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Prevalence and antimicrobial resistance of Salmonella and Escherichia coli from Australian cattle populations at slaughter.

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Abstract

Antimicrobial agents are used in cattle production systems for the prevention and control of bacterial associated diseases. Australia is the world's third largest exporter of beef; however it does not have an ongoing surveillance system for antimicrobial resistance (AMR) in cattle or foods derived from these animals. This study examined 910 beef cattle, 290 dairy cattle and 300 veal calf faecal samples collected at slaughter for the presence of E. coli and Salmonella and determined the phenotypic AMR of 800 E. coli and 217 Salmonella. The results of AMR testing demonstrate a low level of AMR. Infrequent detection of multi-drug resistance (MDR) in Salmonella from beef cattle did occur, however the resistances observed were to antimicrobials of low importance to human medicine. Although some differences in AMR between animal groups was observed, there is minimal evidence that specific production practices are responsible for disproportionate contributions to AMR development and in general resistance to antimicrobials of critical and high importance in human medicine was low regardless of the isolate source. The low level of antimicrobial resistance in bacteria from Australian cattle is likely a result of strict regulation of antimicrobials in food animals in Australia and animal management systems that do not favour bacterial disease.

Executive Summary

Antimicrobial agents are used in cattle production systems for the prevention and control of bacterial associated diseases. A consequence of the use of antimicrobials is the potential for antimicrobial resistance (AMR) to develop in bacteria, including zoonotic pathogens which can be transferred to the human population via the food chain or by direct exposure to animals. Novel resistance phenotypes continue to emerge in zoonotic foodborne pathogens and commensal bacteria isolated from food production animals. Consequently, understanding, assessing and mitigating the risks of non-human use of antimicrobials on human health outcomes remains a high priority. The World Health Organisation (WHO) has developed and maintains criteria and ranks antimicrobials based on their importance to human medicine. These lists can be used by regulators and stakeholders to develop risk management strategies for the use of antimicrobials in food animal production systems.

The aim of this study was to determine the prevalence and phenotypic AMR status of Salmonella and E. coli isolates from Australian cattle populations.

Salmonella was isolated from 105 (11.5%) beef cattle, 75 (25.9%) dairy cattle and 36 (12.0%) veal calf faecal samples for an overall prevalence in Australian cattle of 14.4%
Attempts were made to isolate E. coli from samples that had concentrations of E. coli >1.00 $\log_{10}$ CFU/g with E. coli recovered from 1385 (92.3%) of all samples.

All 217 distinct Salmonella isolates were submitted for AMR analysis. When all isolates are considered the rates of resistance were low with resistance to any one antimicrobial not exceeding 3.7%. The majority (91.5%) of beef cattle isolates and all veal calf and dairy cattle isolates remained susceptible to all antimicrobials except florfenicol.

A total of 800 *E. coli* isolates were randomly selected from a pool of 1385 isolates and submitted for AMR analysis. The group comprised *E. coli* from 469 beef cattle, 155 dairy cattle and 176 veal calves. AMR was generally low across the three animal groups with 92.1%, 96.8% and 93.2% of *E. coli* from beef cattle, dairy cattle and veal calves susceptible to all antimicrobials tested.

This study has determined the AMR status of Salmonella and E. coli isolates from Australian cattle populations. Overall, the results corroborate previous Australian based animal and retail food surveys that have shown a low level of AMR, relatively small proportions of MDR and most importantly the maintenance of susceptibility to most antimicrobials of critical and high importance to human health. It must also be noted that this study investigated isolates collected from cattle faeces and not from beef primal or boxes of boneless beef entering the food chain. The transfer of AMR E. coli and Salmonella to humans via the food chain would be dependent on these organisms being regularly present in beef products entering commerce. Importantly, it would appear that the production practices adopted in the Australian cattle industry are not generating pools of resistance that are likely to result in the inability to treat human infections caused by Salmonella and E. coli. Similarly, although some differences in AMR levels were noted between production systems, there is minimal evidence that specific production practices are responsible for disproportionate contributions to AMR development. Furthermore, comparisons with AMR data from the EU and USA shed a favourable light on Australia's ability to meet any proposed regulations relating to the presence of MDR bacteria in exported beef products. Nevertheless, it is necessary to maintain strict guidelines and controls around the use of antimicrobials in food-production animals in Australia and monitoring the effects of all antimicrobial use is required to support Australia's reputation as a supplier of safe and healthy food

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1 Background

Antimicrobial agents are used in cattle production systems for the prevention and control of bacterial associated diseases. A consequence of the use of antimicrobials is the potential for antimicrobial resistance (AMR) to develop in bacteria, including zoonotic pathogens which can be transferred to the human population via the food chain or by direct exposure to animals (8, 16). Novel resistance phenotypes continue to emerge in zoonotic foodborne pathogens and commensal bacteria isolated from food production animals (23, 25). Consequently, understanding, assessing and mitigating the risks of non-human use of antimicrobials on human health outcomes remains a high priority. The World Health Organisation (WHO) has developed and maintains criteria and ranks antimicrobials based on their importance to human medicine (28). These lists can be used by regulators and stakeholders to develop risk management strategies for the use of antimicrobials in food animal production systems (9).

Australia is one of the world's most efficient producers of cattle and third largest exporter of beef, exporting 67% of its total beef and veal production in 2012-13 (19). Australia has taken a conservative approach to the registration of antimicrobials for use in food-producing animals. Antimicrobials that are highly valued in human clinical medicine such as fluoroquinolones and gentamicin have never been registered for use in food-producing animals and only one 3rd or 4th generation cephalosporin (ceftiofur) has been registered (4). There is currently no ongoing surveillance for AMR in Australia although there have been attempts to assess the AMR status of bacteria of food animal origin (11, 13). Isolates of Salmonella and E. coli from cattle at slaughter and/or in retail beef products demonstrated that phenotypic resistance to the antimicrobials tested was generally low. More specifically the resistances observed were to antimicrobials of lesser importance to human medicine (11). Similarly, genotypic investigations of AMR determined that resistance to fluoroquinolones or third-generation cephalosproins was absent in Salmonella from Australian cattle populations (1). Furthermore, the presence of class 1 and class 2 integrons was not correlated with specific production practices such as feed-lotting and the gene cassettes harboured by the integrons mostly encoded resistance to antimicrobials not considered to be of critical or high importance to human medicine (2, 3).

In contrast to Australia, a number of countries do have established AMR surveillance programs in place. Multi-focus surveillance programs enable trends in AMR development to be further evaluated with respect to production systems, animal type and clinical use and are particularly useful in addressing concerns about the overall impact of antimicrobial use. Indeed, countries such as the United States through their NARMS program could evaluate the impact that a petition aimed at declaring specific strains of AMR Salmonella as adulterants in beef and poultry products might have (6). Countries that do not have ongoing surveillance programs in place instead rely on relatively short-term intensive surveys to evaluate the prevalence and AMR status of bacteria from an animal type, production practice or as a result of clinical use.

2 Projective objectives

The aim of this study was to determine the prevalence and phenotypic AMR status of Salmonella and E. coli isolates from Australian cattle populations.

3 Methodology

3.1 Sample collection

Australian cattle being processed to supply the export beef market can be classified into three animal groups: beef cattle, dairy cattle, and veal calves. A total of 31 abattoirs representing >85% of total beef exports agreed to participate in the survey. The number of cattle to be sampled at each abattoir was stratified based on animal group and slaughter volumes. Sample collection targets of 900, 300 and 300 were established for beef cattle, dairy cattle and veal calves, respectively. Samples were collected across two sampling windows with sample numbers collected from each participating abattoir ranging from 8-80 (mean 24) per sampling window. Systematic random sampling was used to collect the samples across a consecutive two day period in each of the sampling windows. A sampling day consisted of eight hours of production with each abattoir expected to sample evenly across the day. Abattoirs were expected to collect up to a maximum of 40 samples per sampling day therefore all samples were expected to be collected a minimum of 12 minutes apart. Each sampling window occurred over an eight week period with the first window occurring in February and March, 2013 and the second sampling window occurring in August and September, 2013. Faecal samples were collected post-evisceration by cutting the intestine 15-30 cm from the rectal end and expressing at least 40 g of material into a sterile jar. Samples were kept chilled and returned to the laboratory by overnight courier for processing. Participating abattoirs were asked to provide details on product type (beef, dairy, veal) and feed type. For the purpose of assessing the effect of feed type on the prevalence of resistance, animals listed as dairy or veal were assigned to individual groups while beef cattle were divided into grass or grain-fed groups.

3.2 Salmonella isolation

Faecal slurries prepared by diluting 10 g of faeces 1 in 10 with buffered peptone water (BPW; Oxoid, UK) and homogenising for 1 min were then incubated at 42±1°C for 6 h and subsequently tested for the presence of Salmonella using automated immunomagnetic separation (AIMS) with Dynabeads anti-Salmonella (Invitrogen, Norway) following the manufacturer's instructions. Following AIMS, Dynabeads were inoculated into 10 mL of Rappaport-Vassiliadis soy broths (RVS; BioMerieux, France) and incubated for 20 h at 42±1°C. A loopful of RVS broth was plated onto brilliant green agar (BGA; Oxoid) and xylose lysine desoxycholate (XLD; BioMerieux) agar and incubated at 37±1°C for 24 h. Following incubation, plates were examined for the presence of Salmonella using the Salmonella latex agglutination test kit (Oxoid). Colonies that agglutinated with the latex agglutination test kit were plated onto 5% sheep blood agar (SBA; BioMerieux) and confirmed as Salmonella by invA PCR (7) and biochemical tests (Microbact 24E; Oxoid). Up to two confirmed Salmonella isolates were stored at -80°C using Microbank (Pro-Lab Diagnostics, USA). A multiplex PCR-based method capable of identifying and discriminating common clinical serovars of Salmonella was used to determine the identity of Salmonella serovars (18). Conventional

serotyping of isolates not identified using the molecular serotyping approach was conducted by Queensland Health (Brisbane, Australia).

3.3 E. coli isolation

E. coli were isolated by plating 1 mL of serial dilutions of the unenriched faecal slurries onto Petrifilm *E. coli*/coliform count plates (3M; St. Paul, Minnesota, USA). Presumptive *E. coli* were recovered by plating representative colonies onto eosin methylene blue agar (EMB; Oxoid) and incubating at 37 ± 2°C for 18 h. Colonies displaying the typical metallic green sheen were subsequently plated onto 5% sheep blood agar (SBA; BioMerieux, France) and incubated at 37 ± 2°C for 18 h. The resulting isolates were confirmed as *E. coli* using the Microbact 12E or 24E system (Oxoid) and stored at -80°C using MicroBank (Pro-Lab Diagnostics, USA).

3.4 Phenotypic detection of antimicrobial resistance

The antimicrobial resistance phenotype of isolates was determined using the broth microdilution method and the Sensititre apparatus. Custom susceptibility panels for *E. coli* and *Salmonella* (AUSVN2; TREK Diagnostic Systems, UK) were used to test all isolates. The dilution ranges and breakpoints for each antimicrobial are shown in Table1. Interpretation of the MIC values was based on CLSI interpretive criteria when available; otherwise EUCAST and NARMS values were used. The breakpoint listed for florfenicol is the susceptible breakpoint. Isolates that exceeded the MIC value of the susceptible breakpoint were reported as non-susceptible. *Salmonella* Typhimurium ATCC 14028 and *Escherichia coli* ATCC 25922 were used as the control strains.

3.5 Univariate analyses

Simple univariate analysis was performed to assess the effect of various factors on the prevalence of *Salmonella* and *E. coli* in cattle at slaughter. Selected univariate analysis based on comparison of proportions were performed to match biologically plausible hypotheses with significance assessed using Fisher's Exact Test. A similar approach confined to beef cattle was used to assess simple relationships between "feed type", "sampling window" and detection of *Salmonella*. For the assessment of statistical variation in the prevalence of resistance phenotypes exact binomial confidence intervals were derived.

3.6 Salmonella multivariate analyses

The analysis for *Salmonella* prevalence was then extended to a multivariate analysis to account for the potential bias arising from confounding and interaction as well as over dispersion due to the cluster-based method of sampling. A generalised linear model (GLM) approach for binomial data was applied. One GLM model was used to estimate how the prevalence of *Salmonella* was influenced by the factors "class of animal" and "sampling window" including the effects of all of their possible interactions. The output was used to produce a table of the mean effects of the combination of each level of "class of animal" and "sample window" with corresponding 95% confidence intervals. A second GLM model was

similarly used to assess the effect of “feed type” and “sampling window” on *Salmonella* detection in beef cattle. All analysis was performed in Stata 13.1 (Stata Corporation, College Station, TX, USA)

4 Results

4.1 Prevalence and identity

In total, 1500 faecal samples comprising 910 beef cattle faeces, 290 dairy cattle faeces and 300 veal calf faeces were tested for the presence of Salmonella and E. coli.

4.2 Salmonella

Salmonella was isolated from 105 (11.5%) beef cattle, 75 (25.9%) dairy cattle and 36 (12.0%) veal calf faecal samples for an overall prevalence in Australian cattle of 14.4%. Of the 31 abattoirs participating in the survey, 29 provided samples in sampling window 1 and 30 provided samples in sampling window 2. Salmonella was isolated from 25 of 29 (86.2%) abattoirs in sampling window 1 and 19 of 30 (63.3%) in sampling window 2. The predicted mean effects of ‘class of animal’ and ‘sampling window’ on the prevalence of Salmonella is shown in Table 2. Most notably, the prevalence of Salmonella in dairy cattle samples is substantially greater than other classes when the effect of ‘sampling window’ and various interactions are accounted for. In addition, the prevalence of Salmonella in veal calf samples in sampling window 2 is high. The overall prevalence of Salmonella in grain-fed beef cattle samples (9.6%) was lower than grass-fed beef cattle samples (13.0%). Interestingly, grain-fed beef cattle samples from sampling window 1 were three times more likely to yield Salmonella than grain-fed beef cattle samples from sampling window 2. A similar relationship was observed between grain-fed beef cattle samples and grass-fed beef cattle samples in sampling window 2.

Serotyping using the multiplex PCR approach determined the identity of Salmonella in 161 of 216 (74.5%) samples. The identity of the remaining isolates was determined using conventionally serotyping. With the exception of one beef cattle sample harbouring both Saintpaul and Chester, all samples contained a single Salmonella serovar. A total of 37 different serovars were identified across the three animal groups. The distribution of Salmonella serovars for each animal group is shown in Figure 1. The most frequently detected serovar for each animal group was Typhimurium comprising between 28% and 45% of all isolates. The next most prevalent serovars were Anatum (13%) in beef cattle, Bovismorbificans (9%) in dairy cattle and Saintpaul (11%) and Infantis (11%) in veal calves. The serovar Newport was found in one (1.3%) dairy cattle sample and similarly Heidelberg was found in just one (2.8%) veal calf faecal sample. The serovar Hadar was not recovered from any animal group.

4.3 E. coli

Attempts were made to isolate E. coli from samples that had concentrations of E. coli >1.00 $\log_{10}$ CFU/g with E. coli recovered from 1385 (92.3%) of all samples. Veal samples were most likely to yield E. coli with isolates recovered from 294 (98.0%) of 300 samples. E. coli was recovered from 93.0% of dairy cattle samples and 90.2% of beef cattle samples. When the effect of sampling window or feed type were considered there was no significant difference in E. coli recovery rate for sampling window. However, grain-fed beef cattle were

significantly ($p < 0.05$) more likely to yield E. coli from samples with E. coli concentrations >1.00 log10 CFU/g than grass-fed cattle.

4.4 Salmonella antimicrobial susceptibility testing

All 217 distinct Salmonella isolates were submitted for AMR analysis. The distribution of minimum inhibitory concentrations (MICs) for each antimicrobial and animal group is shown in Table 3. When all isolates are considered the rates of resistance were low with resistance to any one antimicrobial not exceeding 3.7%. The majority (91.5%) of beef cattle isolates and all veal calf and dairy cattle isolates remained susceptible to all antimicrobials except florfenicol. Non-susceptibility to florfenicol was observed in 29.2%, 34.7% and 38.9% of isolates from beef cattle, dairy cattle and veal calves, respectively. Resistance to streptomycin (7.5%), ampicillin (7.5%), trimethoprim / sulfamethoxazole (7.5%) and tetracycline (6.6%) were the most common resistances identified in beef cattle isolates. Resistance to cephalosporins and fluorquinolones was not observed in any beef cattle, dairy cattle or veal calf isolates. Multiple resistance (MDR) to three or more classes of antimicrobials was only observed in beef cattle isolates and represented a total of eight (7.5%) of 106 beef cattle isolates. The antimicrobial resistance patterns observed are outlined in Table 5. The antimicrobial resistance pattern AMP-STR-TET-SXT accounted for six of the MDR isolates and was only found in Salmonella isolated from beef cattle which were grain-fed, three of which were the serovar Typhimurium. The remaining MDR isolates included a grain-fed beef cattle isolate with an antimicrobial resistance pattern of AMP-TET-SXT and one MDR grass-fed beef cattle isolate with an antimicrobial resistance pattern of AMP-STR-SXT.

4.5 E. coli antimicrobial susceptibility testing

A total of 800 *E. coli* isolates were randomly selected from a pool of 1385 isolates and submitted for AMR analysis. The group comprised *E. coli* from 469 beef cattle, 155 dairy cattle and 176 veal calves. The distribution of MICs for each antimicrobial and animal group is shown in Table 4. AMR was generally low across the three animal groups with 92.1%, 96.8% and 93.2% of *E. coli* from beef cattle, dairy cattle and veal calves susceptible to all antimicrobials tested. Non-susceptibility to florfenicol was observed in 55.4%, 58.7% and 59.7% of beef cattle, dairy cattle and veal calf isolates, respectively. With the exception of tetracycline in beef cattle *E. coli*, resistance to any one antimicrobial did not exceed 5.0%. Tetracycline resistance was present in 48 (6.0%) of all *E. coli* tested but was significantly ($p < 0.05$) more likely to be present in *E. coli* from grain-fed cattle (15.0%) than any other animal group (2.6 – 4.6%). Resistance of *E. coli* to fluoroquinolones was not observed in any animal group and resistance to 3rd and 4th generation cephalosporins was not present in isolates from grass- or grain-fed beef cattle and dairy cattle. Resistance to amoxicillin / clavulanic acid (1.1%), kanamycin (1.1%), gentamicin (0.6%) and ceftiofur (0.6%) although infrequent, were only observed in *E. coli* from veal calves. Resistance to three or more antimicrobials was not observed in any beef cattle *E. coli* but was present in two (1.3%) and seven (4.0%) dairy cattle and veal calf *E. coli*, respectively. TET alone was the most common antimicrobial resistance pattern identified with STR-TET the only other resistance pattern present in more than two isolates (Table 5).

5 Discussion

Zoonotic bacteria that are resistant to antimicrobials are of increased concern to public health officials throughout the world as they may compromise the ability of various treatment regimes to address disease and infection in human medical settings. Knowledge and understanding of the types of AMR present in food production animals is key to determining the ongoing risk that AMR bacteria pose to human health. Australia currently does not have a nationally coordinated program for the ongoing surveillance and analysis of AMR bacteria in animals, bacteria in food derived from animals, or bacteria from humans. Consequently it relies heavily on routine testing of human and animal clinical isolates as well as infrequent surveys of isolates from animals or from food of animal origin to understand AMR development and trends. Australia most recently conducted surveys for AMR in bacteria of animal origin and in retail foods in 2003/4 and 2007/8. Both studies concluded that resistance to critically and highly important antimicrobials such as 3rd and 4th generation cephalosporins as well as fluoroquinolones was non-existent or very low regardless of animal, food or bacterial type (11). The study detailed here was conducted as an adjunct to a broader survey of microorganisms in Australian cattle populations and therefore solely focuses on isolates from cattle at slaughter. Nevertheless, the large volume of isolates being analysed ensures it provides a comprehensive snapshot assessment of AMR in Australian cattle.

Despite the potential limitations that point prevalence surveys have in comparison to ongoing surveillance programs, the methodology used in this study does allow for the results to be placed in a global context and contrasted with international data. Of prime importance to this study is the prevalence of AMR in Salmonella from Australian cattle populations. This importance is in response to the petition submitted to USDA that requests that specific serovars of MDR Salmonella be classified as adulterants of raw, non-intact beef products (6). Salmonella were isolated from 14.4% of samples which represents a substantial increase from a previous Australian cattle survey that detected Salmonella in 6.8% of samples (14). Importantly, this survey included dairy cattle faecal samples and these were shown to have a substantially greater prevalence of Salmonella than beef cattle or veal calf samples. However, even after taking into account this difference between the surveys, the prevalence of Salmonella in beef cattle samples in this survey was 11.5% and remains higher than previously estimated.

The prevalence of resistance in Salmonella to any of the antimicrobials tested in this study is low with 91.5% of beef cattle isolates and all dairy cattle and veal calf isolates susceptible to all antimicrobials tested. Resistance to streptomycin, ampicillin, chloramphenicol and tetracycline were well below the rates observed in the European Union (EU) and the USA (NARMS). Resistance to the abovementioned antimicrobials in any animal group did not exceed 7.5% in this study compared with >25% resistance in the 2010 NARMS study and 29.1% and 31.1% for ampicillin and tetracycline, respectively in the EU study (12, 24). Resistance to the cephems and fluorquinolones was absent from all Salmonella isolates tested in this study. Globally, cephem resistance varies substantially between the EU where resistance levels are very low and the USA where resistance levels have steadily increased during the last decade to exceed 20% across all Salmonella in 2010 (12, 24). MDR Salmonella were most likely to be recovered from grain-fed beef cattle, however the resistances observed are to antimicrobials that would not be considered of critical or high

importance to human medicine. Nonetheless, presentation of a group of cattle for slaughter with MDR Salmonella, particularly in the serovar Typhimurium is undesirable and exerts additional pressure on existing hygiene controls to maintain a safe food supply.

The monitoring of AMR in E. coli is a common component of all surveillance programs as E. coli have been shown to routinely act as reservoirs of resistance genes that can then spread horizontally to other bacteria. Previous Australian surveys that have investigated phenotypic and genotypic AMR in E. coli from cattle have all indicated that resistance to all antimicrobial classes is low and in particular resistance to antimicrobials of critical and high importance is generally absent (2, 11). The pattern of low levels of resistance in E. coli has continued in this survey with >92% of isolates remaining susceptible to all antimicrobials tested regardless of animal class. E. coli that did exhibit AMR were most likely to do so to older antimicrobials such as tetracycline, streptomycin, ampicillin and trimethoprim / sulfamethoxazole. Additionally, tetracycline resistance was significantly more likely to be associated with grain-fed cattle than grass-fed cattle, dairy cattle or veal calves and may be a result of specific production practices employed during feed-lotting of animals. Similar observations around AMR to older antimicrobials, albeit at increased frequencies, have been made in E. coli from cattle in EU member states (12, 26). NARMS does not perform susceptibility testing on E. coli isolates from live cattle; however the levels of AMR present in E. coli collected from dairy cattle during this study contrast heavily with a retrospective analysis of 3373 US dairy cattle E. coli isolates collected between 2004 and 2011 where 71% of isolates were resistant to two or more antimicrobials (10). Resistance to antimicrobials of critical and high importance to human medicine such as amoxicillin / clavulanic acid, gentamicin and ceftiofur although infrequent, were only observed in E. coli from veal calves. Some member states of the EU have reported increased AMR in isolates from younger animals, mainly fattening calves, compared to older animals (12). Whist similar observations have been made in North American studies (15) it has been suggested that the prevalence of AMR E. coli in calves may not be a function of antimicrobial use and instead related to AMR neonate-adapted bacteria (17). There is no evidence for the persistence of neonate-adapted AMR E. coli in veal calf populations in Australia and in general, resistance in E. coli does not appear to be linked to the age of the animal or the production system from which the isolate was obtained.

Non-susceptibility of Salmonella and E. coli to florfenicol was a notable feature of this study. Florfenicol is a broad-spectrum antimicrobial approved for use in Australian cattle to treat a range of infections including bovine respiratory disease. A range of mechanisms for florfenicol resistance have been identified and they have been shown to be associated with MICs greater than 16 µg/mL (20, 22, 27). EUCAST support this observation and the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) have used an epidemiological cut-off (ECOFF) value of >16 µg/mL for the purposes of conducting proficiency testing (5). The MICs observed for E. coli and Salmonella in this study do not exceed 16 µg/mL and remain consistent with the wild-type populations on which EUCAST based their ECOFF value. When taken together it would indicate that the results of this study are not suggestive of widespread acquisition of florfenicol resistance genes as a result of industry practices.

6 Conclusions/recommendations

This study has determined the AMR status of Salmonella and E. coli isolates from Australian cattle populations. Overall, the results corroborate previous Australian based animal and retail food surveys that have shown a low level of AMR, relatively small proportions of MDR and most importantly the maintenance of susceptibility to most antimicrobials of critical and high importance to human health. It must also be noted that this study investigated isolates collected from cattle faeces and not from beef primal or boxes of boneless beef entering the food chain. The transfer of AMR E. coli and Salmonella to humans via the food chain would be dependent on these organisms being regularly present in beef products entering commerce. A recent survey of the microbiological quality of Australian beef determined that E. coli was not identified in 97.9% of 1,165 boneless beef samples and Salmonella could not be recovered from 1,144 primal cut samples or 1,165 boneless beef samples (21). Importantly, it would appear that the production practices adopted in the Australian cattle industry are not generating pools of resistance that are likely to result in the inability to treat human infections caused by Salmonella and E. coli. Similarly, although some differences in AMR levels were noted between production systems, there is minimal evidence that specific production practices are responsible for disproportionate contributions to AMR development. Furthermore, comparisons with AMR data from the EU and USA shed a favourable light on Australia's ability to meet any proposed regulations relating to the presence of MDR bacteria in exported beef products. Nevertheless, it is necessary to maintain strict guidelines and controls around the use of antimicrobials in food-production animals in Australia and monitoring the effects of all antimicrobial use is required to support Australia's reputation as a supplier of safe and healthy food.

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Table 1. Dilution ranges and breakpoints for antimicrobial susceptibility testing

Antimicrobial	*E. coli* & *Salmonella*	
	Range	**Breakpoint**
Amoxicillin / clavulanic acid	1/0.5 - 32/16	≥32/16
Ampicillin	2-64	≥32
Cefazolin	2-16	≥8
Cefotaxime	0.032-8	≥4
Cefoxitin	0.5–32	≥32
Ceftiofur	0.5–16	≥8
Ceftriaxone	0.125-4	≥4
Chloramphenicol	2-32	≥32
Ciprofloxacin	0.0625-4	≥1
Florfenicol	2-64	
Gentamicin	0.5-16	≥16
Kanamycin	8-64	≥64
Meropenem	0.0625-0.5	8
Nalidixic Acid	1-32	≥32
Streptomycin	16-64	≥64
Tetracycline	2-16	≥16
Trimethoprim / sulfamethoxazole	0.12/2.38 - 4/76	≥4/76

Table 2. *Salmonella* prevalence in Australian cattle groups across sampling windows

Group	Window 1 samples (n=)	*Salmonella* +ve	95% CI	Window 2 samples (n=)	*Salmonella* +ve	95% CI	Total
Beef cattle	469	59 (12.6)[a]	8.1 – 17.0	441	46 (10.4)	3.8 – 17.1	105 (11.5)
Dairy cattle	146	42 (28.8)	6.5 – 51.0	144	33 (22.9)	15.4 – 30.4	75 (25.9)
Veal calves	138	7 (5.1)	0.0 – 10.1	162	29 (17.9)	11.8 – 24.0	36 (12.0)
Total	753	108 (14.3)		747	108 (14.5)		216 (14.4)

[a] figures in parentheses are percent

Table 3. Distribution of MICs and occurrence of resistance among *Salmonella* isolates from beef cattle, dairy cattle and veal calf faecal samples

Class	Antimicrobial	Group	N =	% Resistant	95% CI	Antimicrobial concentration (µg/ml)[b]												
						0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Amino-glycosides	Gentamicin	Beef	106	0.0	0.00 - 3.42					52.8	45.3	1.9						
		Dairy	75	0.0	0.00 - 4.80					70.7	29.3							
		Veal	36	0.0	0.00 - 9.74					72.2	25.0	2.8						
	Kanamycin	Beef	106	0.0	0.00 - 3.42									96.2	3.8			
		Dairy	75	0.0	0.00 - 4.80									100.0				
		Veal	36	0.0	0.00 - 9.74									100.0				
	Streptomycin	Beef	106	7.5	3.31 - 14.33										86.8	5.7	1.9	5.7
		Dairy	75	0.0	0.00 - 4.80										92.0	8.0		
		Veal	36	0.0	0.00 - 9.74										91.7	8.3		
b-lactam/b-lactamase inhibitor combinations	Amoxicillin / Clavulanic acid	Beef	106	0.0	0.00 - 3.42						85.8	6.6	3.8	3.8				
		Dairy	75	0.0	0.00 - 4.80						97.3	2.7						
		Veal	36	0.0	0.00 - 9.74						94.4	5.6						
Carbapenem	Meropenem	Beef	106	0.0	0.00 - 3.42		97.2	0.9	0.9		0.9							
		Dairy	75	0.0	0.00 - 4.80		97.3	1.3			1.3							
		Veal	36	0.0	0.00 - 9.74		100.0											
Cephems	Cefazolin	Beef	106	0.0	0.00 - 3.42							92.5	7.5					
		Dairy	75	0.0	0.00 - 4.80							97.3	2.7					
		Veal	36	0.0	0.00 - 9.74							91.7	8.3					
	Cefotaxime	Beef	106	0.0	0.00 - 3.42		61.3	34.0	3.8	0.9								
		Dairy	75	0.0	0.00 - 4.80		60.0	37.3	2.7									
		Veal	36	0.0	0.00 - 9.74		77.8	16.7	2.8	2.8								
	Cefoxitin	Beef	106	0.0	0.00 - 3.42						3.8	61.3	30.2	3.8	0.9			
		Dairy	75	0.0	0.00 - 4.80							61.3	36.0	2.7				
		Veal	36	0.0	0.00 - 9.74						5.6	61.1	27.8	5.6				

	Ceftiofur	Beef	106	0.0	0.00 - 3.42					45.3	50.9	3.8						
		Dairy	75	0.0	0.00 - 4.80					56.0	44.0							
		Veal	36	0.0	0.00 - 9.74					58.3	41.7							
	Ceftriaxone	Beef	106	0.0	0.00 - 3.42		91.5	6.6			1.9							
		Dairy	75	0.0	0.00 - 4.80		96.0	2.7			1.3							
		Veal	36	0.0	0.00 - 9.74		97.2	2.8										
Folate pathway inhibitors	**Trimethoprim / Sulfamethoxazole**	Beef	106	7.5	3.31 - 14.33		84.9	3.8	1.9	0.9	0.9	7.5						
		Dairy	75	0.0	0.00 - 4.80		96.0	4.0										
		Veal	36	0.0	0.00 - 9.74		100.0											
Penicillins	**Ampicillin**	Beef	106	7.5	3.31 - 14.33						89.6	0.9	0.9	0.9		0.9	6.6	
		Dairy	75	0.0	0.00 - 4.80						100.0							
		Veal	36	0.0	0.00 - 9.74						100.0							
Phenicols	**Chloramphenicol**	Beef	106	0.0	0.00 - 3.42							12.3	85.8	1.9				
		Dairy	75	0.0	0.00 - 4.80							5.3	93.3	1.3				
		Veal	36	0.0	0.00 - 9.74						2.8	8.3	88.9					
	Florfenicol[a]	Beef	106	NA	NA							70.8	29.2					
		Dairy	75	NA	NA							65.3	34.7					
		Veal	36	NA	NA						2.8	58.3	38.9					
Quinolones	**Ciprofloxacin**	Beef	106	0.0	0.00 - 3.42	96.2	1.9	1.9										
		Dairy	75	0.0	0.00 - 4.80	97.3	1.3	1.3										
		Veal	36	0.0	0.00 - 9.74	100.0												
	Nalidixic Acid	Beef	106	0.0	0.00 - 3.42						6.6	85.8	7.5					
		Dairy	75	0.0	0.00 - 4.80						5.3	88.0	6.7					
		Veal	36	0.0	0.00 - 9.74						2.8	91.7	5.6					
Tetracyclines	**Tetracycline**	Beef	106	6.6	2.70 - 13.13						87.7	3.8	1.9	0.9	5.7			
		Dairy	75	0.0	0.00 - 4.80						98.7	1.3						
		Veal	36	0.0	0.00 - 9.74						100.0							

[a]Only a susceptible breakpoint (≤4µg/ml) has been established for florfenicol. Isolates with an MIC ≥8µg/ml are reported as non-susceptible
[b]Solid vertical lines indicate breakpoints for resistance. The white fields indicate the dilution range tested for each antimicrobial. Values in the shaded area indicate MIC values greater than the highest concentration tested.

Table 7. Distribution of MICs and occurrence of resistance among *E. coli* isolates from beef cattle, dairy cattle and veal calf faecal samples

Class	Antimicrobial	Group	N =	% Resistant	95% CI	Antimicrobial concentration (µg/ml)[b]												
						0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Amino-glycosides	Gentamicin	Beef	469	0.0	0.00 - 0.78					21.1	72.1	6.2	0.6					
		Dairy	155	0.0	0.00 - 2.35					29.0	60.0	10.3	0.6					
		Veal	176	0.6	0.01 - 3.12					23.9	68.8	5.1	1.1	0.6		0.6		
	Kanamycin	Beef	469	0.0	0.00 - 0.78									99.4	0.4	0.2		
		Dairy	155	0.0	0.00 - 2.35									98.1	1.9			
		Veal	176	1.1	0.14 - 4.04									97.2	1.1	0.6		1.1
	Streptomycin	Beef	469	1.1	0.35 - 2.47										97.9	1.1	0.2	0.9
		Dairy	155	1.9	0.40 - 5.55										98.1			1.9
		Veal	176	4.0	1.61 - 8.02										96.0		1.1	2.8
b-lactam/b-lactamase inhibitor combinations	Amoxicillin / Clavulanic acid	Beef	469	0.0	0.00 - 0.78						5.3	23.7	59.5	11.5				
		Dairy	155	0.0	0.00 - 2.35						7.7	17.4	56.8	16.8	1.3			
		Veal	176	1.1	0.14 - 4.04						2.3	16.5	69.3	9.7	1.1	0.6	0.6	
Carbapenem	Meropenem	Beef	469	0.0	0.00 - 0.78		99.6	0.2	0.2									
		Dairy	155	0.0	0.00 - 2.35		100.0											
		Veal	176	0.0	0.00 - 2.07		99.4	0.6										
Cephems	Cefazolin	Beef	469	0.2	.01 - 1.18							96.4	3.4		0.2			
		Dairy	155	0.0	0.00 - 2.35							96.8	3.2					
		Veal	176	1.7	0.35 - 4.90							93.8	4.5	0.6		1.1		
	Cefotaxime	Beef	469	0.0	0.00 - 0.78	26.2	63.8	9.4	0.4	0.2								
		Dairy	155	0.0	0.00 - 2.35	27.1	62.6	10.3										
		Veal	176	0.0	0.00 - 2.07	22.2	68.2	6.3	2.3	0.6		0.6						
	Cefoxitin	Beef	469	0.0	0.00 - 0.78						6.4	36.9	46.5	9.6	0.6			
		Dairy	155	0.0	0.00 - 2.35						5.8	43.2	44.5	6.5				
		Veal	176	0.0	0.00 - 2.07						1.7	41.5	47.7	8.5	0.6			
	Ceftiofur	Beef	469	0.0	0.00 - 0.78					99.1	0.9							
		Dairy	155	0.0	0.00 - 2.35					99.4	0.6							

		Veal	176	0.6	0.01 - 3.12					97.2	2.3			0.6					
		Beef	469	0.0	0.00 - 0.78			98.3	1.5		0.2								
	Ceftriaxone	Dairy	155	0.0	0.00 - 2.35			98.7	1.3										
		Veal	176	0.0	0.00 - 2.07			96.6	2.3	0.6	0.6								
Folate pathway inhibitors		Beef	469	0.2	0.01 - 1.18			97.7	1.1	0.9		0.2		0.2					
	Trimethoprim /Sulfamethoxazole	Dairy	155	1.3	0.16 - 4.58			98.1	0.0	0.6				1.3					
		Veal	176	2.3	0.62 - 5.72			95.5	1.1	0.6	0.6			2.3					
		Beef	469	0.0	0.00 - 0.78							37.7	56.1	5.8	0.4				
Penicillins	**Ampicillin**	Dairy	155	2.6	0.71 - 6.48							32.3	54.8	10.3		0.6			1.9
		Veal	176	4.5	1.98 - 8.76							34.7	57.4	2.3	1.1		0.6		4.0
		Beef	469	0.0	0.00 - 0.78							2.6	26.2	65.0	6.2				
	Chloramphenicol	Dairy	155	0.0	0.00 - 2.35							0.6	20.6	70.3	8.4				
Phenicols		Veal	176	0.0	0.00 - 2.07							2.8	22.2	72.2	2.8				
		Beef	469	NA	NA							5.5	39.0	51.6	3.8				
	Florfenicol[a]	Dairy	155	NA	NA								41.3	55.5	3.2				
		Veal	176	NA	NA							5.1	35.2	59.1	0.6				
		Beef	469	0.0	0.00 - 0.78		99.8		0.2										
	Ciprofloxacin	Dairy	155	0.0	0.00 - 2.35		100.0												
Quinolones		Veal	176	0.0	0.00 - 2.07		100.0												
		Beef	469	0.0	0.00 - 0.78						7.5	63.3	27.9	1.1	0.2				
	Nalidixic Acid	Dairy	155	0.0	0.00 - 2.35						6.5	66.5	25.8	1.3					
		Veal	176	0.0	0.00 - 2.07						8.0	69.3	22.2	0.6					
		Beef	469	7.7	5.43 - 10.47							83.8	8.1	0.4	0.9	6.8			
Tetracyclines	**Tetracycline**	Dairy	155	2.6	0.71 - 6.48							83.2	14.2			2.6			
		Veal	176	4.5	1.98 - 8.76							91.5	4.0			4.5			

[a]Only a susceptible breakpoint (≤4µg/ml) has been established for florfenicol. Isolates with an MIC ≥8µg/ml are reported as non-susceptible

[b]Solid vertical lines indicate breakpoints for resistance. The white fields indicate the dilution range tested for each antimicrobial. Values in the shaded area indicate MIC values greater than the highest concentration tested.

Table 5. Antimicrobial resistance patterns of *E. coli* and *Salmonella* from beef cattle, dairy cattle and veal calf faecal samples

Antimicrobial Resistance Patterns[a]	***E. coli***			***Salmonella***			
	Beef (N=469)	**Dairy (N=155)**	**Veal (N=176)**	**Beef (N=106)**	**Dairy (N=75)**	**Veal (N=36)**	**Major serovars present**
ALL SENSITIVE	432 (92.1)[b]	150 (96.8)	164 (93.2)	97 (91.5)	75 (100)	36 (100)	Typhimurium
AMP		1 (0.6)	1 (0.6)				
STR	1 (0.2)		1 (0.6)	1 (0.9)			Adelaide
TET	30 (6.4)		1 (0.6)				
AMP FAZ			1 (0.6)				
AMP TET		1 (0.6)					
FAZ TET	1 (0.2)						
STR TET	4 (0.9)	1 (0.6)	1 (0.6)				
TET SXT	1 (0.2)						
AMP STR TET			1 (0.6)				
AMP STR SXT				1 (0.9)			Meleagridis
AMP TET SXT				1 (0.9)			Dublin
AUG2 AMP FAZ			1 (0.6)				
AMP STR TET SXT		2 (1.3)	1 (0.6)	6 (5.7)			Typhimurium (3), Orion (2), Anatum (1)
GEN STR TET SXT			1 (0.6)				
AMP KAN STR TET SXT			2 (1.1)				
AUG2 AMP FAZ XNL TET			1 (0.6)				

[a] AMP – ampicillin , STR – streptomycin, TET – tetracycline, FAZ – cefazolin, SXT – trimethoprim / sulfamethoxazole, AUG2 – amoxicillin / clavulanic acid, GEN – gentamicin, KAN – kanamycin, XNL – ceftiofur

[b] Figures in parentheses are percent

Figure 1.

Distribution of *Salmonella* serovars in beef cattle (white columns), dairy cattle (black columns) and veal calf (grey columns) faecal samples.