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Antimicrobial resistance in commensal bacteria in bovine faeces at slaughter

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Abstract

Australia does not have a national surveillance program for the monitoring of AMR in the animal sector through ongoing surveillance but instead conducts periodic assessments of AMR. In this study 1001 faecal samples were collected from healthy beef cattle (n=591), dairy cattle (n=194) and veal calves (n=216). Attempts were made to isolate *E. coli*, *Salmonella* and the *Enterococcus* species *faecium*, *faecalis* and *hirae* which were then assessed for their response to antimicrobials. Epidemiological cut off (ECOFF) values were used to distinguish wild-type (WT) non-wild type (NWT) populations. The study determined that 94.0% of *Salmonella*, 83.8% of *E. coli* and 75.8% of *Enterococcus* isolates were WT for all antimicrobials tested. However, small numbers of isolates were deemed NWT for highly or critically important antimicrobials such as 3rd generation cephalosporins, quinolones and oxazolidinones. The outcomes of the study permit the Australian beef industry to arrive at the same conclusion as the previous MLA funded 2013 study. That is, populations of NWT isolates to antimicrobials considered highly or critically important to human medicine are low and there is limited evidence of specific production practices, such as grain-feeding, leading to widespread disproportionate development of NWT isolates.

Executive summary

Antimicrobials have been utilised by the Australian cattle industry to prevent infection and treat disease of animals since the 1950s. Comparatively speaking, the use of antimicrobials in Australia has been conservative with respect to other countries, with restrictions in place for those antimicrobials deemed critically important for human health. Nevertheless, the development of antimicrobial resistance is an unintended consequence of antimicrobial use with the potential for resistance to impact human health a key concern. Australia does not have a national surveillance program for the monitoring of AMR in the animal sector through ongoing surveillance but instead conducts periodic assessments of AMR. Prior to this study, the most recent and largest study of healthy cattle at slaughter occurred in 2013 when 1500 beef and dairy cattle and veal calves were analysed for AMR. The study corroborated previous AMR evaluations with low levels of AMR observed, relatively low levels of multi-drug resistance (MDR), and most importantly ongoing susceptibility to antimicrobials of critical and high importance to human health.

In this study 1001 faecal samples were collected from a total of 25 beef and veal processing establishments located across six Australian states and representing ~77% of total Australian beef exports. Samples were collected from healthy beef cattle (n=591), dairy cattle (n=194) and veal calves (n=216) with analysis of beef cattle samples determining that 235 (39.8%) were from feedlot cattle, 71 (12.0%) were grain-assisted, grass-fed cattle and 285 (48.2%) were grass-fed cattle. Attempts were made to isolate *E. coli*, *Salmonella* and the *Enterococcus* species *faecium*, *faecalis* and *hirae* and these were then assessed for their response to antimicrobials. Epidemiological cut off (ECOFF) values were used to distinguish wild-type (WT) non-wild type (NWT) populations. The overall outcomes of this study reinforce the findings of the 2013 study with 94.0% of *Salmonella*, 83.8% of *E. coli* and 75.8% of *Enterococcus* isolates considered WT for all antimicrobials tested. Furthermore, NWT isolates were most likely to demonstrate reduced susceptibility to older antimicrobials such as aminoglycosides, tetracyclines and phenicols.

Notwithstanding, there were small numbers of isolates that were deemed NWT for highly or critically important antimicrobials such as 3rd generation cephalosporins, quinolones and oxazolidinones. Of note was the isolation of bla_{CMY-2} containing *Salmonella* from three grain-fed beef cattle and one dairy cow that had reduced susceptibility to ceftriaxone and ampicillin. The three grain-fed beef cattle isolates harbouring bla_{CMY-2} were all isolated on a single sampling day from the same processing plant. For *E. coli*, a single grass-fed beef animal, one dairy cow and two veal calf isolates were considered NWT for ciprofloxacin and an additional five isolates that were NWT for ceftriaxone. Three of the five isolates were associated with grass-fed beef cattle and the remaining two with dairy cows with a single isolate from each animal class also deemed NWT for ceftiofur. Six *E. faecium* isolates from across all three animal classes were NWT for linezolid with three of these isolates also demonstrating reduced susceptibility to multiple classes of antimicrobials. The beef cattle isolates that were NWT for linezolid were isolated from feedlot cattle, as were 18/180 (10%) isolates that were NWT for virginamycin, an antimicrobial used in grain-fed production systems to restrict acidosis. *E. faecalis* isolates were more likely to be NWT for daptomycin with 11 isolates across three animal classes having this phenotype. Eight of the NWT daptomycin isolates were from beef cattle and six were from grass-fed animals. Three grass-fed beef cattle and three dairy cattle *E. faecalis* isolates were NWT for linezolid with one of the dairy isolates also NWT for vancomycin.

Limited ECOFF values exist for *E. hirae* and consequently the NWT phenotypes observed in this group of isolates were unremarkable.

This study was conducted to determine if the development of NWT populations of bacteria from beef cattle, dairy cattle or veal calves at slaughter is occurring. The outcomes of the study permit the Australian beef industry to arrive at the same conclusion as the 2013 study. That is, populations of NWT isolates to antimicrobials considered highly or critically important to human medicine are low and there is limited evidence of specific production practices, such as grain-feeding, leading to widespread disproportionate development of NWT isolates.

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1 Project objectives

- Collect 1000 faecal samples from beef cattle (n=600), dairy cattle (n=200) and veal calves (n=200) and attempt isolation of *E. coli*, *Salmonella* and *Enterococcus*.
- Perform microbroth dilution assays to determine the minimum inhibitory concentration (MIC) of each isolate against a recommended panel of antimicrobials.
- Resolve any novel or unexpected findings by performing genotyping on affected isolates.
- Determine the prevalence of AMR for each bacterium-antimicrobial combination, determine the rate of multidrug resistance (MDR; 3 or more classes), and perform direct comparison with the 2013 survey data to identify changes in AMR prevalence.
- Prepare survey outcomes for publication in peer reviewed journals and presentation at international conferences.

2 Success in achieving project objectives

The project objectives of V.MFS.0432 have been successfully completed. A total of 25 beef and veal processing establishments located across six Australian states and representing ~77% of total beef exports participated in the study. A total of 1001 faecal samples were collected from three animal groups: beef cattle (n=591), dairy cattle (n=194) and veal calves (n=216) with analysis of beef cattle samples determining that 235 (39.8%) were from feedlot cattle, 71 (12.0%) were grain-assisted, grass-fed cattle and 285 (48.2%) were grass-fed cattle. *E. coli* was isolated from 969 (96.8%), *Salmonella* from 184 (18.4%) and *Enterococcus* from 907 (90.6%) samples. All *E. coli*, *Salmonella*, *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus hirae* were tested for their phenotypic response to a panel of antimicrobials. This document details the AMR status of 1872 isolates analysed during this study and provides direct comparison to the previous MLA-funded AMR survey conducted in 2013.

3 Draft publication for Journal submission – Antimicrobial resistance of *E. coli*, *Salmonella* and *Enterococcus* from Australian cattle populations at slaughter

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3.1 Introduction

Antimicrobials have been utilised by the Australian cattle industry to prevent infection and treat disease of animals since the 1950s. Comparatively speaking, the use of antimicrobials in Australia has been conservative with respect to other countries, with restrictions in place for those antimicrobials deemed critically important for human health. Notably, the use of fluroquinolones has never been permitted for use in the animal sector and heavy restrictions are in place for the use of 3rd generation cephalosporins [1]. A 2013 report commissioned by Meat & Livestock Australia into the use of antibiotics in Australia found the use of antibiotics across the Australian cattle industry to be low, with ceftiofur and virginiamycin noted as the only antimicrobials likely to select for resistance of public health importance [2]. Nevertheless, any use of antimicrobials poses a risk to the development of antimicrobial resistance with the ability of bacteria to acquire and/or transfer antimicrobial mechanisms requiring a concerted approach from human, animal, health, agricultural, environmental, and food sectors to minimise the development and spread of AMR. These concerns and call for a co-ordinated one health approach are echoed in the recently released Australian government 2020 and beyond national strategy on AMR [3]. The findings of the 1999 JETACAR report are still encompassed in the strategy; with emphasis placed on the importance of ongoing surveillance and monitoring antibiotic resistance patterns [4].

Australia does not have a national surveillance program in place for the monitoring of AMR in the animal sector in the way that other countries, for example, Denmark (DANMAP), Canada (CIPARS) and the United States (NARMS) do. Instead it conducts periodic assessments of AMR in food-producing animals with the pork and chicken industries providing recent examples [5, 6]. Studies into the prevalence of AMR in cattle were carried out as early as the 1970s and 80s and although direct comparisons to modern day data are difficult due to a lack of a standardised approaches, they demonstrate a low-level resistance to tetracycline and streptomycin in *E. coli* and *Salmonella* [7]. In 2007, a study of AMR in bacteria of animal origin concluded that resistance in *E. coli* and *Enterococcus* from cattle was low with resistance to erythromycin and virginiamycin the only notable resistances at 10%. Importantly, resistance to fluroquinolones or 3rd generation cephalosporins was not observed nor were there obvious differences between cattle from differing production systems [8]. The most recent and largest study of healthy cattle at slaughter occurred in 2013 when 1500 beef and dairy cattle and veal calves were analysed for AMR. The study corroborated previous AMR evaluations with low levels of AMR observed, relatively low levels of mutli-drug resistance (MDR),

and most importantly ongoing susceptibility to antimicrobials of critical and high importance to human health [9, 10].

Australia produces just 4% of the world's beef supply yet is typically amongst the top three global beef exporters by volume and value [11]. Beef production in Australia is backed by several integrity and quality assurance programs that enable it to trade globally as a provider of clean, green and wholesome beef products. Maintaining this status is critical to the ongoing profitability of the industry and therefore verifying key claims around appropriate use of antimicrobials and low levels of AMR are central to that goal. Therefore, the aim of this study is to determine the AMR status of *E. coli*, *Salmonella* and *Enterococcus* isolates from healthy Australian cattle at slaughter.

3.2 Materials and Methods

3.2.1 Establishment participation and target animal groups

Australian beef and veal processors were invited to contribute to the nationwide collection of 1000 faecal samples from three beef production classes: beef cattle (n=600), dairy cattle (n=200) and veal calves (n=200). Proportionate stratified sampling based on the production class and slaughter volumes was applied to allocate the number of samples collected from each plant. Sample collection was carried out across four sampling windows in February, March, June and August 2019. Each sampling window comprised a two-week period in each of the months listed.

3.2.2 Sample collection and preparation

Participating establishments were asked to collect between six and 30 faecal samples per sampling window. Faecal samples were collected post-evisceration by cutting the intestine ~60 cm from the rectal end and squeezing the faecal contents into a sterile jar. Samples were returned to the laboratory on ice by overnight courier using chiller boxes. Following arrival at the laboratory, samples were stored chilled for a maximum of 48 hours prior to being prepared for *E. coli*, *Salmonella* or *Enterococcus* isolation. *E. coli* and *Salmonella* enrichments were prepared by diluting 25 g of faeces (1 in 10) in buffered peptone water (BPW; Oxoid, UK). Each enrichment was stomached for 60 s prior to enriching at 42 ± 1°C for 18 ± 2 h. *Enterococcus* enrichments were prepared by diluting 1 g of faeces (1 in 10) in Enterococcosel broth (BD, USA). Each sample was vortexed briefly prior to enriching at 35 ± 1°C for 18 ± 2 h.

3.2.3 Generic *E. coli* isolation

E. coli were isolated by plating BPW enriched faecal samples onto eosin methylene blue (EMB; Oxoid, UK) agar. EMB plates were incubated at 37 ± 1°C for 18 -24 h. Following incubation, two colonies displaying the typical metallic green sheen of *E. coli* were plated onto sheep blood agar (SBA). The resultant colonies were confirmed as *E. coli* using the spot indole test and MALDI-ToF

mass spectrometry (VITEK 2 BioMerieux: Bruker Microflex) and stored at -80°C using Protect bacterial preservers (Technical Service Consultants LTD, UK).

3.2.4 *Salmonella* isolation

The presence of *Salmonella* was assessed 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 SBA and confirmed as *Salmonella* by biochemical tests (Microbact 24E; Oxoid). Up to two confirmed *Salmonella* isolates were stored at -80°C using Protect bacterial preservers.

3.2.5 *Enterococcus* isolation

The prevalence of *Enterococcus* in each animal group was determined by plating the enterococcosel broth enrichments onto enterococcosel agar (BD, USA) and Slanetz and Bartley agar (Oxoid, UK). Enterococcosel agar plates were incubated at 35 ± 1°C for 24-48 h whereas Slanetz and Bartley agar plates were initially incubated at 35 ± 1°C for 4h followed by 44 ± 1°C for 44 h. Following incubation, five presumptive *Enterococcus* colonies from each plate (10 colonies in total) were patched onto SBA and incubated at 37 ± 1°C for 18 – 24 h. The resulting isolates were pooled into groups of five isolates and tested by PCR for the presence of *E. faecium* and *E. faecalis* using previously published protocols [12]. If a pooled group of isolates tested positive for either *E. faecium* or *E. faecalis* then further PCR testing of the individual isolates would occur. Recovered isolates were retained at -80°C using Protect bacterial preservers and confirmed using MALDI-ToF. In samples that yielded both an *E. faecium* and *E. faecalis* isolate both isolates were retained for AMR testing. Isolates from samples that tested negative for *E. faecium* and *E. faecalis* were then confirmed as *Enterococcus spp.* by PCR [13], identified to species level using MALDI-ToF and retained at -80°C using Protect bacterial preservers.

3.2.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on all MALDI-ToF confirmed *E. coli*, *Salmonella*, *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus hirae* isolates by micro-broth dilution using custom made antimicrobial panels. The antimicrobials and the concentration ranges evaluated are shown in Table 1. Epidemiological cut-off values (ECOFFs; EUCAST(https://mic.eucast.org/Eucast2/)) were used to identify wild-type (WT) and non wild-type (NWT) isolates. All phenotypic AMR assessments were completed following incubation at 37°C ± 1°C for 22 ± 2 h. Isolates unable to display turbid growth in the growth control well were considered

invalid and repeated. Repeated isolates that failed to display turbid growth were removed from the analysis.

Table 1. List of antimicrobials, class, dilution range and ECOFFs for *E. coli* and *Salmonella* AMR testing

Antimicrobial	Class	Dilution range	*E. coli* ECOFF[a]	*Salmonella* ECOFF
Amoxicillin-Clavulanate	Beta lactams (bla)	1-32	-[b]	-
Ampicillin	Beta lactams (bla)	2-64	8	8
Azithromycin	Macrolides (mac)	0.125-16	-	-
Cefoxitin	Cephems 1g (c1g)	0.5-32	8	8
Ceftiofur	Cephems 3g (c3g)	0.5-16	1	2
Ceftriaxone	Cephems 3g (c3g)	0.125-4	0.125	-
Chloramphenicol	Phenicols (phe)	2-32	16	16
Ciprofloxacin	Quinolones (qui)	0.063-4	0.063	0.063
Colistin	Polymixins (pol)	0.0125-8	2	-
Florfenicol	Phenicols (phe)	2-64	16	16
Gentamicin	Aminoglycosides (ami)	0.5-16	2	2
Meropenem	Carbapenems (car)	0.063-2	0.125	0.125
Nalidixic acid	Quinolones (qui)	1-32	8	8
Streptomycin	Aminoglycosides (ami)	16-64	16	16
Tetracycline	Tetracyclines (tet)	2-16	8	8
Trimethoprim/ sulfamethoxazole	Folate pathway inhibitors (fpi)	0.125-4	0.25	-

[a] If an organism has an MIC exceeding the ECOFF it is classified as non-wild type; [b] An ECOFF does not exist for this antimicrobial-bacteria combination

Table 2. List of antimicrobials, class, dilution range and ECOFFs for *Enterococcus* AMR testing

Antimicrobial	Class	Dilution range	*E. faecium* ECOFF[a]	*E. faecalis* ECOFF	*E. hirae* ECOFF
Ampicillin	Beta lactams (bla)	0.5-16	4	4	-
Benzylpenicillin	Beta lactams (bla)	0.5-16	16	16	-
Chloramphenicol	Phenicols (phe)	2-32	32	32	8
Daptomycin	Lipopeptides (lip)	0.125-4	8	4	-
Erythromycin	Macrolides (mac)	0.25-8	4	4	2
Gentamicin	Aminoglycosides (ami)	32-1024	32	64	32
Kanamycin	Aminoglycosides (ami)	128-1024	-[b]	-	-
Lincomycin	Lincosamides (lin)	1-32	-	-	-
Linezolid	Oxazolidinones (oxa)	0.5-8	4	4	-
Quinupristin-dalfopristin	Streptogramins (str)	0.25-8	-	-	-
Streptomycin	Aminoglycosides (ami)	256-1024	-	512	-
Teicoplanin	Glycopeptides (gly)	0.125-4	2	2	-
Tetracycline	Tetracyclines (tet)	2-16	4	4	4
Tigecycline	Glycylcycline (glc)	0.016-0.5	0.25	0.5	-
Vancomycin	Glycopeptides (gly)	0.25-32	4	4	-
Virginiamycin	Streptogramins (str)	1-32	4	32	-

[a] If an organism has an MIC exceeding the ECOFF it is classified as non-wild type; [b] An ECOFF does not exist for this antimicrobial-bacteria combination

3.3 Results and Discussion

3.3.1 Sample collection

Twenty-five Australian beef and veal processing establishments representing ~77% of Australian beef and veal exports agreed to take part in the study. A total of 1001 faecal samples were collected from three animal production classes; beef (n=591), dairy (n=194) and veal (n=216) across four sampling windows. A breakdown of the number and types of samples collected from each establishment is shown in Appendix 1. Samples were collected from all Australian states with the largest number of beef cattle samples (59.2%) originating in Queensland establishments, dairy samples were most numerous from Victorian establishments (61.3%) and veal samples originating from New South Wales (47.2%) or Queensland (45.8%) establishments. Further analysis of beef cattle samples determined that 235 (39.8%) were from feedlot cattle, 71 (12.0%) were grain-assisted, grass-fed cattle and 285 (48.2%) were grass-fed cattle.

3.3.2 *Salmonella* isolation

Salmonella was isolated from 83/591 (14.0%) of beef cattle, 34/194 (17.5%) of dairy cattle and 67/216 (31.0%) of veal calf faecal samples for an overall prevalence in Australian cattle of 18.4%. When compared to the previous 2013 study [9], the prevalence of *Salmonella* from veal calf faeces was significantly higher ($P < 0.05$), increasing by 19%. This increase was influenced by a high incidence of *Salmonella* at a single establishment. This establishment collected 80/216 (37%) of all veal samples yet contributed towards 51/67 (76.1%) of *Salmonella* isolates from veal cattle. This was unlikely the effect of clustering with *Salmonella* isolated across all eight sampling days at that establishment. Conversely the prevalence of *Salmonella* isolated from dairy cattle was significantly lower ($P < 0.05$) in this study when compared with the 2013 study [9], dropping by 8.4%. The overall prevalence of *Salmonella* in beef cattle samples was higher in this study (14.0%) compared to the 2013 study (11.5%), however this was not considered to be statistically significant ($P < 0.05$). Analysis of *Salmonella* prevalence by feed type revealed that the prevalence was slightly lower in grass-fed beef cattle (40/285 (14.0%)) compared to feedlot cattle (36/235 (15.3%)), however this difference was considered significant ($p < 0.05$). The prevalence of *Salmonella* varied across all four windows with 16.8% positive in window one, 25.5% in window two, 21.5% in window three with significantly less ($P < 0.05$) in window four with just 9.2% positive samples (Table 3). *Salmonella* was isolated from 21/25 (84.0%) plants that sampled beef, 5/7 (71.4%) plants that sampled dairy and 3/4 (75.0%) plants that sampled veal. The total number of plants having a positive sample also decreased in window 4 with 16 ,18, 15 and 9 plants yielding a *Salmonella* in windows 1, 2, 3 and 4 respectively. The reasons for the decreased prevalence of *Salmonella* in sampling window 4 are not understood, however the sampling window did commence at the end of August which is typically one of Australia's coldest months.

Table 3. *Salmonella* prevalence in Australian cattle groups across sampling windows

Animal Class	Window 1		Window 2		Window 3		Window 4	
	n =	+	n =	+	n =	+	n =	+
Beef Cattle	148	16 (10.3) [a]	142	27 (19.0)	151	28 (18.5)	150	12 (8.0)
Dairy Cattle	54	9 (16.7)	55	15 (27.3)	51	6 (11.8)	34	4 (11.8)
Veal	54	18 (33.3)	54	22 (40.7)	54	21 (38.9)	54	6 (11.1)
Overall	256	43 (16.8)	251	64 (25.5)	256	55 (21.5)	238	22 (9.2)

[a] figures in parentheses are percent

All *Salmonella* isolates were sequenced, assembled and analysed for sequence type (ST) and serovar. In total the ST or serovar was determined in 182/184 (98.9%) of isolates with 37 STs representing 34 serovar classifications identified (Table 4). *Salmonella* serovars Typhimurium, Saintpaul, Anatum and Hindmarsh/Bovismorbificans represent almost half (48.4%) of the isolates and is the same collection of four serovars that dominated the 2013 study [9], with *Salmonella* Typhimurium the most prevalent serovar across all animal groups. A recent petition to the United States Department of Agriculture Food Safety and Inspection Service requested a total of 31 *Salmonella* serovars be classified as adulterants in poultry and meat products [14]. Thirteen of the serovars representing 109/184 (59.2%) *Salmonella* identified in this study are included in the list of 31 proposed adulterants. Whilst it is important to recognise that this study sampled beef faeces and not red meat products, to which such a ruling would apply, it is necessary to accept that these serovars are present in the Australian cattle population and any regulation change in the USA could impact Australian beef exporters.

3.3.3 *E. coli* Isolation

E. coli was isolated from 969/1001 (96.8%) of all samples collected. No difference was seen between animal class with *E. coli* isolated from 574/591 (97.1%) of beef cattle, 186/194 (95.9%) of dairy cattle and 209/216 (96.8%) of veal. No differences were seen between the isolation rate of *E. coli* based on animal class, feed type or window sampled.

Table 4. Sequence types, serovars and frequency of isolation of *Salmonella*

Sequence type	Serovar	Number of isolates	Percentage
19	Typhimurium	36	19.6
50	Saintpaul	22	12.0
64	Anatum	16	8.7
377 or 1499	Hindmarsh or Bovismorbificans[a]	15	8.2
138	Montevideo	8	4.3
466	Zanzibar	8	4.3
93	Reading	8	4.3
580	Orion	7	3.8
16	Virchow	6	3.3
32	Infantis	6	3.3
329	Ohio	5	2.7
413	Mbandaka	5	2.7
Unknown	Virginia or Muenchen[a]	4	2.2
14 or 185	Senftenberg or Dessau[a]	4	2.2
309, 1792 or 3548	Unknown	4	2.2
516	Newington	3	1.6
1370	Oslo	2	1.1
319	II 6,7:z29:[z42] or Tennessee[a]	2	1.1
343	Chester	2	1.1
408	Potsdam	2	1.1
426	Aberdeen	2	1.1
515	Johannesburg	2	1.1
367 or 3548	Cerro or II 18:z4,z23:- or IIIa 18:z4,z23:- [a]	2	1.1
Not typed	Unknown	2	1.1
1069	Poona	1	0.5
13	Agona	1	0.5
15	Heidelberg	1	0.5
1959	Liverpool	1	0.5
440	Adelaide	1	0.5
4491	Pakistan or Litchfield[a]	1	0.5
462	Singapore	1	0.5
523	Wangata	1	0.5
582	Chailey	1	0.5
588	Havana	1	0.5
Unknown	Newport or Bardo[a]	1	0.5

[a] Highly related serovars that should be considered the same serovar

3.3.4 Enterococcus Isolation

Enterococcus faecium and *faecalis* are of greatest importance when consideration is given to the development of antimicrobial resistance (AMR) in Enterococci. In this study, up to 10 isolates from each sample were tested by PCR to determine if they were *E. faecium* or *E. faecalis*. In instances where *E. faecium* or *E. faecalis* could not be detected, presumptive isolates were confirmed by PCR as *Enterococcus* spp. and later identified by MALDI-ToF mass spectrometry. *Enterococcus* was isolated from 546/591 (92.4%) of beef cattle, 182/194 (93.8%) of dairy cattle and 182/216 (84.3%) of veal calf faecal samples for an overall prevalence in Australian cattle of 90.9%. Attempts to isolate *Enterococcus faecium* and *Enterococcus faecalis* produced differing results across the three animal groups with *E. faecium* isolated from 31.5 to 43.3% of samples and *E. faecalis* isolated from 5.6 to 12.5% of samples. These isolation rates are substantially higher than the 2013 study where *E. faecium* and *E. faecalis* were isolated from 8.0% and 6.4% of samples, respectively [10]. The differences observed are most likely due to the revised *Enterococcus* isolation method used in the current study. A further breakdown of isolates per animal group is shown in Table 5. *Enterococcus hirae*, *E. faecium* and *E. faecalis* were the most commonly isolated and represent 846/934 (90.6%) of all *Enterococcus* isolates. Whilst most samples yielded a single species on *Enterococcus*, there were 16 beef cattle samples, 5 dairy cattle samples and 3 veal calf samples from which *E. faecium* and *E. faecalis* were both recovered.

Table 5. Prevalence of *Enterococcus* species for each animal group as determined by MALDI-ToF

***Enterococcus* species**	**Beef cattle (n=591)**		**Dairy cattle (n=194)**		**Veal calves (n=216)**	
	No. of samples	**%**	**No. of samples**	**%**	**No. of samples**	**%**
Enterococcus faecalis	74	12.5	11	5.7	12	5.6
Enterococcus faecium	186	31.5	84	43.3	84	38.9
Enterococcus casseliflavus	21	3.6	8	4.1	10	4.6
Enterococcus hirae	260	44.0	68	35.1	67	31.0
Enterococcus mundtii	20	3.4	14	7.2	6	2.8
Enterococcus gallinarum	0	0.0	1	0.5	5	2.3
Enterococcus villorum	0	0.0	0	0.0	1	0.5
Enterococcus thailandicus	1	0.2	0	0.0	0	0.0

Enterococcus durans	0	0.0	1	0.5	0	0.0
Negative	45	7.6	12	6.2	34	15.7

3.3.5 *Salmonella* antimicrobial susceptibility testing

AMR analysis was conducted on all 184 *Salmonella* isolated over the four windows which comprised 83 beef cattle isolates, 34 dairy isolates and 67 veal isolates. The distribution of MICs for each antimicrobial and animal group is shown in Table 6. The prevalence of NWT *Salmonella* across all three animal groups was low with NWT percentages remaining below 5.9% for all antimicrobials. In total 173/184 (94.0%) of *Salmonella* isolates were considered WT. Of the 11 NWT *Salmonella* identified, six demonstrated reduced susceptibility to streptomycin alone. Reduced susceptibility to quinolones was observed in a single grass-fed beef cattle isolate with an MIC to ciprofloxacin of 0.13 mg/L. However, it is worth noting that the ECOFF value used in this study is lower than the breakpoint of 1 mg/L used in the 2013 study. Furthermore, genes or mutations that give rise to fluorquinolone resistance were not identified during genomic analysis. A further three beef cattle isolates had the NWT phenotype profile bla-c1g-c3g (Table 8) with reduced susceptibility to streptomycin, cefoxitin, and ceftiofur. The three isolates were recovered from feedlot cattle being processed at a single abattoir on the same day. Further genomic characterisation of these isolates identified the presence of $bla_{\text{CMY-2}}$. CMY-2 is an often reported AmpC β-lactamase that has frequently been found in food-producing animals [15]. CMY-2 was also identified in a single dairy cattle isolate possessing the NWT phenotype profile bla-c1g-c3g-tet. In addition to CMY-2, genomic analysis also identified $bla_{\text{TEM-1B}}$, *dfr*A5 and *tet*A which explains the phenotypic profile observed in this isolate.

Table 6. Distribution of MICs and proportion of non-wild type *Salmonella* isolates from faecal samples collected from beef cattle, dairy cattle and veal calves

						minimum inhibitory concentration (mg/L)[a]												
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	≥128
Aminoglycosides	Gentamicin	beef	83	0.2	0.0 - 4.3						96.4	3.6							
		dairy	34	0.5	0.0 - 10.3						85.3	14.7							
		veal	67	0.0	0.0 - 5.4						92.5	7.5							
	Streptomycin	beef	83	3.6	0.8 - 10.2											96.4	3.6		
		dairy	34	5.9	0.7 - 19.7											94.1	5.9		
		veal	67	1.5	0.0 - 8.0											98.5	1.5		
β-lactams	Amoxicillin-Clavulanate	beef	83	-	-							80.7	15.7					3.6	
		dairy	34	-	-							79.4	11.8		5.9			2.9	
		veal	67	-	-							94.0	6.0						
	Ampicillin	beef	83	3.6	0.8 - 10.2								95.2	1.2					3.6
		dairy	34	2.9	0.1 - 15.3								91.2	5.9					2.9
		veal	67	0.0	0.0 - 5.4								98.5	1.5					
Carbapenem	Meropenem	beef	83	0.0	0.0 - 4.3			100											
		dairy	34	0.0	0.0 - 10.3			100											
		veal	67	0.0	0.0 - 5.4			100											
Cephems	Ceftriaxone	beef	83	-	-				96.4						3.6				
		dairy	34	-	-				94.1	2.9					2.9				
		veal	67	-	-				100										
	Ceftiofur	beef	83	3.6	0.8 - 10.2						55.4	41					3.6		
		dairy	34	2.9	0.1 - 15.3						47.1	50					2.9		
		veal	67	0.0	0.0 - 5.4						77.6	22.4							
	Cefoxitin	beef	83	3.6	0.8 - 10.2								33.7	60.2	2.4			3.6	
		dairy	34	2.9	0.1 - 15.3								44.1	50.0	2.9			2.9	
		veal	67	0.0	0.0 - 5.4								59.7	38.8	1.5				
Macrolide	Azithromycin	beef	83	-	-									25.3	74.7				
		dairy	34	-	-									52.9	47.1				
		veal	67	-	-									59.7	40.3				
Phenicols	Chloramphenicol	beef	83	0.0	0.0 - 4.3									6.0	94				
		dairy	34	0.0	0.0 - 10.3									8.8	91.2				
		veal	67	0.0	0.0 - 5.4														
	Florfenicol	beef	83	0.0	0.0 - 4.3								3.6	80.7	15.7				
		dairy	34	0.0	0.0 - 10.3								5.9	88.2	5.9				

Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	minimum inhibitory concentration (mg/L)[a] 0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	≥128
		veal	67	0.0	0.0 - 5.4								13.4	86.6					
Polymixins	Colistin	beef	83	-	-							2.4	83.1	13.3	1.2				
		dairy	34	-	-							2.9	88.2	5.9	2.9				
		veal	67	-	-							1.5	83.6	14.9					
Quinolones	Ciprofloxacin	beef	83	1.2	0.0 - 6.5			98.8	1.2										
		dairy	34	0.0	0.0 - 10.3			100											
		veal	67	0.0	0.0 - 5.4			100											
	Nalidixic Acid	beef	83	0.0	0.0 - 4.3								9.6	89.2	1.2				
		dairy	34	0.0	0.0 - 10.3									100					
		veal	67	0.0	0.0 - 5.4								1.5	94	4.5				
Tetracycline	Tetracycline	beef	83	0.0	0.0 - 4.3								100						
		dairy	34	2.9	0.1 - 15.3								97.1				2.9		
		veal	67	0.0	0.0 - 5.4								100						
Folate pathway inhibitor	Trimethoprim/ sulfamethoxazole	beef	83	-	-				100										
		dairy	34	-	-				97.1			2.9							
		veal	67	-	-				100										

[a] Solid vertical lines indicate ECOFF values for designating WT and NWT isolates. Shaded areas indicate the dilution range tested for each antimicrobial.

Values outside of the shaded area indicate MICs greater than the highest concentration tested.

3.3.6 *E. coli* antimicrobial susceptibility testing

All 969 *E. coli* isolates recovered from beef cattle, dairy cattle and veal calf samples were submitted for AMR analysis. The distribution of MICs for each antimicrobial and animal group is shown in Table 7. In general, the percentage of isolates considered WT for all antimicrobials evaluated was high with 80.8%, 87.6% and 88.5% of beef cattle, dairy cattle and veal calf isolates, respectively, having this status. When individual antimicrobials are assessed within each animal group only four antimicrobial-animal group combinations had percentages of NWT organisms exceeding 5.0%. These included streptomycin in veal calf isolates (5.7%), tetracycline in beef cattle (15.9%), tetracycline in dairy cattle (8.1%) and tetracycline in veal calves (9.1%). The value for streptomycin NWT in veal calf isolates is higher than the percentage resistance observed in the 2013 study (4.0%), however the breakpoint for streptomycin in this study was lower (16 mg/L) compared with the 2013 study (32 mg/L). The breakpoint for tetracycline was consistent between this study and the 2013 study with analysis determining that the percentage NWT for tetracycline in all animal groups exceeds the 95% confidence intervals of the 2013 study [9]. Further analysis of beef cattle isolates determined that feedlot animals (48/231) were significantly ($P < 0.05$) more likely to yield a tetracycline NWT isolate than grass-fed animals (28/274).

Elevated MICs to fluoroquinolones or 3rd generation cephalosporins are of importance to beef production systems. A total of 4/969 (0.4%) comprising one grass-fed beef cattle, one dairy cattle and two veal calf isolates were considered NWT for fluoroquinolones. Similarly, just 5/969 (0.5%) of all *E. coli* isolates were NWT for 3rd generation cephalosporins. Three of the five isolates were associated with grass-fed beef cattle and the remaining two with dairy cattle. All five isolates were recovered from separate establishments across three Australian states. Further molecular investigation is required to determine the basis of the NWT status. The antimicrobial phenotype profiles are shown in Table 8. Isolates that were NWT to three of more antimicrobial classes were recovered from 23/574 (4%) beef cattle isolates, 6/186 (3.2%) dairy cattle isolates and 8/209 (3.8%) with AMI_BLA_TET, AMI_BLA_FPI and AMI_BLA_FPI_TET most commonly observed. No relationship was observed between the type of beef cattle production system and the presence of NWT *E. coli* to three or more antimicrobials.

Table 7. Distribution of MICs and proportion of non-wild type *E. coli* isolates from faecal samples collected from beef cattle, dairy cattle and veal calves

						minimum inhibitory concentration (mg/L)[a]												
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	≥128
Aminoglycosides	Gentamicin	beef	574	0.2	0.0 - 1.0						52.8	44.6	2.4	0.2					
		dairy	186	0.5	0.0 - 3.0						55.4	40.3	3.8	0.5					
		veal	209	0.0	0.0 - 1.7						51.2	45	3.8						
	Streptomycin	beef	574	4.5	3.0 - 6.6											95.5	0.3	1.2	3.0
		dairy	186	3.8	1.5 - 7.6											96.2	3.2		0.5
		veal	209	5.7	3.8 - 9.8											94.3	1.9	0.5	3.3
β-lactams	Amoxicillin-Clavulanate	beef	574	-	-							1.9	15.3	61.5	20.2	0.9	0.2		
		dairy	186	-	-							2.2	13.4	64.5	18.3	1.6			
		veal	209	-	-							1.4	12	65.6	20.1	1.0			
	Ampicillin	beef	574	4.7	3.1 - 6.8								23.5	66.5	5.8	0.1	0.2	0.4	3.5
		dairy	186	3.8	1.5 - 7.6								26.8	61.8	6.6			0.5	4.2
		veal	209	3.3	1.4 - 6.8								19.9	73.1	3.2	0.5	0.5	0.5	2.2
Carbapenem	Meropenem	beef	574	0.0	0.0 - 0.6			100											
		dairy	186	0.0	0.0 - 2.0			100											
		veal	209	0.0	0.0 - 1.7			100											
Cephems	Ceftriaxone	beef	574	0.5	0.1 - 1.5				99.5	0.2			0.2		0.1				
		dairy	186	1.1	0.1 - 3.8				98.9	0.5		0.5							
		veal	209	0.0	0.0 - 1.7				100										
	Ceftiofur	beef	574	0.2	0.0 - 1.0						99.7	0.2			0.2				
		dairy	186	0.5	0.0 - 3.0						98.9	0.5	0.5						
		veal	209	0.0	0.0 - 1.7						99.5	0.5							
	Cefoxitin	beef	574	1.2	0.5 – 2.5						0.2	1.2	17.4	57.5	22.5	1.0	0.2		
		dairy	186	1.1	0.1 – 3.8						0.5	1.6	14	67.7	15.1	1.1			
		veal	209	1.0	0.1 – 3.4								10	71.3	17.7	1.0			
Macrolide	Azithromycin	beef	574	-	-							0.9	6.1	59.2	33.1	0.7			
		dairy	186	-	-				0.5		0.5	0.5	3.8	54.8	37.6	2.2			
		veal	209	-	-							0.5	3.3	60.3	35.4	0.5			
Phenicols	Chloramphenicol	beef	574	0.0	0.0 - 0.6								2.1	19.3	77	1.6			
		dairy	186	0.0	0.0 - 2.0								2.2	16.7	76.3	4.8			
		veal	209	0.0	0.0 - 1.7								0.5	16.7	78.5	4.3			
	Florfenicol	beef	574	0.0	0.0 - 0.6								8.2	49.1	41.5	1.2			
		dairy	186	0.0	0.0 - 2.0								5.9	45.2	47.3	1.6			

Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	minimum inhibitory concentration (mg/L)[a] 0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	≥128
		veal	209	0.0	0.0 - 1.7								4.8	43.5	50.2	1.4			
Polymixins	Colistin	beef	574	0.2	0.0 - 1.0						10.6	65.5	23.7	0.2					
		dairy	186	1.6	0.3 - 4.6				0.5		4.3	65.1	28.5	1.1	0.5				
		veal	209	0.5	0.0 - 2.6						5.7	67.5	26.3		0.5				
Quinolones	Ciprofloxacin	beef	574	0.2	0.0 - 1.0			99.8		0.2									
		dairy	186	0.5	0.0 - 3.0			99.5		0.5									
		veal	209	1.0	0.1 - 3.4			99.0	0.5	0.5									
	Nalidixic Acid	beef	574	0.2	0.0 - 1.0							1.9	58.9	38.3	0.7			0.2	
		dairy	186	0.0	0.0 - 2.0							3.2	50.5	44.6	1.6				
		veal	209	0.0	0.0 - 1.7							1.0	52.2	46.4	0.5				
Tetracycline	Tetracycline	beef	574	15.9	13 - 19.1								81.2	3.0		1.6	14.3		
		dairy	186	8.1	4.6 -13.0								86.6	5.4			8.1		
		veal	209	9.1	5.6-13.8								84.7	5.3	1.0	0.5	8.6		
Folate pathway inhibitor	Trimethoprim/ sulfamethoxazole	beef	574	2.8	1.6 - 4.5-				96.7	0.5	0.2	0.5			2.1				
		dairy	186	1.1	0.1 - 3.8				96.8	2.2					1.1				
		veal	209	1.4	0.3 - 4.1				96.2	2.4	1.0				0.5				

[a] Solid vertical lines indicate ECOFF values for designating WT and NWT isolates. Shaded areas indicate the dilution range tested for each antimicrobial.

Values outside of the shaded area indicate MICs greater than the highest concentration tested.

Table 8. Antimicrobial phenotype profiles of *E. coli* and *Salmonella* from beef cattle, dairy cattle and veal calf faecal samples

	E. coli			***Salmonella***		
	Beef (N=574)	**Dairy (N=186)**	**Veal (N=209)**	**Beef (N=83)**	**Dairy (N=34)**	**Veal (N=67)**
Wild-type	464 (80.8)[b]	163 (87.6)	185 (88.5)	76 (91.6)	31 (91.2)	66 (98.5)
AMI		1 (0.5)	3 (1.4)	3 (3.6)	2 (5.9)	1 (1.5)
BLA	3 (0.5)					
C1G	5 (0.9)	1 (0.5)	1 (0.5)			
POL	1 (0.2)	3 (1.6)	1 (0.5)			
TET	66 (11.5)	8 (4.3)	7 (3.3)			
QUI				1 (1.2)		
AMI_TET	6 (1.0)	3 (1.6)	3 (1.4)			
BLA_C3G	1 (0.2)	1 (0.5)				
BLA_TET	3 (0.5)					
C1G_C3G	1 (0.2)					
C1G_TET			1 (0.5)			
QUI_TET	1 (0.2)					
AMI_BLA_FPI	7 (1.2)	1 (0.5)				
AMI_BLA_TET	6 (1.0)	2 (1.1)	5 (2.4)			
AMI_FPI_TET	3 (0.5)		1 (0.5)			
BLA_C1G_C3G	1 (0.2)	1 (0.5)		3 (3.6)		
BLA_FPI_TET	1 (0.2)	1 (0.5)				
AMI_BLA_FPI_TET	5 (0.9)					
AMI_BLA_QUI_TET		1 (0.5)				
BLA_C1G_C3G_TET					1 (2.9)	
BLA_FPI_QUI_TET			2 (1.0)			

[a] AMI – aminoglycosides , BLA – beta lactams, C1G – cephems 1g, C3G – cephems 3g, POL – polymixins, QUI – Quinolones, TET – tetracyclines, FPI – folate pathway inhibitors; [b] Figures in parentheses are percent

3.3.7 *Enterococcus* antimicrobial susceptibility testing

Attempts to isolate *Enterococcus* from samples collected in this study determined that 846/934 (90.6%) belong to the species *faecium*, *faecalis* or *hirae*. Evaluation of reduced susceptibility to antimicrobials in *Enterococcus* typically focuses on *E. faecium* and *E. faecalis* due to their increased association with human disease [16]. *Enterococcus hirae* are regularly associated with cattle populations [17] may occasionally cause human disease and may play a role in the development and spread of antimicrobial resistance genes. All *E. faecium*, *E. faecalis* and *E. hirae* isolates were submitted for AMR analysis, however a number of isolates failed to pass the quality control conditions relating to the growth in the control well by 24 hours and were excluded from the overall analysis. Data are presented for 92 *E. faecalis*, 343 *E. faecium* and 284 *E. hirae* isolates.

3.3.7.1 *Enterococcus faecium susceptibility testing*

Antimicrobial susceptibility analysis was conducted on 343 *E. faecium* isolates from beef cattle (n=180), dairy cattle (n=80) and veal calves (n=83). The distributions of MICs for each antimicrobial and animal group are shown in Table 9. The percentage of isolates deemed to be WT for all antimicrobials ranged from 66.1% in beef cattle isolates to 85.5% for veal calf isolates (Table 12). The lower WT percentage in beef cattle isolates was influenced heavily by isolates that were NWT for erythromycin (26.1%), virginiamycin (10.0%) and tetracycline (10.0%). The production system from which the isolate was recovered was a key predictor in the development of NWT *E. faecium* isolates with beef cattle from feedlots contributing 55.3%, 88.9%, and 66.7% of NWT erythromycin, virginiamycin and tetracycline isolates, respectively, despite contributing just 68/180 (37.8%) of the total beef cattle isolates. Conversely, the prevalence of NWT erythromycin, virginiamycin and tetracycline isolates coming from grass-fed beef cattle was 8.0%, 0.0%, and 4.6%, respectively. The association of virginiamycin with feedlot cattle and the absence in grass-fed cattle is unsurprising as virginiamycin is used to prevent acidosis due to grain feeding [18]. Like virginiamycin, quinupristin-dalfopristin also belong the streptogramin class of antimicrobials and until recently was considered critically important for human health. An ECOFF does not exist for quinupristin-dalfopristin and therefore it wasn't possible to distinguish between WT and NWT isolates. However, all 18 beef cattle isolates that were NWT for virginiamycin had reduced susceptibility to quinupristin-dalfopristin with MICs of 16 mg/L. Further research to develop an ECOFF for quinupristin-dalfopristin is required to assist in the early detection of acquired resistance to streptogramins. Macrolides (erythromycin) are listed as a critically important antimicrobial in human health by WHO, however the focus is on the development of macrolide resistance in *Campylobacter* [18]. Erythromycin is seldom used in the treatment of enterococcal infections [19] and therefore the prevalence of NWT isolates observed in this study are of little importance to human health. Notwithstanding, the prevalence of NWT isolates in this study exceeds the 95% confidence intervals of the 2013 study [10] and highlights a potential need for further education around the judicious use of macrolides in cattle production systems such as those recently published for the Australian cattle feedlot industry [20].

Ampicillin, vancomycin and linezolid are of importance to human medicine for the treatment of uncomplicated enterococcal infections and to maintain optimal treatment options for more complicated scenarios. Seven isolates were determined to be NWT for linezolid (n=6) or ampicillin (n=1) whereas all *E. faecium* isolates were WT for vancomycin. Resistance to linezolid, ampicillin and

vancomycin was not observed in the 2013 study [10], however the NWT percentages of the current study do not exceed the 95% confidence intervals and don't necessarily represent a trend in the development of reduced susceptibility to these antimicrobials. Nevertheless, reduced susceptibility to linezolid or ampicillin was often associated with reduced susceptibility to multiple classes of antimicrobials. Three of the six (50%) isolates that were NWT for linezolid had the antimicrobial phenotype profile of MAC_OXA_TET (n=1) or MAC_OXA_STR_TET (n=2) and similarly the NWT ampicillin isolate had the profile BLA_MAC_TET (Table 12). Further investigation to determine if there is a genetic basis to the reduced susceptibilities and better understand the potential consequences for human health.

3.3.7.2 *Enterococcus faecalis* susceptibility testing

Antimicrobial susceptibility analysis was conducted on 92 *E. faecalis* isolates from beef cattle (n=70), dairy cattle (n=10) and veal calves (n=12). The distributions of MICs for each antimicrobial and animal group are shown in Table 10. The percentage of *E. faecium* deemed to be WT for all antimicrobials was 82.9%, 60.0% and 50.0% for beef cattle, dairy cattle and veal calf isolates, respectively (Table 12). Caution must be given to the relatively low numbers of isolates in the dairy cattle and veal calves groups which make the discussion of NWT percentages impractical. For these groups, all results will discuss isolate numbers instead. For beef cattle isolates, non-wild type isolates were only observed for daptomycin (11.4%), linezolid (4.3%), tetracycline (4.3%) and streptomycin (2.9%). The percentage of daptomycin NWT isolates is consistent with the percentage of resistance observed in the 2013 study [10]. NWT daptomycin isolates were three times more likely to be isolated from grass-fed beef cattle than cattle from feedlots and were usually WT for all other antimicrobials, however two of the isolates from grass-fed animals were also considered NWT for linezolid. The remaining NWT linezolid isolate was also recovered from a grass-fed animal and although the number of isolates from beef cattle that were NWT for a single antimicrobial were low, they were more likely to be derived from grass-fed animals which is in total contrast to the results observed for *E. faecium* isolates. Of the four dairy cattle isolates considered NWT for an antimicrobial, three were NWT for linezolid and includes an isolate with the phenotypic profile GLY_OXA_TET meaning that it was also NWT for vancomycin and tetracycline. Further investigation of the genetic basis of the NWT status is required. Six of 12 veal calf isolates were NWT for a single antimicrobial and although much of the NWT status was towards antimicrobials that are not important to human medicine, there were two isolates that were NWT for daptomycin and a single isolate with the phenotype AMI_MAC_PHE_TET.

Table 9 Distribution of MICs and proportion of non-wild type *E. faecium* isolates from faecal samples collected from beef cattle, dairy cattle and veal calves

						minimum inhibitory concentration (mg/L)[a]															
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Aminoglycoside	Gentamicin	beef	180	0.0	0.0 - 2.0											100						
		dairy	80	1.3	0.0 - 6.8											98.8	1.3					
		veal	83	1.2	0.0 - 6.5											98.8	1.2					
	Kanamycin	beef	180	-	-													21.1	40	31.7	7.2	
		dairy	80	-	-													5.0	40	46.3	8.8	
		veal	83	-	-													27.7	39.8	22.9	8.4	1.2
	Streptomycin	beef	180	-	-														98.9		1.1	
		dairy	80	-	-														98.8			1.3
		veal	83	-	-														100			
Beta lactam	Benzylpenicillin	beef	180	0.6	0.0 - 3.1					16.1	19.4	51.7	11.7	0.6		0.6						
		dairy	80	0.0	0.0 - 4.5					11.3	15	56.3	16.3	1.3								
		veal	83	0.0	0.0 - 4.3					13.3	14.5	53.0	18.1	1.2								
Glycopeptides	Teicoplanin	beef	180	0.0	0.0 - 2.0			5.6	5.6	47.2	41.7											
		dairy	80	0.0	0.0 - 4.5			3.8	7.5	42.5	46.3											
		veal	83	0.0	0.0 - 4.3				6.0	59.0	34.9											
	Vancomycin	beef	180	0.0	0.0 - 2.0				5.0	73.3	10.0	11.7										
		dairy	80	0.0	0.0 - 4.5				3.8	82.5	7.5	6.3										
		veal	83	0.0	0.0 - 4.3				8.4	80.7	4.8	6.0										
Lincosamides	Lincomycin	beef	180	-	.						31.1	2.8	7.8	10.0	33	7.2	8.3					
		dairy	80	-	.						57.5		3.8	7.5	28	1.3	2.5					
		veal	83	-	.						60.2		1.2	4.8	25	7.2	1.2					
Lipopeptide	Daptomycin	beef	180	0.0	0.0 - 2.0			1.1	0.6	1.1	2.2	24.4	47.8	22.8								
		dairy	80	0.0	0.0 - 4.5				1.3		1.3	12.5	32.5	52.5								

						minimum inhibitory concentration (mg/L)[a]																
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
		veal	83	0.0	0.0 - 4.3						3.6	16.9	49.4	30.1								
Macrolide	Erythromycin	beef	180	26.1	19.9-33.2				15.0	5.6	3.3	23.3	26.7	15.6	11.0							
		dairy	80	15.0	8.0 - 24.7				17.5	6.3	7.5	16.3	37.5	10.0	5.0							
		veal	83	7.2	2.7 - 15.1				24.1	1.2	4.8	34.9	27.7	6.0	1.2							
Oxazolidinones	Linezolid	beef	180	1.1	0.1 - 4.0					1.7	2.8	21.7	72.8		1.1							
		dairy	80	2.5	0.3 - 8.7					3.8	3.8	11.3	78.8	2.5								
		veal	83	2.4	0.3 - 8.4					2.4	3.6	20.5	71.1	2.4								
Penicillin	Ampicillin	beef	180	0.6	0.0 - 3.1					21.1	41.1	31.7	5.6			0.6						
		dairy	80	0.0	0.0 - 4.5					15.0	26.3	42.5	16.3									
		veal	83	0.0	0.0 - 4.3					18.1	32.5	42.2	7.2									
Phenicol	Chloramphenicol	beef	180	0.0	0.0 - 2.0							0.6	6.7	62.2	30.0	0.6						
		dairy	80	0.0	0.0 - 4.5							2.5	2.5	55	40.0							
		veal	83	0.0	0.0 - 4.3							1.2	3.6	63.9	31.0							
Streptogramins	Quinupristin-Dalfopristin	beef	180	-	-				1.1	18.3	21.1	34.4	12.2	2.2	11							
		dairy	80	-	-					21.3	42.5	31.3	5.0									
		veal	83	-	-				6.0	32.5	26.5	25.3	7.2		2.4							
	Virginiamycin	beef	180	10.0	6.0 - 15.3						85.0	2.2	2.8	0.6	0.6	7.8	1.1					
		dairy	80	0.0	0.0 - 4.5						97.5	2.5										
		veal	83	2.4	0.3 - 8.4						97.6					1.2	1.2					
Tetracycline	Tetracycline	beef	180	10.0	6.0 - 15.3							88.9	1.1		0.6	9.4						
		dairy	80	6.3	2.1 - 14							93.8			1.3	5.0						
		veal	83	1.2	0 - 6.5							98.8				1.2						

[a] Solid vertical lines indicate ECOFF values for designating WT and NWT isolates. Shaded areas indicate the dilution range tested for each antimicrobial. Values outside of the shaded area indicate MICs greater than the highest concentration tested.

Table 10. Distribution of MICs and proportion of non-wild type *E. faecalis* isolates from faecal samples collected from beef cattle, dairy cattle and veal calves

						minimum inhibitory concentration (mg/L)[a]																
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Aminoglycoside	Gentamicin	beef	70	0.0	0.0 - 5.1											100						
		dairy	10	0.0	0.0 - 30.8											100						
		veal	12	0.0	0.0 - 26.5											100						
	Kanamycin	beef	70	-	-													97.1	2.9			
		dairy	10	-	-													100				
		veal	12	-	-													91.7				8.3
	Streptomycin	beef	70	2.9	0.3 - 9.9														97.1		1.4	1.4
		dairy	10	0.0	0.0 - 30.8														100			
		veal	12	25.0	5.5 - 57.2														75.0		8.3	16.7
Beta lactam	Benzylpenicillin	beef	70	0.0	0.0 - 5.1					14.3	27.1	55.7	2.9									
		dairy	10	0.0	0.0 - 30.8						30.0	70.0										
		veal	12	0.0	0.0 - 26.5					8.3		83.3	8.3									
Glycopeptides	Teicoplanin	beef	70	0.0	0.0 - 5.1			8.6	11.4	71.4	8.6											
		dairy	10	10.0	0.3 - 44.5				10.0	80.0				10.0								
		veal	12	0.0	0.0 - 26.5			8.3	25.0	66.7												
	Vancomycin	beef	70	0.0	0.0 - 5.1				2.9	12.9	60	24.3										
		dairy	10	10.0	0.3 - 44.5					10.0	70.0	10.0				10.0						
		veal	12	0.0	0.0 - 26.5					25.0	58.3	16.7										
Lincosamides	Lincomycin	beef	70	-	-						5.7		2.9	4.3	10.0	63	14.3					
		dairy	10	-	-									10.0	10.0	80						
		veal	12	-	-											58	41.7					
Lipopeptide	Daptomycin	beef	70	11.4	5.1 - 21.3			1.4	2.9	7.1	8.6	28.6	40	11.4								
		dairy	10	10.0	0.3 - 44.5			10			10	30	40	10								
		veal	12	16.7	2.1 - 48.4							41.7	42	17								

						minimum inhibitory concentration (mg/L)[a]															
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Macrolide	Erythromycin	beef	70	0.0	0.0 - 5.1				28.6	15.7	31.4	21.4	2.9									
		dairy	10	0.0	0.0 - 30.8				30.0	20.0	40.0	10.0										
		veal	12	8.3	0.2 - 38.5				8.3	16.7	41.7	25.0			8.3							
Oxazolidinones	Linezolid	beef	70	4.3	0.9 - 12					1.4	7.1	77.1	10	1.4	2.9							
		dairy	10	30.0	6.7 - 65.2							70.0		30.0								
		veal	12	0.0	0.0 - 26.5							91.7	8.3									
Penicillin	Ampicillin	beef	70	0.0	0.0 - 5.1					28.6	38.6	30	2.9									
		dairy	10	0.0	0.0 - 30.8					20.0	30.0	50.0										
		veal	12	0.0	0.0 - 26.5					16.7	33.3	50.0										
Phenicol	Chloramphenicol	beef	70	0.0	0.0 - 5.1								4.3	87.0	8.6							
		dairy	10	0.0	0.0 - 30.8									90.0	10.0							
		veal	12	8.3	0.2 - 38.5								8.3	75.0	8.3		8.3					
Streptogramins	Quinupristin-Dalfopristin	beef	70	-	.				4.3	1.4	5.7	1.4		74.0	13							
		dairy	10	-	.								10	80.0	10.0							
		veal	12	-	.								8.3	75.0	17							
	Virginiamycin	beef	70	0.0	0.0 - 5.1						12.9	5.7	49	33.0								
		dairy	10	0.0	0.0 - 30.8							20.0	50	30.0								
		veal	12	0.0	0.0 - 26.5							8.3	67	25.0								
Tetracycline	Tetracycline	beef	70	4.3	0.9 - 12.0							94.3	1.4	1.4		2.9						
		dairy	10	20.0	2.5 - 55.6							80.0				20.0						
		veal	12	25.0	5.5 - 57.2							75.0				25.0						

[a] Solid vertical lines indicate ECOFF values for designating WT and NWT isolates. Shaded areas indicate the dilution range tested for each antimicrobial. Values outside of the shaded area indicate MICs greater than the highest concentration tested.

Table 11: Distribution of MICs and proportion of non-wild type *E. hirae* isolates from faecal samples collected from beef cattle, dairy cattle and veal calves

						minimum inhibitory concentration (mg/L)[a]															
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Aminoglycoside	Gentamicin	beef	185	1.1	0.1 - 3.9											98.9	1.1					
		dairy	48	2.1	0.1 - 11.1											97.9	2.1					
		veal	51	0.0	0 – 7.0											100						
	Kanamycin	beef	185	-	-													95.7	2.7	0.5	1.1	
		dairy	48	-	-													91.7	6.3	2.1		
		veal	51	-	-													96.1		3.9		1.2
	Streptomycin	beef	185	-	-														97.8	1.6		0.5
		dairy	48	-	-														100			
		veal	51	-	-														100			
Beta lactam	Benzylpenicillin	beef	185	-	-					80	10.8	8.1	0.5	0.5								
		dairy	48	-	-					85.4	8.3	6.3										
		veal	51	-	-					90.2	7.8	2										
Glycopeptides	Teicoplanin	beef	185	-	-			51.4	35.1	11.4	1.1	1.1										
		dairy	48	-	-			54.2	37.5	8.3												
		veal	51	-	-			47.1	47.1	3.9	2.0											
	Vancomycin	beef	185	-	-				16.8	74.6	4.3	4.3										
		dairy	48	-	-				14.6	79.2	6.3											
		veal	51	-	-				23.5	72.5	3.9											
Lincosamides	Lincomycin	beef	185	-	-						14.1	5.4	15.1	20.5	27	5.4	13.0					
		dairy	48	-	-						31.3	14.6	22.9	20.8	8.3	2.1						
		veal	51	-	-						19.6	3.9	3.9	33.3	29	5.9	3.9					
Lipopeptide	Daptomycin	beef	185	-	-			7.0	1.1	10.3	24.3	35.1	16.2	5.9								
		dairy	48	-	-			2.1	2.1	10.4	35.4	35.4	10.4	4.2								
		veal	51	-	-					15.7	29.4	37.3	13.7	3.9								

						minimum inhibitory concentration (mg/L)[a]															
Class	Antimicrobial agent	Group	*n*	% non-wild type	95% CI	0.016	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Macrolide	Erythromycin	beef	185	4.9	2.2 - 9.0				77.3	9.7	5.4	2.7	2.7	0.5	1.6							
		dairy	48	4.2	0.5 - 14.3				89.6	2.1	4.2		4.2									
		veal	51	3.9	0.5 - 13.5				90.2	2	3.9			3.9								
Oxazolidinones	Linezolid	beef	185	-	-					24.9	23.8	30.3	18.4	1.6	1.1							
		dairy	48	-	-					20.8	22.9	37.5	16.7		2.1							
		veal	51	-	-					25.5	21.6	25.5	25.5	2								
Penicillin	Ampicillin	beef	185	-	-					84.9	11.9	3.2										
		dairy	48	-	-					93.8	4.2	2.1										
		veal	51	-	-					94.1	5.9											
Phenicol	Chloramphenicol	beef	185	6.5	3.4 - 11.1							37.8	21.1	34.6	6.5							
		dairy	48	4.2	0.5 - 14.3							33.3	20.8	41.7	4.2							
		veal	51	7.8	2.2 - 18.9							27.5	19.6	45.1	7.8							
Streptogramins	Quinupristin-Dalfopristin	beef	185	-	-				24.3	27	15.1	16.8	8.6	4.3	3.8							
		dairy	48	-	-				31.3	39.6	12.5	14.6		2.1								
		veal	51	-	-				25.5	33.3	11.8	27.5	2.0									
	Virginiamycin	beef	185	-	-						86.5	4.3	2.7	4.3	1.6	0.5						
		dairy	48	-	-						95.8		2.1	2.1								
		veal	51	-	-						98	2.0										
Tetracycline	Tetracycline	beef	185	16.8	11.7 - 22.9							82.7	0.5	0.5	3.2	13.0						
		dairy	48	2.1	0.1 - 11.1							97.9		2.1								
		veal	51	9.8	3.3 - 21.4							90.2		2.0		7.8						

[a] Solid vertical lines indicate ECOFF values for designating WT and NWT isolates. Shaded areas indicate the dilution range tested for each antimicrobial. Values outside of the shaded area indicate MICs greater than the highest concentration tested.

Table 12. Antimicrobial phenotype profiles of *E. faecium*, *E. faecalis* and *E. hirae* from beef cattle, dairy cattle and veal calf faecal samples

	E. faecium			***E. faecalis***			***E. hirae***		
	Beef (N=180)	**Dairy (N=80)**	**Veal (N=83)**	**Beef (N=70)**	**Dairy (N=10)**	**Veal (N=12)**	**Beef (N=185)**	**Dairy (N=48)**	**Veal (N=51)**
Wild-type	119 (66.1)[b]	62 (77.5)	71 (85.5)	58 (82.9)	6 (60.0)	6 (50.0)	138 (74.6)	44 (91.7)	41 (80.4)
AMI[a]		1 (1.3)	1 (1.2)			1 (8.3)	1 (0.5)	1 (2.1)	
MAC	29 (16.1)	11 (13.8)	6 (7.2)				4 (2.2)		2 (3.9)
OXA		1 (1.3)	2 (2.4)	1 (1.4)	2 (20.0)				
STR	6 (3.3)		2 (2.4)						
TET	7 (3.9)	4 (5.0)	1 (1.2)	1 (1.4)		1 (8.3)	26 (14.1)	1 (2.1)	4 (7.8)
LIP				6 (8.6)		2 (16.7)			
PHE							10 (5.4)		3 (5.9)
MAC_STR	8 (4.4)								
MAC_TET	6 (3.3)						4 (2.2)		
STR_TET	1 (0.6)								
AMI_TET				2 (2.9)		1 (8.3)			
LIP_OXA				2 (2.9)					
LIP_TET					1 (10.0)				
MAC_PHE							1 (0.5)	2 (4.2)	
PHE_TET									1 (2.4)
BLA_MAC_TET	1 (0.6)								
MAC_OXA_TET		1 (1.3)							
MAC_STR_TET	1 (0.6)								
GLY_OXA_TET					1 (10.0)				
AMI_PHE_TET							1 (0.5)		
MAC_OXA_STR_TET	2 (1.1)								
AMI_MAC_PHE_TET						1 (8.3)			

[a] AMI – aminoglycosides , MAC – macrolides, OXA – oxazolidinones, STR - streptogramins, TET – tetracyclines, LIP – lipopeptides, PHE – phenicols, BLA – Beta lactam, GLY – glycopeptides; [b] Figures in parentheses are percent

3.3.7.3 *Enterococcus hirae* susceptibility testing

Traditionally clinical resistant breakpoints were unavailable for *Enterococcus* isolates other than *E. faecium* and *E. faecalis*, however ECOFF values are now available for four antimicrobials and *E. hirae*. Antimicrobial susceptibility analysis was conducted on 284 *E. hirae* isolates from beef cattle (n=185), dairy cattle (n=48) and veal calves (n=51). The distributions of MICs for each antimicrobial and animal group are shown in Table 11. NWT percentages >5.0% were observed for tetracycline and chloramphenicol in beef cattle and veal calves with NWT tetracycline isolates in beef cattle (16.8%) the only combination to exceed 10.0%. Nine NWT isolates had phenotypic profiles comprised of two or more antimicrobial classes. The most common combination was MAC_TET, which was found in four beef cattle isolates, with the longest combination being AMI_PHE_TET which was observed in a single beef cattle isolate. Comparisons with the 2013 study are not possible as *Enterococcus* isolates other than *E. faecium* and *E. faecalis* were not speciated as part of that study.

3.4 Conclusion

Antimicrobials continue to be used in beef production systems for the treatment and prevention of disease. The development of antimicrobial resistance is an unintended consequence of antimicrobial use with the potential for resistance to impact human health a key concern. Whilst the recently published review of AMR in food in Australia and New Zealand [21] continues the call for food AMR surveillance to be integrated into a nationally coordinated active surveillance program, Australia continues to rely on infrequent surveys of isolates from food or food-producing animals. Meat & Livestock Australia previously funded a survey of AMR in healthy Australian cattle at slaughter in 2013 which concluded that resistance to clinically significant antimicrobials was seldom observed and resistance to most antimicrobials was low by international comparisons [9, 10]. This report details the outcomes of a similarly designed that was conducted across four sampling windows in 2019. Changes to the design of this survey in comparison to the 2013 survey included a reduction in overall sample number from 1500 to 1000, the inclusion of a targeted *Enterococcus* method to increase the proportion of *E. faecium* and *E. faecalis* isolates, and a switch from CLSI clinical breakpoints to ECOFF values. The use of ECOFF values is recommended for AMR monitoring programs on healthy animals and food of animal origin to facilitate early detection of acquired resistance [22]. Their use, however, does make direct comparisons with previous studies that used clinical breakpoints more difficult due to changes in breakpoint values. Nevertheless, when combined with efforts to conduct antimicrobial susceptibility testing on all *E. coli*, *Salmonella, E. faecium, E. faecalis* and *E. hirae* isolates the present study provides analysis on an increased number of isolates from the 2013 study.

The overall outcomes of this study reinforce the findings of the 2013 study with 94.0% of *Salmonella*, 83.8% of *E. coli* and 75.8% of *Enterococcus* isolates considered WT for all antimicrobials tested. Furthermore, NWT isolates were most likely to demonstrate reduced susceptibility to older antimicrobials such as aminoglycosides, tetracyclines and phenicols. Notwithstanding, there were small numbers of isolates that were deemed NWT for highly or critically important antimicrobials such as 3rd generation cephalosporins, quinolones and oxazolidinones [23]. Of note was the isolation of bla_{CMY-2} containing *Salmonella* from grain-fed beef cattle and dairy cattle that had reduced susceptibility to ceftriaxone and ampicillin. For *E. coli*, a single grass-fed beef cattle, one dairy cattle and two veal calf isolates were considered NWT for ciprofloxacin and an additional five isolates that were NWT for ceftriaxone. Three of the five isolates were associated with grass-fed beef cattle and the remaining two with dairy cattle with a single isolate from each animal class also deemed NWT for ceftiofur. Six *E. faecium* isolates from across all three animal classes were NWT for linezolid with

three of these isolates also demonstrating reduced susceptibility to multiple classes of antimicrobials. The beef cattle isolates that were NWT for linezolid were isolated from feedlot cattle, as were isolates that were NWT for virginamycin, an antimicrobial used in grain-fed production systems to restrict acidosis [18]. *E*. *faecalis* isolates were more likely to be NWT for daptomycin with 11 isolates across three animal classes having this phenotype. Interestingly, there was an association with grass-fed beef cattle and contrasts with the trend of NWT *E. faecium* isolates. Three *E. faecalis* isolates were NWT for linezolid with the single dairy isolate in this group also NWT for vancomycin. Limited ECOFF values exist for *E. hirae* and consequently the NWT phenotypes observed in this group of isolates were unremarkable.

This study was conducted to determine if trends can be identified that suggest that the development of NWT populations of bacteria from beef cattle, dairy cattle or veal calves at slaughter is occurring. The bacterial-antimicrobial NWT populations worthy of further investigation were identified and although minor differences were observed, this study permits the Australian beef industry to arrive at the same conclusion as the 2013 study. That is, populations of NWT isolates to antimicrobials considered highly or critically important to human medicine are low and there is limited evidence of specific production practices, such as grain-feeding, leading to widespread disproportionate development of NWT isolates.

3.5 References

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4 Appendix 1

Table A1-1. Breakdown of sample number and type collected from each establishment

	Beef	Beef	Dairy	Dairy	Veal	Veal
Establishment Number	Weekly Production (%)	Total Samples Collected (%)	Weekly Production (%)	Total Samples Collected (%)	Weekly Production (%)	Total Samples Collected (%)
1	6200 (6.0)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2	5400 (5.3)	18 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
3	13500 (13.2)	72 (12.2)	1800 (45.3)	27 (13.9)	0 (0.0)	0 (0.0)
4	2000 (1.9)	11 (1.9)	0 (0.0)	13 (6.7)	0 (0.0)	0 (0.0)
5	1500 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1000 (36.9)	80 (37.0)
6	1750 (1.7)	0 (0.0)	1750 (44.0)	107 (55.2)	0 (0.0)	0 (0.0)
7	Not provided	11 (1.9)	Not provided	1 (0.5)	Not provided	0 (0.0)
8	3040 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	160 (5.9)	24 (11.1)
9	5500 (5.4)	1 (0.2)	100 (2.5)	26 (13.4)	50 (1.8)	12 (5.6)
10	5643 (5.5)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
11	2400 (2.3)	12 (2.0)	100 (2.5)	12 (6.2)	0 (0.0)	0 (0.0)

	Beef	Beef	Dairy	Dairy	Veal	Veal
Establishment Number	Weekly Production (%)	Total Samples Collected (%)	Weekly Production (%)	Total Samples Collected (%)	Weekly Production (%)	Total Samples Collected (%)
12	2950 (2.9)	24 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
13	4200 (4.1)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
14	2640 (2.6)	24 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
15	2500 (2.4)	28 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
16	2508 (2.4)	24 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
17	3800 (3.7)	0 (0.0)	100 (2.5)	0 (0.0)	1500 (55.4)	100 (46.3)
18	4168 (4.1)	30 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
19	5000 (4.9)	28 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
20	4500 (4.4)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
21	4250 (4.1)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
22	8000 (7.8)	40 (6.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
23	6500 (6.3)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
24	2400 (2.3)	36 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
25	2295 (2.2)	16 (2.7)	125 (3.1)	8 (4.1)	0 (0.0)	0 (0.0)

5 Appendix 2: Comparison of 2013 and 2019 surveys

It is useful to compare AMR studies of equivalent robustness to identify shifts in the susceptibility of isolates to selected antimicrobials. Direct comparison of the results provided in this report with the outcomes of the 2013 study are not possible as the 2013 used clinical breakpoints and the 2019 study used ECOFFs. Clinical breakpoints represent the concentration of an antimicrobial at which clinical treatment is likely to be ineffective (i.e. clinical resistance) whereas ECOFFs represent microbiological resistance. The following sections use the 2019 ECOFFs on the 2013 data and the 2013 clinical breakpoints on the 2019 data to provide a comparison between the studies.

5.1 *Salmonella*

Table A2-1 Comparison of antimicrobial susceptibility testing of *Salmonella* from the 2013 and 2019 surveys using the 2019 ECOFF values

Antimicrobial	Class	ECOFF 2019	2013 NWT %			2019 NWT %		
			Beef (n=106)	Dairy (n=75)	Veal (n=36)	Beef (n=83)	Dairy (n=34)	Veal (n=67)
Ampicillin	Beta lactams (bla)	8	8.4	0.0	0.0	3.6	5.9	1.5
Cefoxitin	Cephems 1g (c1g)	8	0.9	0.0	0.0	3.6	2.9	0.0
Ceftiofur	Cephems 3g (c3g)	2	0.0[a]	0.0	0.0	3.6	2.9	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	Quinolones (qui)	0.063	3.2	3.2	0.0	1.2	0.0	0.0
Florfenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	Aminoglycosides (ami)	2	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	Carbapenems (car)	0.125	1.8	1.3	0.0	0.0	0.0	0.0
Nalidixic acid	Quinolones (qui)	8	0.0	0.0	0.0	0.0	0.0	0.0
Streptomycin	Aminoglycosides (ami)	16	13.3[b]	8.0	8.3	3.6	5.9	1.5
Tetracycline	Tetracyclines (tet)	8	6.6	0.0	0.0	0.0	2.9	0.0

[a] Cells shaded green indicate percent non-wild-type that is lower than the 95% CI quoted in the 2019 study; [b] Cells shaded red indicate percent non-wild-type that is higher than the 95% CI quoted in the 2019 study.

Table A2-2 Comparison of antimicrobial susceptibility testing of *Salmonella* from the 2013 and 2019 surveys using the 2013 clinical breakpoints

Antimicrobial	Class	Breakpoint 2013	2013 Resistance %			2019 Resistance %		
			Beef (n=106)	Dairy (n=75)	Veal (n=36)	Beef (n=83)	Dairy (n=34)	Veal (n=67)
Amoxicillin-Clavulanate	Beta lactams (bla)	16	0.0	0.0	0.0	3.6[b]	2.9	0.0
Ampicillin	Beta lactams (bla)	16	7.5	0.0	0.0	0.0[a]	0.0	0.0
Cefoxitin	Cephems 1g (c1g)	16	0.0	0.0	0.0	3.6	2.9	0.0
Ceftiofur	Cephems 3g (c3g)	4	0.0	0.0	0.0	3.6	2.9	0.0
Ceftriaxone	Cephems 3g (c3g)	2	0.0	0.0	0.0	3.6	2.9	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	Quinolones (qui)	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	Aminoglycosides (ami)	8	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	Carbapenems (car)	4	0.0	0.0	0.0	0.0	0.0	0.0
Nalidixic acid	Quinolones (qui)	16	0.0	0.0	0.0	0.0	0.0	0.0
Streptomycin	Aminoglycosides (ami)	32	7.5	0.0	0.0	0.0	0.0	0.0
Tetracycline	Tetracyclines (tet)	8	6.6	0.0	0.0	0.0	2.9	0.0
Trimethoprim/ sulfamethoxazole	Folate pathway inhibitors (fpi)	2	7.5	0.0	0.0	0.0	0.0	0.0

[a] Cells shaded green indicate percent resistance that is lower than the 95% CI quoted in the 2013 study; [b] Cells shaded red indicate percent resistance that is higher than the 95% CI quoted in the 2013 study.

Additional notes:

- Amoxicillin / clavulanate, azithromycin, ceftriaxone and trimethoprim / sulfamethoxazole removed from NWT table as no ECOFF available.
- Cefazolin, cefotaxime, kanamycin removed from breakpoint Table as not used in 2019 survey.
- 2013 survey had 9/106 beef cattle Salmonella that were resistant to 1 or more antimicrobials. Six were AMP-STR-TET-SXT.
- 2019 survey had 7/83 beef cattle, 3/34 dairy and 1/67 veal Salmonella that were NWT to 1 or more antimicrobials.
- Three feedlot cattle and a dairy cattle Salmonella harboured CMY2 and had the profiles BLA-C1G-C3G or BLA-C1G-C3G-TET.
- The three feedlot Salmonella harbouring CMY2 were collected at a single abattoir on a single day.
- All Salmonella with CMY2 would be considered resistant to amoxicillin / clavulanate using 2013 breakpoints.

Multidrug resistance:

- 2013 - observed in beef cattle isolates only with 8/106 (7.5%) classified as MDR. Majority were from feedlot cattle.
- 2019 - observed in 3/83 (3.6%) beef cattle and 1/34 (2.9%) of dairy isolates. Not seen in Salmonella from veal. Beef cattle isolates were from feedlot cattle but are clustered to a single plant on a single day

5.2 *E. coli*

Table A2-3 Comparison of antimicrobial susceptibility testing of *E. coli* from the 2013 and 2019 surveys using the 2019 ECOFF values

Antimicrobial	Class	ECOFF 2019	2013 NWT %			2019 NWT %		
			Beef (n=469)	Dairy (n=155)	Veal (n=176)	Beef (n=574)	Dairy (n=186)	Veal (n=209)
Ampicillin	Beta lactams (bla)	8	0.4[a]	2.6	5.7	4.7	3.8	3.3
Cefoxitin	Cephems 1g (c1g)	8	0.6	0.0	0.6	1.2	1.1	1.0
Ceftiofur	Cephems 3g (c3g)	1	0.0	0.0	0.6	0.2	0.5	0.0
Ceftriaxone	Cephems 3g (c3g)	0.125	1.7[b]	1.3	3.5	0.5	1.1	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	Quinolones (qui)	0.063	0.2	0.0	0.0	0.2	0.5	1.0
Florfenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	Aminoglycosides (ami)	2	0.6	0.6	2.3	0.2	0.5	0.0
Meropenem	Carbapenems (car)	0.125	0.2	0.0	0.0	0.0	0.0	0.0
Nalidixic acid	Quinolones (qui)	8	0.2	0.0	0.0	0.2	0.0	0.0
Streptomycin	Aminoglycosides (ami)	16	2.2	1.9	4.0	4.5	3.8	5.7
Tetracycline	Tetracyclines (tet)	8	7.7	2.6	4.5	15.9	8.1	9.1
Trimethoprim/ sulfamethoxazole	Folate pathway inhibitors (fpi)	0.25	1.3	1.9	3.5	2.8	1.1	1.4

[a] Cells shaded green indicate percent non-wild-type that is lower than the 95% CI quoted in the 2019 study; [b] Cells shaded red indicate percent non-wild-type that is higher than the 95% CI quoted in the 2019 study.

Table A2-4 Comparison of antimicrobial susceptibility testing of *E. coli* from the 2013 and 2019 surveys using the 2013 clinical breakpoints

Antimicrobial	Class	Breakpoint 2013	2013 Resistance %			2019 Resistance %		
			Beef (n=469)	Dairy (n=155)	Veal (n=176)	Beef (n=574)	Dairy (n=186)	Veal (n=209)
Amoxicillin-Clavulanate	Beta lactams (bla)	16	0.0	0.0	1.1	0.2	0.0	0.0
Ampicillin	Beta lactams (bla)	16	0.0	2.6	4.5	4.7[b]	3.2	3.3
Cefoxitin	Cephems 1g (c1g)	16	0.0	0.0	0.0	0.2	0.0	0.0
Ceftiofur	Cephems 3g (c3g)	4	0.0	0.0	0.6	0.2	0.0	0.0[a]
Ceftriaxone	Cephems 3g (c3g)	2	0.0	0.0	0.0	0.1	0.0	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	Quinolones (qui)	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	Aminoglycosides (ami)	8	0.0	0.0	0.6	0.0	0.0	0.0
Meropenem	Carbapenems (car)	4	0.0	0.0	0.0	0.0	0.0	0.0
Nalidixic acid	Quinolones (qui)	16	0.0	0.0	0.0	0.2	0.0	0.0
Streptomycin	Aminoglycosides (ami)	32	1.1	1.9	4.0	4.2	0.5	3.8
Tetracycline	Tetracyclines (tet)	8	7.7	2.6	4.5	15.9	8.1	9.1
Trimethoprim/ sulfamethoxazole	Folate pathway inhibitors (fpi)	2	0.0	1.3	2.3	2.1	1.1	0.5

[a] Cells shaded green indicate percent resistance that is lower than the 95% CI quoted in the 2013 study; [b] Cells shaded red indicate percent resistance that is higher than the 95% CI quoted in the 2013 study.

5.3 *Enterococcus faecium*

Table A2-5 Comparison of antimicrobial susceptibility testing of *E. faecium* from the 2013 and 2019 surveys using the 2019 ECOFF values

Antimicrobial	Class	ECOFF 2019	2013 NWT % *E. faecium* (n=120)	2019 NWT % Beef (n=180)	Dairy (n=80)	Veal (n=83)
Ampicillin	Beta lactams (bla)	4	0.0	0.6	0.0	0.0
Benzylpenicillin	Beta lactams (bla)	16	0.0	0.6	0.0	0.0
Chloramphenicol	Phenicols (phe)	32	0.0	0.0	0.0	0.0
Daptomycin	Lipopeptides (lip)	8	0.0	0.0	0.0	0.0
Erythromycin	Macrolides (mac)	4	8.3[a]	26.1	15.0	7.2
Gentamicin	Aminoglycosides (ami)	32	0.8	0.0	1.3	1.2
Linezolid	Oxazolidinones (oxa)	4	0.0	1.1	2.5	2.4
Teicoplanin	Glycopeptides (gly)	2	0.0	0.0	0.0	0.0
Tetracycline	Tetracyclines (tet)	4	13.4	10.0	6.3	1.2
Vancomycin	Glycopeptides (gly)	4	0.8	0.0	0.0	0.0
Virginiamycin	Streptogramins (str)	4	0.0	10.0	0.0	2.4

[a] Cells shaded green indicate percent non-wild-type that is lower than the 95% CI quoted in the 2019 study

Table A2-6 Comparison of antimicrobial susceptibility testing of *E. faecium* from the 2013 and 2019 surveys using the 2013 clinical breakpoints

Antimicrobial	Class	Breakpoint 2013	Resistance % *E. faecium* (n=120)	2019 Resistance % Beef (n=180)	Dairy (n=80)	Veal (n=83)
Ampicillin	Beta lactams (bla)	8	0.0	0.6	0.0	0.0
Benzylpenicillin	Beta lactams (bla)	8	0.0	0.6	0.0	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.6	0.0	0.0
Daptomycin	Lipopeptides (lip)	4	2.5	22.8[b]	52.5	30.1
Erythromycin	Macrolides (mac)	4	8.3	26.1	15.0	7.2
Gentamicin	Aminoglycosides (ami)	256	0.0	0.0	0.0	0.0
Kanamycin	Aminoglycosides (ami)	512	0.8	7.2	8.8	9.6
Lincomycin	Lincosamides (lin)	4	94.2	58.5[a]	39.3	38.2
Linezolid	Oxazolidinones (oxa)	4	0.0	1.1	2.5	2.4
Streptomycin	Aminoglycosides (ami)	512	0.0	1.1	1.3	0.0
Teicoplanin	Glycopeptides (gly)	2	0.0	0.0	0.0	0.0
Tetracycline	Tetracyclines (tet)	8	11.7	10.0	6.3	1.2
Vancomycin	Glycopeptides (gly)	16	0.0	0.0	0.0	0.0
Virginiamycin	Streptogramins (str)	4	0.0	10.0	0.0	2.4

[a] Cells shaded green indicate percent resistance that is lower than the 95% CI quoted in the 2013 study; [b] Cells shaded red indicate percent resistance that is higher than the 95% CI quoted in the 2013 study.

Additional notes:

- Kanamycin, lincomycin, quinupristin / dalfopristin and streptomycin removed from NWT table as not analysed or no ECOFF in 2019.
- Quinupristin /dalfoprisitn was removed from the 2013 analysis due to a lack of concordance with follow up disc-susceptibility testing.
- Erythromycin breakpoint and ECOFF is the same for both studies therefore 2-3-fold increases for dairy cattle and beef cattle isolates, respectively in 2019.
- Daptomycin resistance increases significantly in 2019 if 2013 breakpoint applied. Investigations of isolates in 2013 could not identify genetic basis for resistance and the shift to an ECOFF of 8 more accurately represents a cut-off where AMR genes are likely to be present.
- Virginiamycin breakpoint and ECOFF is the same for both studies therefore an increase in resistant or NWT isolates was observed in 2019.
- Isolates that were NWT for erythromycin, daptomycin and tetracycline were most often from feedlot cattle.
- Six isolates (2 from each animal group) were NWT for linezolid. The two beef cattle isolates were from feedlot cattle.

Multidrug resistance:

- 2013 - observed in 5/120 (4.2%) of all E. faecium isolates.
- 2019 - observed in 26/180 (14.4%) beef cattle, 10/80 (12.5%) of dairy isolates and 5/83 (6.0%) of veal calf isolates. Beef cattle MDR isolates were most likely from feedlot cattle 19/26 (73.1%).

5.4 Enterococcus faecalis

Table A2-7 Comparison of antimicrobial susceptibility testing of *E. faecalis* from the 2013 and 2019 surveys using the 2019 ECOFF values

Antimicrobial	Class	ECOFF 2019	2013 NWT % *E. faecalis* (n=96)	2019 NWT % Beef (n=70)	Dairy (n=10)	Veal (n=12)
Ampicillin	Beta lactams (bla)	4	0.0	0.0	0.0	0.0
Benzylpenicillin	Beta lactams (bla)	16	0.0	0.0	0.0	0.0
Chloramphenicol	Phenicols (phe)	32	0.0	0.0	0.0	8.3
Daptomycin	Lipopeptides (lip)	4	9.4	11.4	10.0	16.7
Erythromycin	Macrolides (mac)	4	10.4[b]	0.0	0.0	8.3
Gentamicin	Aminoglycosides (ami)	32	0.0	0.0	0.0	0.0
Linezolid	Oxazolidinones (oxa)	4	0.0[a]	4.3	30.0	0.0
Streptomycin	Aminoglycosides (ami)	512	1.0	2.9	0.0	25.0
Teicoplanin	Glycopeptides (gly)	2	0.0	0.0	10.0	0.0
Tetracycline	Tetracyclines (tet)	4	11.5	4.3	20.0	25.0
Vancomycin	Glycopeptides (gly)	4	2.1	0.0	10.0	0.0
Virginiamycin	Streptogramins (str)	32	0.0	0.0	0.0	0.0

[a] Cells shaded green indicate percent non-wild-type that is lower than the 95% CI quoted in the 2019 study; [b] Cells shaded red indicate percent non-wild-type that is higher than the 95% CI quoted in the 2019 study.

Table A2-8 Comparison of antimicrobial susceptibility testing of *E. faecium* from the 2013 and 2019 surveys using the 2013 clinical breakpoints

Antimicrobial	Class	Breakpoint 2013	2013 Resistance % *E. faecalis* (n=96)	2019 Resistance % Beef (n=70)	Dairy (n=10)	Veal (n=12)
Ampicillin	Beta lactams (bla)	8	0.0	0.0	0.0	0.0
Benzylpenicillin	Beta lactams (bla)	8	0.0	0.0	0.0	0.0
Chloramphenicol	Phenicols (phe)	16	0.0	0.0	0.0	8.3
Daptomycin	Lipopeptides (lip)	4	9.4	11.4	10.0	16.7
Erythromycin	Macrolides (mac)	4	10.4	0.0	0.0	8.3
Gentamicin	Aminoglycosides (ami)	256	0.0	0.0	0.0	0.0
Kanamycin	Aminoglycosides (ami)	512	1.0	0.0	0.0	0.0
Lincomycin	Lincosamides (lin)	4	85.4	91.4	100.0	100.0
Linezolid	Oxazolidinones (oxa)	4	0.0	4.3	30.0	0.0
Streptomycin	Aminoglycosides (ami)	512	1.0	2.9	0.0	25.0
Teicoplanin	Glycopeptides (gly)	2	0.0	0.0	10.0	0.0
Tetracycline	Tetracyclines (tet)	8	7.3	2.9	20.0	25.0
Vancomycin	Glycopeptides (gly)	16	0.0	0.0	10.0	0.0

[a] Cells shaded green indicate percent resistance that is lower than the 95% CI quoted in the 2013 study; [b] Cells shaded red indicate percent resistance that is higher than the 95% CI quoted in the 2013 study.

Additional notes:

- Kanamycin and lincomycin removed from NWT table as not analysed or no ECOFF in 2019.
- Small numbers of isolates from dairy and veal do not allow robust comparisons to be made for these animal groups. Beef isolates from 2019 were compared with overall findings from 2013.
- Erythromycin breakpoint and ECOFF is the same for both studies therefore the 2019 study has lower resistance or NWT percentages than the 2013 study.
- Six isolates (3 beef cattle and 3 dairy) are NWT for linezolid. The beef isolates were all from grass-fed animals.

Multidrug resistance:

- 2013 - observed in 12/96 (12.5%) of all E. faecalis isolates.
- 2019 - observed in 3/70 (4.3%) beef cattle, 2/10 (20%) of dairy isolates and 3/12 (25%) of veal calf isolates. Of the beef cattle MDR isolates, two were from grass-fed cattle and one from feedlot cattle.