically resistant, limited to antimicrobial agents tested in both survey periods). Proportions are plotted with 95% confidence intervals based on the binomial distribution. *P* values were calculated using Fisher's exact test to compare resistance to each antimicrobial across different food-producing animals; * indicates $P < 0.05$ and *** indicates $P < 0.001$.

human health and antimicrobials used to treat animals, particularly those that may confer resistance to antimicrobials used in human health. The 2018–2022 survey described in this paper characterized carcass samples from poultry, pigs, very young calves, and dairy cattle, whereas the 2009–2010 study (Heffernan et al., 2011) characterized carcass samples from poultry, pigs, very young calves, and fresh produce. Changes were made to the antimicrobials tested between the 2009–2010 and 2018–2022 studies, based on recommendations from the AMR Surveillance Advisory Group. Specifically, for the Gram-negative plate cephalothin, ceftiofur, chloramphenicol, nalidixic acid, spectinomycin, and neomycin were excluded from the 2018–2022 study and colistin was added; for the Gram-positive plate chloramphenicol and nitrofurantoin were excluded; and for the *Campylobacter* plate, chloramphenicol was excluded. All Sensititre plates included changes to the antimicrobial concentrations tested, due to the changes in AST interpretive standards and the availability of more relevant susceptibility data.

It is important to monitor antimicrobial resistance in animal populations to inform treatment options for sick animals as well as to identify possible risks to humans if animal-human transmission was to occur. All antimicrobials tested as part of this study are used to treat infections in animals and/or humans. The presence of resistance suggests that treatment failure may occur if these antimicrobials were used, although for all bacteria tested in this survey, there were other treatment options that remain effective.

Among *E. coli* isolates, the highest resistance levels were found for streptomycin, sulfamethoxazole, and tetracycline in bacteria from all food animal species, except dairy cattle. The highest resistance levels

in enterococci isolates from all animal species were found for bacitracin, erythromycin, and tetracycline. Resistance to erythromycin, and tetracycline among enterococci isolates from dairy cattle was significantly less than observed in other food animals (Fig. 2). Resistance in *C. coli* isolates from poultry was low or absent, although 16.6% of *C. jejuni* isolates were coresistant to ciprofloxacin, nalidixic acid, and tetracycline. Resistance to some antimicrobials included in this study showed a significant increase compared to the 2009–2010 baseline survey. In pig samples, resistance to ampicillin and tetracycline among *E. coli* isolates had significantly increased, as had resistance to erythromycin among *E. faecalis* isolates, and erythromycin and tetracycline among *E. faecium* isolates. In poultry, *C. jejuni* isolates were significantly more resistant to ciprofloxacin, nalidixic acid, and tetracycline in the current study compared to the 2009–2010 baseline survey.

Resistance was not observed among *Salmonella* isolates from very young calves although there were too few isolates to draw any robust conclusions regarding antimicrobial susceptibility among *Salmonella* isolates on carcasses from NZ food animals or make any meaningful comparisons to international data. *Salmonella* has been detected at a very low prevalence in meat products sampled under the National Microbiological Database program since its inception in 1997 (Cook, 2023). The low number of *Salmonella* isolates recovered over the AMR survey period is therefore reflective of this ongoing low prevalence, and indicative of potentially negligible exposure to antimicrobial-resistant foodborne pathogens through meat given that it is also usually cooked prior to consumption. Although we interpret the absence of resistance with cautious optimism due to the small sample size, this result is consistent with a 2019 report on AMR among nontyphoidal *Salmonella* isolates, which showed only low levels of resistance to cotrimoxazole, streptomycin, and tetracycline among nonhuman sources, which included samples from animals, environment, and food (Institute of Environmental Science and Research, 2019).

International comparisons. Direct comparisons of AMR between countries are difficult due to variations in sampling sources, antimicrobial testing methods, and interpretive standards used across different surveillance systems and research programs. However, resistance levels can be compared when sampling designs are similar and interpretative standards are harmonized. Denmark is seen as a world leader in controlling AMR and the methodology for this survey was based on the DANMAP system for monitoring AMR in bacteria from food animals (Statens Serum Institut et al., 2013), making DANMAP data the most suitable for international comparisons. Data from this study were also compared with average AMR surveillance data reported by the European Food Safety Authority (EFSA) for European Union (EU) countries (EFSA (European Food Safety Authority) et al., 2023), and AMR data from various research publications from the past decade concerning AMR in Australian poultry (Abraham et al., 2019) (Abraham et al., 2020) (O'Dea et al., 2019), pigs (Kidsley et al., 2018) (Lee et al., 2021), and cattle (including both dairy cattle and veal) (Barlow et al., 2015) (Sahibzada et al., 2022).

Resistance levels to critically important antimicrobials ampicillin, ciprofloxacin, colistin, cefotaxime, and gentamicin were generally lower in *E. coli* isolates from NZ food animals compared to comparable animals from Australia, Denmark, and the EU although a few notable examples were observed. For example, resistance to ampicillin in NZ very young calves was higher than in Australian veal and Danish young calves but comparable to the average among EU young calves (27.0% in NZ vs. 5.7% in Australia, 5.0% in Denmark, and 30.6% in EU). Resistance to the highly important antimicrobial tetracycline was higher in *E. coli* isolates from NZ pigs (72.3% in NZ vs. 66.2% in Australia, 35.0% in Denmark, and 44.0% in the EU). Resistance to tetracycline was also higher among isolates from NZ very young calves compared to Australian veal and Danish young calves but comparable to the average among EU young calves (42.7% in NZ vs. 4.5% in Australia, 8.0% in Denmark, 43.2% in EU). AMR data for *E. coli* isolates from dairy cattle were only available for NZ and Australian animals. No *E. coli* isolates from NZ or Australian dairy cattle were resistant to ciprofloxacin, cefotaxime, or gentamicin, and resistance was low to ampicillin (2.7% in NZ vs. 2.6% in Australia) and tetracycline (1.0% vs. 2.6%).

Enterococci (*E. faecalis* and *E. faecium*) isolates from NZ pigs, very young calves, and dairy cattle were compared only with isolates from Australian animals. DANMAP (National Food Institute et al., 2021) only reported AMR data for *E. faecalis* and *E. faecium* isolates from poultry and reporting of AMR data for *E. faecalis* and *E. faecium* in food animals is voluntary (EFSA (European Food Safety Authority) et al., 2023) and consequently not included in any recent publicly available EFSA reports or supplementary materials. *E. faecalis* isolates from NZ poultry had extremely high resistance to tetracycline whereas isolates from Australian poultry had high resistance and isolates from Danish isolates had very high resistance (77.4% vs 46.2% vs 62.0%, respectively). Gentamicin resistance was low (<6.0%, MIC>128 mg/L) for *E. faecalis* isolates from all food animals in NZ and Australia. Only *E. faecalis* isolates from Australian poultry were resistant to ampicillin (9.8%) and only *E. faecalis* isolates from Australian dairy were resistant to vancomycin (10.0%, MIC>4 mg/L). *E. faecalis* isolates from NZ pigs had lower resistance levels to tetracycline (40.4% vs 82.4%) than Australian pigs. *E. faecalis* isolates from NZ very young calves also had considerably lower resistance to tetracycline (25.0% vs 66.4%) compared to Australian veal. Resistance levels were moderate for tetracycline (10.7% vs 20.0%) in both NZ and Australian dairy cattle.

E. faecium isolates from NZ poultry had lower resistance levels to the critically important antimicrobials ampicillin, erythromycin, and vancomycin and the highly important antimicrobials quinupristin/dalfopristin and tetracycline. *E. faecium* isolates from NZ poultry had moderate resistance to erythromycin whereas isolates from Australian poultry had high resistance and isolates from Danish isolates had low resistance (21.7% vs 39.0% vs 9.0%, respectively). Resistance to quinupristin/dalfopristin was low in isolates from NZ poultry and high in isolates from both Australian and Danish poultry (3.9% vs 46.8% vs 44.0%, MIC>4mg/L). Tetracycline- resistance was high in isolates from NZ and Australian poultry and low in isolates from Danish poultry (27.1% vs 40.3% vs 12.0%). No resistance to vancomycin was observed in *E. faecium* from NZ poultry but was observed at very low levels in Australian and Danish poultry (0.0% vs 1.2% vs 1.0%). *E. faecium* isolates from NZ pigs had lower resistance levels to ampicillin (0.0% vs 6.0%), quinupristin/dalfopristin (4.3% vs 42.9%, MIC>4 mg/L), and tetracycline (56.4% vs 91.7%). Very low resistance to vancomycin was only in isolates from Australian pigs (0.0% vs 1.2%, MIC>4) (MICs available, (Lee et al., 2021)). *E. faecium* isolates from NZ very young calves had much higher resistance levels to tetracycline (49.7% vs. 1.2%)), and somewhat higher resistance levels to erythromycin (12.6% vs 6.1%) compared to isolates from Australian veal. No vancomycin-resistant *E. faecium* isolates were observed among either NZ very young calves or Australian veal. *E. faecium* isolates from NZ dairy cattle had higher resistance levels to tetracycline (14.6% vs 6.3%) compared to isolates from Australian dairy cattle (Sahibzada et al., 2022). Resistance to gentamicin was very low across all food animals in NZ and Australia.

C. coli isolates from NZ and Australian poultry had very low or low resistance to the critically important antimicrobials ciprofloxacin, erythromycin, and gentamicin (all ≤5.2%). In contrast, the average *C. coli* isolates from the EU were very highly resistant to ciprofloxacin (61.9%) and tetracycline (67.3%) (EFSA (European Food Safety Authority) et al., 2023). *C. jejuni* isolates from NZ, Australian, Danish, and EU poultry had no or very low resistance to erythromycin and gentamicin. In contrast, resistance to nalidixic acid (16.6% vs 14.8% vs 38.0% vs 69.2%), ciprofloxacin (16.6% vs 14.8% vs 38.0% vs 72.8%), and tetracycline (18.2% vs 22.2% vs 34.0% vs 52.7%) was moderate in isolates from NZ and Australian poultry, high in isolates

from Danish poultry, and very high or extremely high in isolates from EU poultry.

Variations of the resistance levels observed between countries, for example, the greater resistance to tetracycline observed in isolates from Australian food animals, likely mostly reflect differences in veterinary antimicrobial usage patterns across countries. However, it is not possible to prove this association due to a lack of standardized data on antimicrobial use at the animal species level in each country, including NZ (Agricultural Compounds and Veterinary Medicines team, 2023; Beckett et al., 2022). Variations in antimicrobial use are also unlikely to explain the emergence of resistance to antimicrobials not currently registered for use in food animals in these countries. For example, the NZ poultry industry does not use fluoroquinolones (Agricultural Compounds and Veterinary Medicines team, 2023) therefore, the increased resistance observed among *C. jejuni* isolates from NZ poultry likely emerged via other means. It is hypothesized that this observed resistance is due to the emergence of a single fluoroquinolone and tetracycline coresistant *C. jejuni* lineage (ST6964) that emerged concurrently in 2014 in poultry from several North Island poultry producers and as a major cause of campylobacteriosis in humans (French et al., 2019). It is likely that this same lineage is responsible for the larger proportion of *C. jejuni* isolates coresistant to fluoroquinolones and tetracycline in the 2018–2022 study compared to the 2009–2010 baseline survey. We did not conduct genomic subtyping of *C. jejuni* isolates in our study, so it is not possible to verify which lineages are prevalent in our survey. However, the persistence of this lineage is a plausible explanation for the presence of isolates coresistant to fluoroquinolones and tetracycline in poultry. Recent studies of AMR *Campylobacter* from poultry in Australia, where fluoroquinolones are also not registered for use, have suggested that bacteria resistant to these critically important antimicrobials were likely introduced via reverse zoonosis (human-animal transmission) or transmission from wild birds or pest animals (Abraham et al., 2020).

Overall, results from this study suggest that the levels of resistant *E. coli*, enterococci, and *Campylobacter* isolates from food animals were equal to or lower than observed in comparable studies of food animals in Australia, Denmark, and the EU, with a few notable exceptions such as erythromycin-, streptomycin-, and tetracycline-resistant enterococci in pigs, very young calves, and dairy cattle; tetracycline-resistant *E. coli* in pigs; and ampicillin-, streptomycin-, and tetracycline-resistant *E. coli* in very young calves. High levels of bacitracin-resistant enterococci were observed in all food animals. Testing for this antimicrobial was not included in comparator studies of food animals in Denmark or Australia. Resistance in *Salmonella* isolates was not observed, although the low numbers of isolates available limit what conclusions can be drawn. While resistance decreased for several antimicrobials between 2009 and 2010 and the current study, there are examples where a significant increase in resistance was observed, and examples where resistance has remained high, very high, or extremely high.

These observations, together with international data showing similar trends, highlight the importance of continued monitoring of resistance in bacteria from food animals. Ideally, future work would include regular monitoring of AMR in food animals. These observations, together with international data showing similar trends, highlight the importance of continued monitoring of resistance in bacteria from food animals. Despite the potential limitations that point prevalence surveys have in comparison to ongoing surveillance programs, the methodology used in this study has allowed for contemporary data on AMR in NZ food animals to be compared to a 2009–2010 baseline study and compared with contemporary international data. Future work in this area should include regular monitoring of AMR in NZ food animals to ensure timely detection of AMR with potential impacts on animal and human health. More regular monitoring and reporting of AMR in NZ food animals could also enable more robust trend analyses to be performed. For example, limited conclusions could be drawn in this study regarding seasonal differences in AMR prevalence when comparing contemporary results to AMR data collected during the 2009–2010 baseline study. Future surveillance activities could also be expanded to include other food animals (e.g., sheep, deer) to confirm the perceived low prevalence is accurate, and current phenotypic surveillance could be complemented with targeted genomic characterization to better understand how resistance is emerging and has been transmitted in bacteria from food animals.

As well as expanding the monitoring and reporting of AMR in food animals, future surveillance activities should consider antimicrobial use in each animal species as a potential driver of resistance. Currently, MPI monitors and reports on sales of agricultural compounds containing antimicrobials for use in animals but does not track when and how antimicrobials are used (Agricultural Compounds and Veterinary Medicines team, 2023). Due to the multispecies licensing of medicines, the sales analysis reports generated by MPI do not provide information on the amounts of antimicrobials sold specifically for use in the individual animal species included in this survey, instead only aggregated sales data are provided (Agricultural Compounds and Veterinary Medicines team, 2023). MPI also recognizes that sales analysis reports do not fully account for the stockpiling of antimicrobials in anticipation of later use, veterinarians authorizing the use of human preparations, importing antimicrobials if a suitable registered veterinary medicine is not available in NZ, or using their professional discretion to employ antimicrobials for “off-label” uses (Agricultural Compounds and Veterinary Medicines team, 2023). Comprehensive, coordinated, and effective surveillance of antimicrobial use and AMR in NZ food animals should continue to include input from food animal industries and regulators, and encourage research into, and implementation of, effective strategies to prevent and control future resistance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was funded by the New Zealand Ministry for Primary Industries. The authors wish to thank the processors and Recognised Laboratory Programme laboratories for providing samples for this project. The staff of the ESR Christchurch Microbiology and Molecular Biology Laboratories and the Antibiotic Reference Laboratory are thanked for their dedication to quality in carrying out the laboratory analyses associated with the project. We also thank the staff of the ESR Enteric Reference Laboratory for supplying the *Salmonella* isolates.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2024.100245.

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