Final report

Longitudinal analysis of antimicrobial resistance of E.coli, Salmonella, and Enterococcus species during pre-feedlot, feedlot and slaughter periods

Project code: B.FLT.3003

Prepared by: Yohannes E. Messele, Mauida A. Khallawi, Tania Veltman, Darren Trott, Stephen P. Kidd, Wai Y. Low, Kiro R. Petrovski
The University of Adelaide

Date published:

PUBLISHED BY
Meat and Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

Identification of antimicrobial-resistant bacteria in agricultural settings may at times create issues for both producers and consumers. Selection pressure from the direct use of different antimicrobial classes for treatment, metaphylaxis and prophylaxis is the main factor contributing to the development of antimicrobial resistance (AMR) in food-producing animals, but is unlikely to be the only factor. Australia has followed a strict approach on the registration of antimicrobials for use in food-producing animals and conducts periodic assessments on the status of AMR bacteria in healthy livestock at slaughter. Although previous research conducted in Australia reported that the enteric bacteria isolated from cattle harboured a low number of AMR determinants, continuing surveillance is important. This report outlines the status of AMR in *E. coli*, *Salmonella* and *Enterococcus* species isolated from cattle cohorts in Southern Australia, commencing at the entry into the beef feedlot ('entry'), and, following animals through to slaughter ('exit'). The target bacteria were isolated from a variety of sample sources including the individual faecal samples at entry and exit, pen level fresh faecal pats (home and hospital pens), carcase swabs and lymph nodes. A total of 409 *Escherichia coli*, 24 *Salmonella* sp. and 696 *Enterococcus* spp. isolates were obtained and tested for resistance to 14-16 antimicrobials, including those used in both human and veterinary medicine. At entry into the feedlot (i.e. faeces collected per rectum), little resistance was observed among *E. coli* isolates, with the highest prevalence obtained for ampicillin and clavulanic acid-potentiated amoxicillin (both 1.5%). At exit (i.e. faeces collected per rectum after slaughter), the highest prevalence of resistance among *E. coli* was obtained for tetracycline (20.7%), followed by ampicillin (9.6%), streptomycin (7.4%), sulfisoxasole (6.7%), and ceftiofur (4.4%). All *E. coli* isolates were sensitive to ciprofloxacin and gentamycin. The potential associations between resistance phenotypes and resistance genotype was also explored by whole genome sequencing ('WGS'), incorporating screening for known genetic AMR determinants in CARD and ResFinder databases. The most common AMR genes identified in resistant isolates were *tet(B)* (48.5%), *aph(3'')-Ib* and *aph(6)-Id* (both 36.4%), $bla_{\text{TEM-1B}}$ (27.3%), and *sul2* (27.3%), responsible for resistance to tetracyclines, aminoglycosides, β-lactams and sulfonamides, respectively. Overall, observed AMR genes were correlated with phenotypic resistance in almost all cases. Pre-harvest interventions have reduced microbial contamination on carcases, evident because no E. coli detected from carcase samples. Three S*almonella* serovars, *S. infantis* (n=20), *S. bovismorbificans* (n=3) and *S. anatum* (n=1), were isolated from faecal pats (10/126; 7.9%) and lymph nodes (14/290; 4.8%). All *Salmonella* isolates were sensitive to all antimicrobial tested. Overall, 12 species within the *Enterococcus* genus were identified with *Enterococcus faecium* (53.6%), *Enterococcus hirae* (34.2%), *Enterococcus gilvus* (3.6%), *Enterococcus faecalis* (2.4%), and *Enterococcus mundtti* (2.3%) most common. Based on National Antimicrobial Resistance Monitoring System (NARMS) and Clinical and Laboratory Standards Institute (CLSI) breakpoints, the highest prevalence of resistance among enterococci isolated from rectal faeces at entry was observed to lincomycin (60.6%), followed by daptomycin (25.0%), nitrofurantoin (8.7%), ciprofloxacin (6.7%), tetracycline (4.8%), tigecycline (3.9%), and quinupristin/dalfopristin (2.9%). Among these, *E. faecium* isolates (n=9) were resistant to ciprofloxacin (77.8%), lincomycin (33.3%), tetracycline (33.0%), quinupristin/dalfopristin, and nitrofurantoin (22.2% each). At exit, resistance to lincomycin (84.0%), nitrofurantoin (53.5%), daptomycin (22.9%), quinupristin/dalfopristin (18.1%), ciprofloxacin (7.6%), and tetracycline (6.9%) was observed among the 144 *Enterococcus* spp. isolates. From these, a significant

proportion of *E. faecium* (n=117) were found to be resistant to lincomycin (82.9%), nitrofurantoin (61.5%), quinupristin/dalfopristin (21.4%), daptomycin (17.9%), and ciprofloxacin (9.4%). Ciprofloxacin and quinupristin/dalfopristin resistance were detected only in E. faecium isolates and the majority were observed at the entry into the feedlot. In contrast, higher daptomycin and tigecycline resistance was observed in E. hirae isolates.

The shift in specific *Enterococcus* spp. isolated, with a higher prevalence of *E. faecium* identified at exit compared to entry, was a noteworthy finding of the study. All *Enterococcus* isolates were sensitive to chloramphenicol, gentamycin, linezolid, penicillin, and vancomycin. The ciprofloxacin, daptomycin, erythromycin, quinupristin/dalfopristin, and tigecycline-resistant *E. faecium* (n=62) and *E. hirae* (n=47) isolates from rectal faeces were selected for further molecular analysis by WGS. Overall, 14 AMR genes including (*aac(6')Ii*, *aac(6')-Iid*, *ant(6)-Ia*, *pbp5*, *eatAv*, *lnu(G)*, *vat(E)*, *msr(C)*, *erm(B)*, *efmA*, *tet(45), tet(L)*, *tet(M),* and *tet(S)* were observed in *E. faecium* isolates submitted for WGS with a high proportions of isolates carrying *aac (6')-Ii* and *msr(C)* (95.2%), *eatAv* (75.8%), and *efmA* (33.9%) genes, responsible for resistance to aminoglycosides, macrolides, streptogramin, and fluoroquinolones, respectively. However, none of the daptomycin, nitrofurantoin and tigecycline-resistant strains harboured any recognised AMR genes responsible for the phenotype, possibly suggesting that this may be a testing aberration or unknown resistance mechanism rather than encoded by previously reported resistance genes. Further research is required to identify the molecular mechanism of resistance of those antimicrobials. The proportion of E. coli and Enterococcus spp. exhibiting AMR from treated versus non-treated cattle was not different. This result may have been associated with the exposure of cattle to individuals admitted to the hospital pen but may also be due to changes in the environment and feeding cycle within the feedlot itself.

The results from this study would indicate that further research areas or recommendations to the industry

1. Continuous surveillance of feedlot indicator bacteria is essential
2. Larger sample size and number of pens should be included in the future research
3. Most newly-acquired antimicrobial resistance seems to be associated with exposure to the hospital pen and this needs further investigation, particularly in cattle exposed to high rates of treatment
4. Understanding of backgrounding antimicrobial resistance patterns may be important for feedlot operations and needs to be investigated
5. The origin and basis for daptomycin and nitrofurantoin resistance in *E. faecium* need to be investigated
6. No Salmonella resistance was detected from samples collected. A potential source for humans may be ground beef where large carcase lymph nodes are usually incorporated and testing of ground beef is recommended.
7. Changes in resistance associated with the use of macrolides in hospitalised cattle needs to be examined further (e.g. the risk of changing the azithromycin resistance)
8. To prevent further development of Quinpristin/Dafloprístin resistance in *E. faecium,* the industry should continue focussing on the antimicrobial stewardship, particularly with streptogramin antimicrobials

Table of Contents

Project objectives

- Conduct a longitudinal analysis of AMR of *Escherichia coli*, *Salmonella* and *Enterococcus* species during pre-feedlot, feedlot and slaughter periods to medically important antimicrobials, and determine relationships of prevalence of resistance to antimicrobial usage.
- Determine if bacteria can be cultured from feedlot dust samples downwind of the hospital and home pen facilities and the AMR of those bacteria

1. Introduction

The global need for high quality animal protein derived from food-producing animals has increased dramatically. This rapidly growing demand for animal products has led to the intensification of animal production in many countries, and associated with it, the increased use of antimicrobials. Antimicrobials play a significant role in animal health and welfare, food safety, and public health (Agga et al., 2019). They have been widely used in livestock production systems for different purposes including therapeutic, metaphylactic, prophylactic, or growth promotion, though it is important to note the rapid global decline in the latter type of use, following the withdrawal of avoparcin (Economou and Gousia, 2015). The use of antimicrobials in both humans and animals has been associated with the risk of development of antimicrobial resistance (AMR) in key microorganisms including *Escherichia coli, Salmonella spp.,* and *Enterococcus faecium* (Moudgil et al., 2018; Sharma et al., 2017).

The frequent use of antimicrobials may result in selection pressure which initiates resistance evolution in bacteria, either through mutational changes in antimicrobial target genes or via the horizontal acquisition of specific AMR genes. Bacteria may also be intrinsically resistant to certain antimicrobial classes. Both commensals and pathogenic microbes can act as a reservoir of AMR gene elements. The spread of AMR genes by mobile genetic elements can be mediated by environment and host factors, or the properties of the genetic elements themselves (van Hoek et al., 2011). The most significant mobile genetic elements involved in horizontal gene transfer are those that use the process of conjugation to move to another host (e.g. plasmids and integrative conjugative elements). Bacteriophages also play a vital role in the dissemination of DNA between bacteria in a process called transduction (Johnson and Grossman, 2015). Gene transfer is favoured between closely related microorganisms, but it can also occur between different species and genera (Wiedenbeck and Cohan, 2011). For example, enterococci may transmit vancomycin resistance to more pathogenic microorganisms such as methicillin-resistant *Staphylococcus aureus* (Kos et al., 2012).

The potential for transfer of AMR from enteric bacteria in animals to humans is a global public health concern (de Jong et al., 2018). Genetic similarity has been reported to exist between certain animal bacterial strains and those causing infections in humans, in particular the zoonotic foodborne pathogens *Salmonella* and *Campylobacter* (Hermanovská et al., 2016; Yılmaz et al., 2016). The transfer of resistance determinants between bacteria derived from animal and human sources has also been reported (Bourafa et al., 2015; Hammerum, 2012). However, in many recent studies, incorporating whole genome sequencing (**'WGS'**), strains of the same bacterial species isolated from animals and humans have been shown to be unrelated and/or possess distinct AMR genes (O'Dea et al., 2019; Zaheer et al., 2020a). To implement any mitigation strategies, it is necessary to expand knowledge on how AMR is influenced by different risk factors. AMR can be minimised by interventions aimed at the microorganism, resistance gene mechanism, and antimicrobial drug level, as well as directly influencing host-specific factors (e.g. biosecurity and infection control) within the health care, agriculture, and environment sectors (Holmes et al., 2016).

Australia has strict registration and regulation of antimicrobial use in livestock production systems. This minimises the risk of development and spread of resistance to the critically important antimicrobials used in human clinical practice. Despite these restrictions, there is a need for ongoing surveillance of AMR in bacteria that may cause clinical infections in humans and also frequently colonise the gut of healthy livestock (e.g. *E. coli*, *Enterococcus*

spp. and *Salmonella* sp.). In-as-much, it is important to determine the effects of antimicrobial use on the AMR status of these livestock associated micro-organisms, as well as to determine their relatedness to the strains typically causing human infection. Additionally, it is important to determine if antimicrobial use in a feedlot setting contributes to the overall resistance burden in these commensal bacteria and what proportion of animals may be already colonised with resistance phenotypes at feedlot entry. Hence, this project aimed to assess the level of AMR in *E. coli*, *Salmonella* sp. and *Enterococcus* spp. isolated from cattle from entry into the beef feedlot to the end of the chain within the slaughter house.

2. Methodology

2.1. Study animals

A longitudinal study was carried out to determine the AMR status of *E. coli, Salmonella* sp. and *Enterococcus* spp. isolated from cattle in Southern Australia, starting at entry into the beef feedlot (**'entry'**), and, following them to slaughter (**'exit'**). This study was conducted in a large commercial feedlot with a capacity of 17,000 cattle. For this study, a total of 150 cattle that originated from Location A (82), Location B (54), and Location C (14) were used. Cattle arrived at the feedlot in October, 2019 and their average weight was 405 kg. For the duration of the feedlot phase, all cattle were housed in a single pen (the **'target pen'**). The pens left and right from the target pen were the **'neighbouring pen/s'**. Sick cattle were transiently re-located to the pen holding sick individual animals (the **'hospital pen'**).

Antimicrobials used for treatment of sick cattle, for the duration of the study, were tulathromycin, oxytetracycline and ceftiofur. Concurrently, tetracycline-based product was also used for metaphylaxis of cattle arriving to the feedlot from high-risk sources (e.g., cattle bought from saleyards), however it was not used on target pen. When an individual animal from the target pen was exhibiting early signs of illness, it was transferred to the hospital pen for the duration of the treatment period and then returned to the target pen (n=13; termed the '**treated cattle**'). The remainder of the group (n=137) were termed the '**non-treated cattle**'. Among the treated cattle, tulathromycin ceftiofur and oxytetracycline treatment was given to 10, 2 and 1 cattle respectively.

2.2. Sample collection

2.2.1. Faeces sample

Using single use rectal gloves, approximately 15g of faecal sample was collected from the rectum of each incoming animal just before the entry at the feedlot. Fresh faecal deposits (15g) were also collected from the target pen at 2, 4, 6, 8, 10, and 12 weeks after entry. Six fresh faecal pats from healthy animals (never treated with an antimicrobial) were collected per visit from the ground of the pen using single use gloves within 1 min after defecation. Additionally, one sample was pooled from the six samples collected at each sampling point, totaling to 42 faecal pat samples. For the pooled samples, 2 g of faeces from each of the 6 sampled pats were mixed into a sterile falcon tube. At the same time points, fresh faecal deposits were collected from the hospital pen. Similarly, the same amount of sample was collected from the two neighboring pens at monthly intervals. Finally, faecal swab samples were collected following exit from the feedlot at the abattoir using Ames transport media swabs (Copan, Italy). These samples were obtained post-evisceration by incision into the rectum 15–30 cm cranial to the anus following the method described by Abreham et al. (2019). The faecal samples were transported to the laboratory under chilled conditions in EPS box containing a frozen gel packs.

2.2.2. Water sample

Water samples (50mL) were collected from the water trough located at the middle of each pen (target, hospital or neighbouring) starting from the second time point as the collection of

the fresh faecal pats. The water samples were transported to the laboratory under chilled conditions in EPS box containing a frozen gel packs.

2.2.3. Air sample

The air /dust samples were collected one meter high from the ground by Coriolis cyclonic air collector (Bertin technologies, Montigny-le-Bretonneux, France) (time: 5min; capacity: 300L/min; buffer: 10mL 1xPBS 7.2PH; decontamination: 1% H_2O_2). The sample were collected from a distance of 20m and 200m from the target and the hospital pens using slightly modified method described by Tenzin et al. (2019), at the same time points as the collection of the fresh faecal pats. The dust samples were transported to the laboratory under chilled conditions in EPS box containing a frozen gel packs.

2.2.4. Carcase swab

Carcase swab samples were collected immediately after evisceration from the flank and brisket, and hip/round areas using a sterile Puritan's sampling swab (Adelab Scientific, Australia). The sampling was carried out by wiping the swab in vertical and horizontal crossing covering an area of approximately 100 cm^2 for each site of sampling. Two swabs were used for each carcase, with one swab side utilized for the flank and the other side for the brisket area, while the second swab was used for the hip/round area of the carcase. The carcase swabs were transported to the laboratory under chilled conditions in EPS box containing a frozen gel packs.

2.2.5. Lymph node collection

The lymph nodes were also removed immediately after opening the carcase. They were harvested by cutting into the adipose tissue surrounding the node following the method described by Brichta-Harhay et al. (2012). One prescapular and one subiliac lymph node were collected from the chuck and flank, respectively on one half of the carcase. The samples were placed into individual Whirl-Pak sample bags and transported to the laboratory in cool boxes containing frozen ice packs.

2.3. Bacterial isolation

2.3.1. Faecal sample

Isolation of *E. coli* was carried out following the method described by Kidsley et al. (2018). Briefly, ten (10) grams of faeces were added into 7 mL of sterile 0.1% buffered peptone water in a falcon tube. The mixture was vortexed and a sterile cotton tip applicator was used to seed it onto MacConkey agar and Brilliance ESBL agar (Thermofisher Scientific, Australia). A similar approach was used for faecal swab samples collected at the slaughter house. The sample was streaked using a sterile loop and incubated at 37°C ± 2°C for 24 hours. After incubation, one presumptive, well isolated colony was selected from the MacConkey agar and Brilliance ESBL agar, respectively. If colonies grew on ESBL agar and were confirmed to be *E. coli*, the MacConkey agar plate colony was not kept. Similarly, to identify *Enterococcus* spp., the faecal mixture was plated and streaked onto Slanetz and Bartley agar plate (Thermofisher Scientific, Australia) (Vignaroli et al., 2011). The plate was incubated in to 37°C ± 2°C for 48 h. A single, well isolated red, maroon or pink coloured colony was carefully chosen, and subcultured onto

sheep blood agar.The remaining faecal mixture found in the falcon tube was incubated overnight to isolate *Salmonella* according the protocol described previously (Kidsley et al., 2018). After incubation, a sterile cotton tip applicator was used to inoculate into Rappaport Vassiliadis broth and Mannitol selenite broth (Thermofisher Scientific, Australia). After incubating in 37°C ± 2°C for 24h, the broth was briefly vortexed, and aliquots plated onto xylose lysine desoxycholate (XLD) and Salmonella Brilliance agar (Thermofisher Scientific, Australia). The plates were incubated at 37°C ± 2°C for 24 h. Black and purple colonies were selected from XLD and Salmonella Brilliance plates, respectively. All selected colonies were subcultured onto sheep blood agar plates (Thermofisher Scientific, Australia) and incubated at 37° ± 2°C for 24 h. Finally, the identity of all suspected target colonies was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik GMBH, Germany) and stored in -80°C in tryptone soya broth with 20% glycerol.

2.3.2. Water and air samples

The water and air samples were examined for the presence of *E. coli*, *Enterococcus* spp., and *Salmonella* sp. according to a previously described protocol (Beauvais et al., 2018). Briefly, 1 mL of water was taken from the original sample and diluted with 9mL distilled water and filtered by vacuum using 0.45-µm cellulose ester filters. The filters were placed onto the appropriate selective media for each microorganism. After incubation, a single colony was selected from each respective selective agar media. The selected colonies were subcultured onto sheep blood agar plates and incubated at 37°C ± 2°C for 24 h. The identity of the selected colonies was confirmed by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry and pure cultures stored at -80°C in tryptone soya broth plus 20% glycerol.

2.3.3. Lymph node sample

The lymph nodes were processed as described previously by Brichta-Harhay et al. (2012). Briefly, the surrounding fat and fascia were trimmed from lymph nodes, they were submerged into boiling water for 3-5 seconds for surface sterilization, immediately placed into a filtered sample bag and cut into approximately 1 cm^3 with a sterile scalpel blade. Forty mL of peptone water was added in to the bag containing the sample and homogenised using a Stomacher (Lab-Blender 400, Bury St. Edmonds, UK). The homogenised lymph node was incubated at room temperature for 1-2 h. For the identification of *E. coli* and *Enterococcus* spp., a sterile cotton swab was used to immediately inoculate into each respective selective media. For the identification of *Salmonella* sp., the enriched sample was subjected to immune-magnetic separation (IMS) using paramagnetic beads coated with antibodies to *Salmonella* (Dynabeads anti-*Salmonella*, Invitrogen, Oslo, Norway). The IMS product was transferred to Rappaport-vassiliadis broth (bioMerieux, Marcy lE toile, France) and mannitol selenite broth (Thermo Fisher scientific, Australia) followed by incubation at 37°C ± 2°C for 20-24 h. The incubated RVS broth was streaked onto XLD (Thermo Fisher scientific, Australia) and *Salmonella* Brilliance agar (Thermo Fisher Scientific, Australia) prior to incubation at 37°C ± 2°C for 18–20 h. The remainder of the *Salmonella* isolation procedure followed the previously described protocol in section 2.3.1.

2.3.4. Carcase swab

Upon arrival at the laboratory, the samples were processed by adding 5 mL peptone water diluent to each sample prior to homogenisation for 30 s with the help of a stirrer (Alvseike et al., 2019). From the dilution, 100 µL were inoculated onto MacConkey and Brilliance ESBL agars, and Slanetz and Bartley agar (Thermofisher Scientific Australia) to identify *E. coli* and *Enterococcus,* respectively. The remaining sample was incubated overnight and 100 µl was used to inoculate *Salmonella* selective media as described. The plates and broths were incubated at 37°C ± 2°C for 24 hours. Finally, the presumptive identity of the colonies in pure culture was confirmed by MALDI-TOF.

2.4. Antimicrobial susceptibility testing

All isolates of *E. coli, Enterococcus* and *Salmonella* species were subjected to antimicrobial susceptibility testing. Commercially prepared plates were used to test the minimum inhibitory concentration of the isolates, following the Clinical and Laboratory Standards Institute and National Antimicrobial Resistance Monitoring System guidelines (CLSI, 2020; NARMS, 2011). For Gram-negative bacteria, phenotypic susceptibility was determined using the standard Sensititre NARMS Gram-negative CMV3AGNF MIC Plate that included amoxicillin-clavulanate, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole (Table 1). The reference strains were *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *P. Aeruginosa* ATCC 27853. For Gram-positive bacteria, phenotypic susceptibility was determined using the Sensititre NARMS Gram-positive CMV3AGPF Plate that included chloramphenicol, ciprofloxacin, daptomycin, erythromycin, gentamycin, kanamycin, lincomycin, linezolid, nitrofurantoin, penicillin, quinupristin/dalfopristin, streptomycin, tetracycline, tigecycline, tylosin tartrate, and vancomycin (Table2). To date, only a susceptible breakpoint has been established for tigecycline. In this study, ≥ 0.5 µg/mL for tigecycline (NARMS) were used as the resistance cut-off values. The reference strains were *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213.

Table 1. Tested dilution ranges and breakpoints used for the antimicrobial susceptibility testing of *E. coli* and *Salmonella* spp.

Antimicrobial agent	Range	Breakpoints
Amoxicillin/clavulanic acid	1/0.5 - 32/16	≥ 32/16
Ampicillin	1 - 32	≥ 32
Azithromycin	0.12 - 16	> 16
Cefoxitin	0.5 - 32	≥ 32
Ceftiofur	0.12 - 8	≥ 8
Ceftriaxone	0.25 - 64	≥ 4
Chloramphenicol	2 - 32	≥ 32
Ciprofloxacin	0.015 - 4	≥ 1
Gentamycin	0.25 - 16	≥ 16
Nalidixic acid	0.5 - 32	≥ 32
Streptomycin	2 - 64	≥ 64
Sulfisoxazole	16 - 256	> 256
Tetracycline	4 - 32	≥ 16
Trimethoprim/ sulfamethoxazole	0.12/2.38 – 4/76	≥ 4/76

Table 2. Dilution ranges and breakpoints used for antimicrobial susceptibility testing of *Enterococcus* spp. isolates.

Antimicrobial agent	Range	Breakpoints
Chloramphenicol	2 - 32	≥ 32[a]
Ciprofloxacin	0.12 - 4	≥ 4[a]
Daptomycin	0.25 - 16	≥ 8[a]
Erythromycin	0.25 - 8	≥ 8[a]
Gentamicin	128 - 1024	≥ 512[b]
Kanamycin	128 - 1024	≥ 1024[b]
Lincomycin	1 - 8	≥ 8[b]
Linezolid	0.5 - 8	≥ 8[a]
Nitrofurantoin	2 - 64	> 64[a]
Penicillin	0.25 - 16	≥ 16[a]
Streptomycin	512 - 2048	≥ 1024[b]
Quinupristin/dalfopristin	0.5 - 32	≥ 4[a]
Tetracycline	1 - 32	≥ 16[a]
Tigecycline	0.015 – 0.5[c]	≥ 0.5[b]
Tylosin tartrate	0.25 - 32	≥ 32[b]
Vancomycin	0.25 - 32	≥ 32[a]

[a] Clinical and Laboratory Standards Institute guidelines; [b] National Antimicrobial Resistance Monitoring System; [c] only breakpoint for sensitivity established

2.5. DNA extraction and whole genome sequencing

Among isolates obtained from rectal faeces, all antimicrobial-resistant *E. coli* as well as *E. faecium and E. hirae* exhibiting resistance to ciprofloxacin, daptomycin, erythromycin, quinupristin/dalfopristin, and/or tigecycline isolated from rectal faeces were further investigated for the presence of AMR genes by WGS. Pure bacterial culture plates were sent to SA Pathology (Adelaide, Australia). Genomic DNA was extracted with a QIASymphony Virus/Pathogen DSP kit on a QIASymphony instrument per the manufacturer's instructions. WGS was performed using NextSeq 550 platform and NextSeq MID-output (2x150bp) – paired end sequencing kit. Libraries were prepared by following the Nextera XT Library preparation with Nextera XT indices. Reads were trimmed by removing ambiguous nucleotides and those with Phred scores of 30. Assembly was performed *de novo* for each isolate with Shovill (v1.0.9) + contig filtering with seqtk (v1.3-r106). Contig filtering was performed to remove contigs less than 1000bp. The AMR genes were predicted by the Antibiotic Resistance Genes Database (ARDB) the Comprehensive Antibiotic Resistance Database (Alcock et al., 2020), ResFinder 4.0 (Bortolaia et al., 2020) and PointFinder (Zankari et al., 2017).

2.6. Data analysis

A categorical table was generated with either a presence or an absence of a result for each isolate for each sampling point. Isolate susceptibility was first dichotomised as resistant and sensitive (the later included both sensitive and intermediate classifications) and analysed using logistic regression. Tables showing the prevalence of resistance per bacterial species and sample types were prepared. Statistical analysis was performed in STATA version 15.0 (Stata Corporation, College Station, TX, USA) or the R Statistical Package version 4.0.0. The significance level was set at $P \leq 0.05$. A day effect was analysed for a specific sample type for each sampling point and its association with the resistance outcome. The AMR patterns were assessed for each sample type. The effect of antimicrobial use was estimated using a logistic

regression accounting for the effect of treatment on the AMR pattern. The effect of treatment on the AMR pattern was compared between treated and non-treated cattle. The frequency of resistance for each antimicrobial agent was described as rare: <0.1%; very low: 0.1% to 1.0%; low: >1.0% to 10.0%; moderate: >10.0% to 20.0%; high: >20.0% to 50.0%; very high: >50.0% to 70.0%; and extremely high: >70.0%; according to the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (EFSA) (EFSA, 2017).

3. Results

3.1. *Escherichia coli*

No difference in the relative recovery rate of *E. coli* in faecal samples collected from the entry and exit of the feedlot program was observed (Table 3).

Table 3. The number and proportions of *Escherichia coli* isolated from different samples.

Sample source	Type of sample	Number of samples	*E.coli* isolates (%)		
			Non-ESBL	ESBL	Total
Entry	Rectal faeces	150	135 (90.0)	1 (0.7)	136 (90.7)
Feedlot	Target pen faecal pat	42	38 (90.5)	4 (9.5)	42 (100.0)
	Hospital pen faecal pat	42	10 (23.8)	32 (76.2)	42 (100.0)
	Neighboring pens faecal pat	42	40 (95.2)	2 (4.8)	42 (100.0)
	Target pen water	5	0	0	0
	Hospital pen water	5	3 (60.0)	1 (20.0)	4 (80.0)
	Neighboring pens water	6	3 (50.0)	0	3 (50.0)
	Target pen air	12	0	0	0
	Hospital pen air	12	3 (25.0)	0	3 (25.0)
Exit	Rectal faeces	150	129 (86.0)	6 (4.0)	135 (90.0)
	Hip/round swab	150	0	0	0
	flank and brisket swab	149	0	0	0
	Prescapular lymph node	146	1 (0.7)	0	1 (0.7)
	Subiliac lymph node	144	1 (0.7)	0	1 (0.7)

3.1.1. Phenotypic determination of antimicrobial resistance

3.1.1.1. Rectal faeces

A total of 136 (90.7%) isolates of *E. coli* were recovered from 150 beef cattle at entry to the feedlot. Among these, only a single sample (0.7%) yielded an *E. coli* isolate on Brilliance ESBL agar at extremely low abundance (indicating ceftiofur resistance). Overall, the majority of isolates (97.8%) were pan-susceptible to all tested antimicrobials. The highest frequency of resistance was observed to ampicillin and clavulanic acid-potentiated amoxicillin (2/136; 1.5%). Similarly, a total of 135 (90.0%) isolates of *E. coli* were cultured from the faecal samples collected at the slaughterhouse. From these, putative ESBL-producing *E. coli* were isolated from the Brilliance ESBL agar for 6/150 (4.4%) samples only. None of the ESBL producing E. coli isolated at slaughter was from the individual that entered the feedlot with it, however one of the isolates was from cattle which received ceftiofur treatment in the feedlot. Among the *E. coli* isolated from slaughterhouse rectal faeces, the frequency of resistance to at least one antimicrobial was 23.7%. The highest frequency of resistance was observed to tetracycline (20.7%), followed by ampicillin (9.6%), streptomycin (7.4%), sulfisoxasole (6.7%), and ceftiofur (4.4%). However, all isolates were susceptible to ciprofloxacin, gentamicin, and nalidixic acid (Table 4).

Table 4. Results of the antimicrobial susceptibility testing of *Escherichia coli* isolated from rectal faeces at feedlot entry (n=136) and exit (n=135).

Antimicrobial class	Antimicrobial agent	Sample (150 each)	Resistant (%)	95 % CI	Proportion of isolates per MIC value (µg/mL)*															
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Aminoglycosides	Gentamycin	Entry	0.0	-						63.2	36	0.7								
		Exit	0.0	-					3.7	72.6	23			0.7						
	Streptomycin	Entry	0.7	0.10-5.03									55.1	43.4	0.7		0.7			
		Exit	7.4	4.03-13.22									52.6	37.8	1.5	0.7	5.2	2.2		
β-lactam	Ampicillin	Entry	1.5	0.37-5.69							6.6	46.3	43.4	0.7	1.5	1.5				
		Exit	9.6	4.59-14.67							14.8	45.2	27.4	2.2	0.7		9.6			
	Amoxicillin/clavulanic acid	Entry	1.5	0.37-5.69							3.7	15.4	65.4	13.2	0.7	1.5				
		Exit	1.5	0.37-5.73							5.9	34.1	46.7	11.8		1.5				
	Cefoxitin	Entry	0.7	0.10-5.03								26.5	64	8.8		0.7				
		Exit	1.5	0.37-5.73							11.1	30.4	37.8	19.3			1.5			
	Ceftiofur	Entry	0.7	0.10-5.03				0.7	14	78.7	5.1	0.7		0.7						
		Exit	4.4	2.05-9.54				11.8	27.4	50.4	5.9				4.4					
	Ceftriaxone	Entry	0.7	0.10-5.03					96.3	1.5	1.5				0.7					
		Exit	4.4	2.05-9.54					95.6							0.7		3.7		
Folate pathway inhibitor/antagonists	Sulfisoxazole	Entry	0.0	-											88.2	11			0.7	
		Exit	6.7	3.50-12.32											89.6	3.7				6.7
	Trimethoprim/sulfamethoxazole	Entry	0.7	0.10-5.03				97.8	0.7	0.7			0.7							
		Exit	1.5	0.37-5.73				97	1.5					1.5						
Macrolides	Azithromycin	Entry	0.0	0.00-2.76						2.9	15.4	36.8	44.1		0.7					
		Exit	2.2	0.72-6.66							9.6	38.5	49.6			2.2				
Phenicols	Chloramphenicol	Entry	0.0	-								2.9	49.3	47.1	0.7					
		Exit	0.7	0.10-5.07								0.7	31.1	65.2	2.2	0.7				
Quinolones	Ciprofloxacin	Entry	0.0	-	97.1	2.2			0.7											
		Exit	0.0	-	95.6	3.7				0.7										
	Nalidixic acid	Entry	0.0	-						0.7	13.2	80.9	5.1							
		Exit	0.0	-							11.1	76.3	11.8	0.7						
Tetracycline	Tetracycline	Entry	0.7	0.10-5.03									99.3			0.7				
		Exit	20.7	14.72-28.41									78.5	0.7		2.2	18.5			

* The range of MICs tested are indicated in white boxes and the vertical line indicates the resistance breakpoint cut off value

Among entry samples, 3/136 (2.2%) isolates were resistant to at least one antimicrobial class and only one isolate was MDR. The MDR isolate was resistant to ampicillin, streptomycin and trimethoprim-sulfamethoxazole. The single entry (0.7%) isolate exhibiting resistance to β-lactams (amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, and ceftriaxone) had an *ampC* β-lactamase phenotype (resistance to third-generation cephalosporins and amoxicillin/clavulanic acid) and was also resistant to tetracycline. In exit samples, 11 (8.1%) of the isolates were MDR. Among these, resistance to five antimicrobial classes was identified in three (2.2%) of the isolates. Furthermore, an ESBL-producing *E. coli* phenotype (resistance to third-generation cephalosporins and susceptibility to amoxicillin/clavulanic acid) was also observed in six (4.0%) of the isolates. Amongst ESBL-producing isolates they were resistant to one (16.7% of ESBL isolates), three (16.7%), four (16.7%), and five (50.0%) antimicrobial classes (Table 5).

Table 5. The antimicrobial resistance pattern of *E. coli* isolated from entry into the feedlot and exit (slaughter house).

Antimicrobial pattern	**Total no. of isolates (%)**		**Resistance pattern (no. of isolates)**	
	Induction (136)	**Exit (135)**	**Induction**	**Exit**
All susceptible	133 (97.8)	103 (76.3)	133	103
1	1 (0.7)	17 (12.6)	AUG2 (1)	TET (13) FIS (1) AMP (1) AMP-AUG2-AXO-FOX-XNL (1)[a] AUG2-FOX (1)
2	1 (0.7)	4 (3.0)	AUG2-AMP-FOX-TET-XNL-AXO (1)[a]	FIS-TET (1) AMP-TET (3)
3	1 (0.7)	7 (5.2)	AMP-STR-SXT (1)	FIS-STR-TET (3) AMP-STR-TET(3) AMP-AXO-AZI-TET-XNL (1)[a]
4		1 (0.7)		AMP-AXO-FIS-STR-SXT-TET-XNL (1)[a]
5		3 (2.2)		AMP-AXO-AZI-FIS-STR-TET-XNL (2)[a] AMP-AXO-CHL-FIS-STR-SXT-TET-XNL (1)[a]
Non-MDR	2 (1.5)	21 (15.6)		
MDR	1 (0.7)	11 (8.1)		
Resistance	3 (2.2)	23.7		

[a]ESBL producing *E.coli,* **AUG2** (Amoxicillin/clavulanic acid), **AMP** (Ampicillin), **FOX** (Cefoxitin), **XNL** (Ceftiofur), **AXO** (Ceftriaxone), **CHL** (Chloramphenicol), **STR** (Streptomycin), **FIS** (Sulfisoxazole), **TET** (Tetracycline) and **SXT** (Trimethoprim/sulfamethoxazole)

At exit, the frequency of AMR in treated and non-treated cattle was 23.1% and 23.8%, respectively, with no significant difference between the two groups (Table 6). The *E. coli* isolated from antimicrobial-treated cattle faeces were resistant to tetracycline and β-lactams (15.4% each) (Table 7). Among *E. coli* isolated from non-treated cattle, the highest frequency of resistance was observed to tetracycline (18.9%).

Table 6. Comparative analysis of antimicrobial resistance in *E. coli* isolated from antimicrobial-treated and non-treated cattle at feedlot exit

Cattle	No. of *E.coli* isolates	Resistance prevalence (%)	OR	P-value
Non-treated	122	23.8	0.96	0.955
Treated	13	23.1		

Overall, from the treated and non-treated cattle, 76.9% and 76.2% of the *E. coli* isolates were respectively sensitive to all antimicrobials tested. Among resistant isolates, 10 (8.2%) were MDR. A single *E.coli* isolate, obtained from a tulathromycin-treated cattle individual was resistant to five antimicrobial classes (Table 7).

Table 7. The antimicrobial resistance pattern of *E. coli* isolated from antimicrobial treated and non-treated cattle at feedlot exit

Number of antimicrobial classes	Total no. of isolates (%) Non-treated (122)	Treated (13)	Resistance pattern (no. of isolates) Non-treated	Treated
All sensitive	93 (76.23)	10 (76.9)	93	10
1	15 (12.29)	2 (15.4)	TET (12) FIS (1) AMP (1) AUG2-FOX (1)	TET (1)(***) AMP-AUG2-AXO-FOX-XNL (1)[a](**)
2	4 (3.28)		AMP-TET (3) FIS-TET (1)	
3	7 (5.74)		FIS-STR-TET (3) AMP-STR-TET(3) AMP-AXO-AZI-TET-XNL (1)[a]	
5	3 (2.46)	1 (7.7)	AMP-AXO-AZI-FIS-STR-TET-XNL (2)[a] AMP-AXO-CHL-FIS-STR-SXT-TET-XNL (1)[a]	AMP-AXO-FIS-STR-SXT-TET-XNL (1)[a](*)
Resistance (%)	29 (23.8)	3 (23.1)		
P-value	0.955			

[a]ESBL producing *E.coli;* *Draxxin Tulathromycin); **Excede (Ceftiofur); ***Bivatop (Oxytetracycline)
AUG2 (Amoxicillin/clavulanic acid), **AMP** (Ampicillin), **FOX** (Cefoxitin), **XNL** (Ceftiofur), **AXO** (Ceftriaxone), **CHL** (Chloramphenicol), **STR** (Streptomycin), **FIS** (Sulfisoxazole), **TET** (Tetracycline) and **SXT** (Trimethoprim/sulfamethoxazole)

3.11.2. Fresh faecal pats

From the target pen, the prevalence of AMR in *E. coli* isolated from the faecal pat sample of healthy animals (never treated with antimicrobials) collected at the first time point (2 weeks on feed) was high to a number of tested antimicrobials. An increased level of resistance was observed especially for the β-lactam antimicrobial class. Resistance to ampicillin**,** amoxicillin-clavulanate, cefoxitin, ceftiofur, ceftriaxone was detected in 42.8% of isolates cultured. The prevalence of tetracycline resistance was detected in 42.8 % of isolates at 6 weeks and dropped to 28.6% at 10 and 12 weeks after the entry into feedlot. All *E. coli* isolates from all sampling points were susceptible to azithromycin, ciprofloxacin and gentamycin. Generally, AMR was higher at the early sampling points (Figure 1). There was no difference in the relative abundance of AMR in the faecal pats collected from target versus neighbouring pens (data not shown). However, the level of resistance was significantly higher in isolates from the

hospital pen and a considerably larger number of samples yielded *E. coli* growing on ESBL Brilliance Agar and much higher levels of abundance compared to other sample types. Interestingly, the prevalence of AMR followed a similar trend for some of the antimicrobial classes. For instance, resistance to ampicillin, ceftriaxone, ceftiofur and tetracycline was detected in 85.7% of isolates each at the first sampling point (2 weeks) and increased to 100% at 4 and 6 weeks of the feedlot program for cattle in the hospital pen. Overall, at every sampling point, the highest frequency resistance was observed to tetracycline. Tetracycline resistance was observed in 85.7, 100.0, 100.0, 85.7, 85.7, 71.4% of *E. coli* isolates from samples collected at 2, 4, 6, 8, 10, and 12 weeks (Figure 2). Unlike the target pen, high amount of azithromycin resistance E. coli was detected from the hospital pen, most likely is due to the macrolide use in these cattle.

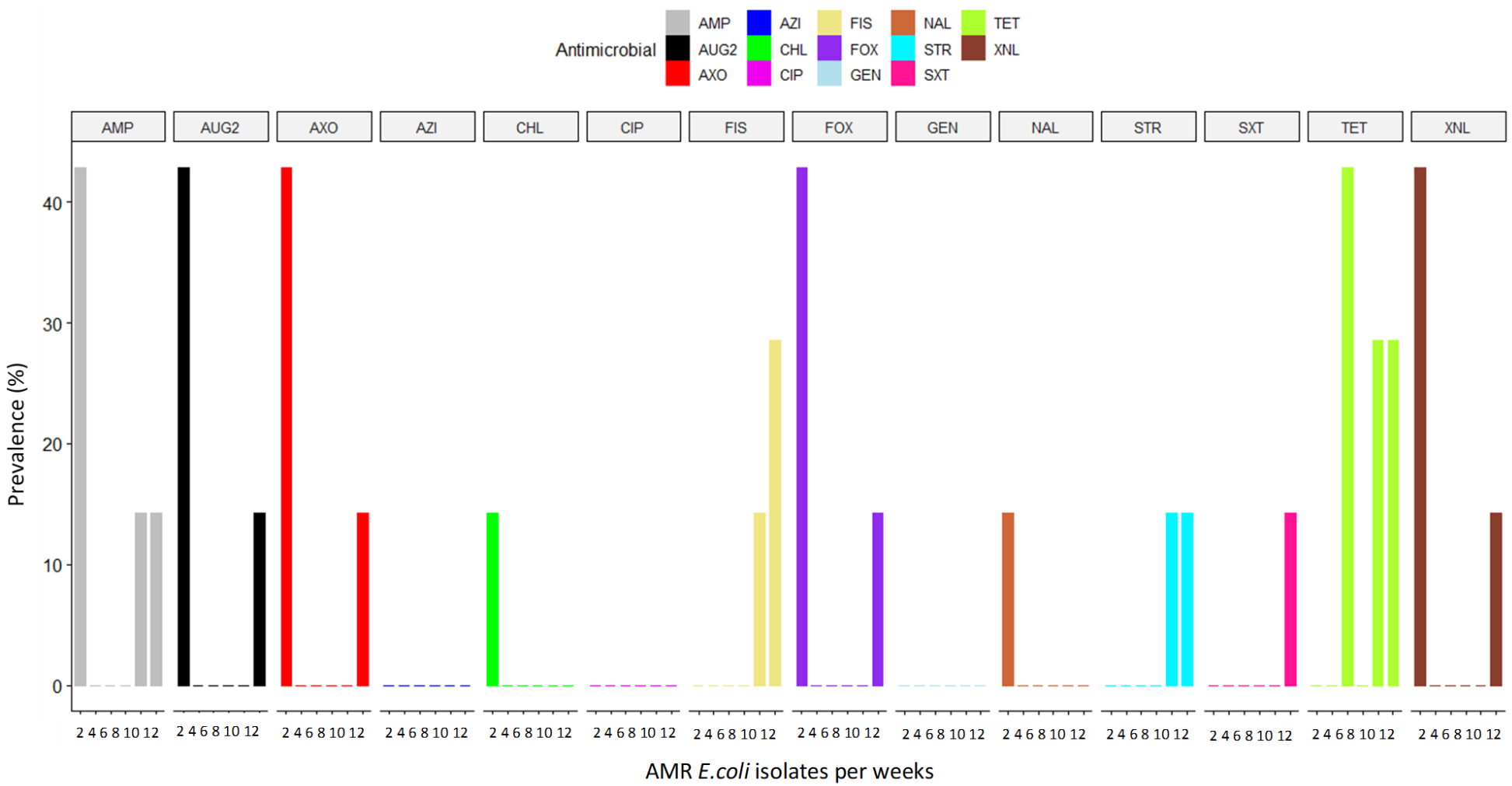

Figure 1. Longitudinal analysis of the antimicrobial resistance prevalance (% of isolates) in *Escherichia coli* isolated from fresh faecal pats collected from the target cattle pen (n=42) collected from non-treated cattle. **AUG2** (Amoxicillin/clavulanic acid), **AMP** (Ampicillin), **AXO** (Ceftriaxone), **AZI** (Azithromycin), **CHL** (Chloramphenicol), **CIP**(Ciprofloxacin), **FIS** (Sulfisoxazole), **FOX** (Cefoxitin), **GEN** (Gentamcin), NAL (Nalidixic acid), **STR** (Streptomycin), **SXT** (Trimethoprim/sulfamethoxazole), **TET** (Tetracycline) and **XNL** (Ceftiofur)

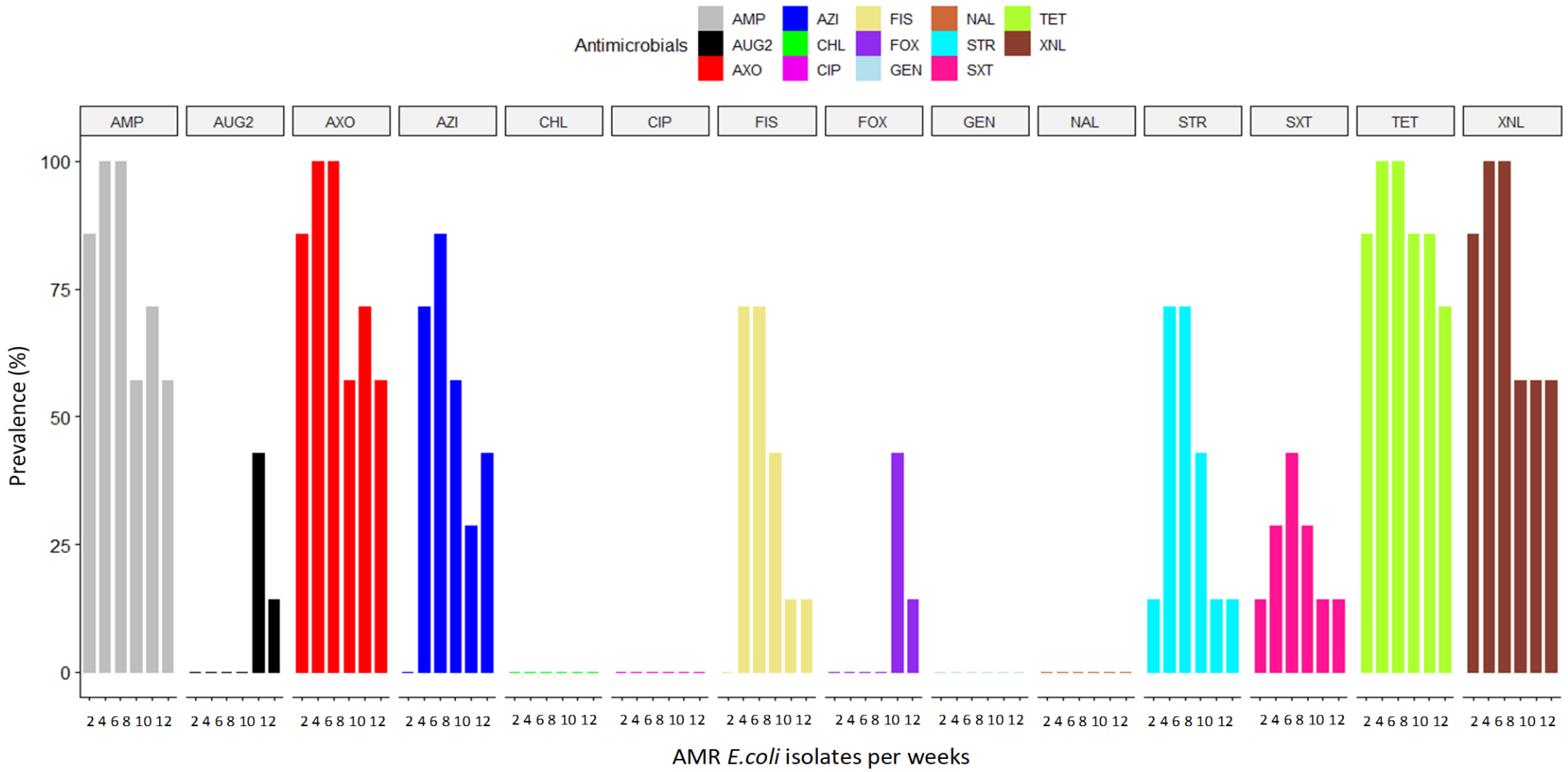

Figure 2. Longitudinal analysis of the antimicrobial resistance prevalance (% of isolates) in *Escherichia coli* isolated from fresh faecal pats collected from the hospital pen (n=42). **AUG2** (Amoxicillin/clavulanic acid), **AMP** (Ampicillin), **AXO** (Ceftriaxone), **AZI** (Azithromycin), **CHL** (Chloramphenicol), **CIP**(Ciprofloxacin), **FIS** (Sulfisoxazole), **FOX** (Cefoxitin), **GEN** (Gentamcin), NAL (Nalidixic acid), **STR** (Streptomycin), **SXT** (Trimethoprim/sulfamethoxazole), **TET** (Tetracycline) and **XNL** (Ceftiofur)

In total, 84 *E. coli* isolates were cultured from fresh faecal samples from the target and the hospital pens (Table 8). Among isolates from the target pen, resistance was observed most frequently to tetracycline (16.7%) followed by ampicillin (11.9%), ceftiofur and ceftriaxone (9.5% each), sulfisoxazole (7.1%), and streptomycin (4.8%). Similarly, among faecal pats from the hospital pen resistance was observed most frequently to tetracycline (88.1%), followed by ceftriaxone (78.6%), ampicillin (78.6%), ceftiofur (76.2%) and azithromycin (47.6%).

Table 8. Results of the antimicrobial susceptibility testing of *Escherichia coli* isolated from fresh faecal pats collected from the target pen (TP) and hospital pen (HP) (n=42)

Antimicrobial class	Antimicrobial agent	Faecal deposit	Resistant (%)	95 % CI	Proportion of the isolates per MIC value (µg/mL)*															
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Aminoglycosides	Gentamycin	TP	0.0	-					11.9	61.9	23.8	2.4								
		HP	0.0	-					2.4	38.1	54.8	4.8								
	Streptomycin	TP	4.8	1.19-17.15								7.1	38.1	42.9	2.4	4.8	4.8			
		HP	33.4	20.84-48.71								2.4	9.5	50.0	2.4	2.4	4.8	28.6		
β-lactam	Ampicillin	TP	11.9	5.04-25.59							21.4	38.1	26.2		2.4	4.8	7.1			
		HP	78.6	63.69-88.46							7.1	7.1	4.8	2.4		14.3	64.3			
	Amoxicillin/clavulanic acid	TP	9.5	3.62-22.78							7.1	28.6	50	4.8		7.1	2.4			
		HP	9.5	3.62-22.78							2.4	2.4	35.7	47.6	2.4	2.4	7.1			
	Cefoxitin	TP	9.5	3.62-22.78							7.1	23.8	54.8	4.8		7.1	2.4			
		HP	9.5	3.62-22.78							2.4	14.3	54.8	14.3	4.8	2.4	7.1			
	Ceftiofur	TP	9.5	3.62-22.78				9.5	28.6	45.2	2.4	2.4	2.4	7.1	2.4					
		HP	76.2	61.13-86.69				4.8	4.8	9.5	2.4		2.4	14.3	61.9					
	Ceftriaxone	TP	9.5	3.62-22.78					88.1	2.4				7.1		2.4				
		HP	78.6	63.69-88.46					19.1	2.4				4.8	2.4	21.4	35.7	14.3		
Folate pathway inhibitor/antagonists	Sulfisoxazole	TP	7.1	2.32-19.94											73.8	14.2			4.8	7.1
		HP	30.9	18.90-46.31											50	16.7			2.4	30.9
	Trimethoprim/sulfamethoxazole	TP	2.4	0.33 -15.07				88.1		4.8		4.8		2.4						
		HP	23.8	13.31-38.87				69.0	7.1				2.4	21.4						
Macrolides	Azithromycin	TP	0.0	-							2.4	33.3	52.4	9.5	2.4					
		HP	47.6	33.16-1-62.49								16.7	16.7		19	47.6				
Phenicols	Chloramphenicol	TP	2.4	0.33 -15.07								2.4	54.8	35.7	4.8	2.4				
		HP	0.0	-								2.4	33.3	64.3						
Quinolones	Ciprofloxacin	TP	0.0	-	90.5	2.4	2.4	4.8												
		HP	0.0	-	76.19	14.3			7.1	2.4										
	Nalidixic acid	TP	2.4	0.33 -15.07							16.7	66.7	9.5	2.4	2.4	2.4				
		HP	0.0								7.1	52.4	33.3	7.1						
Tetracycline	Tetracycline	TP	16.7	8.16-31.05									78.6	4.8			16.7			
		HP	88.1	74.41-94.96									9.5	2.4	4.8	21.4	61.9			

* The range of MICs tested are indicated in white boxes and the vertical line indicates the resistance breakpoint cut off value

Among *E. coli* isolated from pen faecal pats, AMR was identified in 10 (23.8%) target isolates, of which 4 (9.5%) were MDR, and 37 (88.1%) hospital isolates of which 23 (54.8%) were MDR. Putative ESBL-producing *E. coli* isolates were identified in 4 (9.5%) target and 32 (76.2%) hospital pen faecal samples. Eleven *E. coli* isolates from the hospital pen were resistant to five antimicrobial classes (Table 9).

Table 9. Resistance and MDR profiles of *E.coli* isolated from fresh and hospital pen faecal samples

Antimicrobial pattern	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	Target pen (42)	Hospital pen (42)	Target pen	Hospital pen
All susceptible	32 (76.2)	5 (11.9)	32	5
1	6 (14.28)	4 (9.5)	TET (4) AUG2-AMP-FOX-XNL-AXO (2)[a]	TET (4)
2		10 (23.8)	AUG2-AMP-FOX-CHL-NAL-XNL-AXO	AMP-TET-XNL-AXO(7)[a] AUG2-AMP-FOX-TET-XNL-AXO(3)[a]
3	3 (7.14)	9 (21.4)	FIS-STR-TET (1) AUG2-AMP-FOX-CHL-NAL-XNL-AXO (1)[a] AUG2-AMP-FOX-FIS-TET-XNL-AXO-SXT (1)[a]	AMP-AZI-TET-XNL-AXO (8)[a] AUG2-AMP-AZI-FOX-TET-XNL-AXO (1)[a]
4	1 (2.38)	3(7.1)	AMP-STR-FIS-TET (1)	AMP-STR-FIS-TET-XNL-AXO-SXT (1)[a] AMP-STR-TET-XNL-AXO-SXT(1)[a] AMP-STR-FIS-TET-AXO-SXT (1)
5		11 (26.2)		AMP-AZI-STR-FIS-TET-XNL-AXO-SXT (7)[a] AMP-AZI-STR-FIS-TET-XNL-AXO (4)[a]
Non-MDR	6 (14.28)	14 (33.3)		
MDR	4 (9.5)	23 (54.8)		
Resistance (%)	10 (23.8)	37 (88.1)		

[a]ESBL producing isolate

3.1.1.3. Water

No *E. coli* was isolated from the target pen but four were recovered from the hospital pen. The frequency of β-lactams, azithromycin and tetracycline resistance was 50% in *E. coli* isolated from the hospital pen water trough (Appendix 1). One isolate was resistant to ampicillin, azithromycin, ceftiofur, ceftriaxone and tetracycline and the second isolate was resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, azithromycin, and tetracycline.

3.1.1.4. Air

No *E. coli* was isolated from air / dust samples collected from a proximity of 20 and 200 m away from the target pen. However, three *E. coli* were isolated from the air / dust samples from the hospital pen, one from a proximity of 20 m and two from a proximity of 200 m. The one *E. coli* isolated from 20m proximity was resistant to amoxycillin-clavunate, ampicillin, cefoxitin, ceftiofur and ceftriaxone. The one *E. coli* isolated from 200m far from the hospital pen was resistant to ampicillin and tetracycline while the second one was sensitive to all antimicrobials.

3.1.1.5. Carcase

No *E. coli* was isolated from hip (150), and flank and brisket (149) samples.

3.1.1.5. Lymph node

Two *E. coli* were recovered from prescapular (146) and subiliac (144) lymph nodes. Both isolates were sensitive to all tested antimicrobials.

3.1.2. Genotypic determination of antimicrobial resistance

From rectal faecal samples at entry and exit, 33 antimicrobial-resistant and 4 sensitive *E. coli* isolates were selected for further molecular analysis. Using the AMR gene CARD and ResFinder database, WGS data was analysed for the presence of ARGs in *E. coli* (n = 37). Across all isolates, a total of 24 resistance genes were observed which conferred resistance to a range of antimicrobial classes including aminoglycosides, β-lactams, macrolides, folate synthesis inhibitors, phenicols, fluoroquinolones, and tetracyclines (Table 10). The most common AMR genes observed in these isolates were *tet(B)* (48.5%), *aph(3'')-Ib* and *aph(6)-Id* (36.4%), bla_{TEM-1B} (27.3%), and *sul2* (27.3%) which are responsible for resistance to tetracyclines, aminoglycosides, β-lactams and sulfonamides, respectively. The most commonly detected β-lactamase genes were bla_{TEM} (33.3%), bla_{CTX-M} (15.1%) and bla_{CMY} (6.1%). Two isolates harboured both bla_{CTX-M} and bla_{TEM} genes. However, the combination of bla_{CMY} and bla_{TEM} was observed in only one isolate. From the ESBL-producing *E. coli* isolates (n=7), the bla_{CTX} gene were detected in 71.4%, bla_{TEM} in 42.8% and bla_{CMY} in 28.6%. Overall, a gene responsible for reduced susceptibility to ciprofloxacin (*qnrS1*), and resistance to chloramphenicol (*cmlA1*) and gentamycin (*aac(3)-IV*) was detected in a single isolate .

Table 10. The identification of antimicrobial resistance genes in *E. coli* (n=33) isolated at entry to and exit from the feedlot.

Resistance gene	Antimicrobial class	Resistance phenotype	Number of isolates (%)
strA [aph(3'')-Ib]	Aminoglycosides	STR	12 (36.4)
strB [aph(6)-Id]	Aminoglycosides	STR	12 (36.4)
aadA1(ant(3'')-Ia)	Aminoglycosides	STR, KAN	1 (3.0)
aph(3')-Ia	Aminoglycosides	KAN	1 (3.0)
aph(4)-Ia(hph)	Aminoglycosides	HYG	1 (3.0)
aac(3)-IV	Aminoglycosides	GEN	1 (3.0)
bla_{CMY-2}	β-lactam	AUG2,AXO,FOX,XNL	2 (6.1)
$bla_{CTX-M-15}$	β-lactam	AMP,AXO,XNL	3 (9.1)
$bla_{CTX-M-27}$	β-lactam	AMP,AXO,XNL	2 (6.1)
bla_{TEM-1B}	β-lactam	AMP,AXO, XNL	9 (27.3)
bla_{TEM-1C}	β-lactam	AMP	2 (6.1)
mph(A)	Macrolide	AZI, ERY	3 (9.1)
mph(E)	Macrolide	AZI, ERY	1 (3.0)
msr(E)	Macrolide	AZI, ERY	1 (3.0)
sul1	Folate synthesis inhibitors	FIS	1 (3.0)
sul2	Folate synthesis inhibitors	FIS	9 (27.3)
sul3	Folate synthesis inhibitors	FIS	1 (3.0)
dfrA5	Folate synthesis inhibitors	SXT	2 (6.1)
dfrA12	Folate synthesis inhibitors	SXT	1 (3.0)
dfrA14	Folate synthesis inhibitors	SXT	1 (3.0)
cmlA1	Phenicols	CHL	1 (3.0)
qnrS1	Fluoroquinolones	CIP	1 (3.0)
tet(A)	Tetracyclines	TET	12 (36.4)
tet(B)	Tetracyclines	TET	16 (48.5)

AUG2, amoxicillin/clavulanic acid; AMP, ampicillin; AZI, azithromycin; AXO, ceftriaxone; CHL, chloramphenicol; ERY, erythromycin; FIS, sulfisoxazole; FOX, cefoxitin; GEN; gentamycin; HYG, hygromycin; STR, streptomycin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; XNL, ceftiofur

Sequenced isolates contained one (40.5%), two (8.1%), three (27.0%), four (2.7%), five (5.4%) or six (2.7%) identified AMR genes (Table 11).

Table 11. The antimicrobial resistance pattern of *E. coli* isolated at the entry to and exit from the feedlot.

Antimicrobial classes pattern	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	Phenotypic (37)	Genotypic (37)	Phenotypic (MIC)	Genotypic(resistance gene)
All susceptible	4 (10.8)	5 (13.5)	4	5
1	16 (43.2)	15 (40.5)	AMP (1)	*bla*$_{TEM-1B}$ (1)
			AMP-AUG-FOX-XNL-AXO (1)	*bla*$_{CMY-2}$ (1)
			FIS (1)	*sul2* (1)
			TET (13)	*tet(A)*(4)
				tet(B) (8)
2	5 (13.5)	3 (8.1)	AMP-AUG-AXO-FOX-TET-XNL (1)	*bla*$_{TEM-1C}$, *tet(A)* (2)
			AMP-TET (3)	*sul2*,*tet(B*) (1)
			FIS-TET (1)	
3	8 (21.6)	10 (27.0)	AMP-STR-SXT (1)	*bla*$_{CMY-2}$, *bla*$_{TEM-1B}$, *dfrA5*, *tet(A)* (1)
			AMP-STR-TET (3)	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B}$, *tet(B)* (4)
			AMP-AXO-AZI-TET-XNL (1)	*aph(3')-Ia*, *aph(3'')-Ib /strA*, *aph(6)-Id/strB*, *bla*$_{TEM-1B}$, *dfrA5*, *sul2* (1)
			FIS-STR-TET (3)	*aph(3'')-Ib*, *aph(6)-Id*, *sul2*, *tet(B)* (3)
4	1 (2.7)	1 (2.7)	AMP-AXO-FIS-STR-SXT-TET-XNL (1)	aph(3'')-Ib, aph(6)-Id, bla$_{CTX-M-15}$, bla$_{TEM-1B}$,dfrA14, sul2, tet(A) (1)
5	3 (8.1)	2(5.4)	AMP-AXO-AZI-FIS-STR-TET-XNL (2)	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{CTX-M-27}$,*mph(A), sul2*, *tet(A*) (2)
			AMP-AXO-CHL-FIS-STR-SXT-TET-XNL (1)	
6		1 (2.7)		*aac(3)-IV*, *aadA1/ant(3'')-Ia*, *aph(3'')-Ib*, *aph(4)-Ia/hph*, *aph(6)-Id*, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *cmlA1*, *dfrA12* , *qnrS1*, *sul1*, *sul3*, *tet(A*) (1)
Non-MDR	21 (56.7)	18 (48.6)		
MDR	12 (32.4)	14 (37.8)		
Resistance	33 (89.2)	32(86.5)		

AUG2, amoxicillin/clavulanic acid; AMP, ampicillin; AZI, azithromycin; AXO, ceftriaxone; CHL, chloramphenicol; ERY, erythromycin; FIS, sulfisoxazole; FOX, cefoxitin; GEN; gentamycin; HYG, hygromycin; STR, streptomycin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; XNL, ceftiofur

Generally, resistance genotypes were correlated with resistance phenotypes (Table 12). All β-lactams resistance isolates harboured either *bla*$_{TEM}$, *bla*$_{CTX-M}$ or *bla*$_{CMY}$ resistance genes. The same was true for azithromycin and chloramphenicol resistance phenotypes. Among tetracycline-resistant isolates (n=29), 28 (96.5%) possessed either *tet (A)* or *tet (B)* resistance genes. However, some AMR genes were identified in isolates that were susceptible to the phenotype. For instance, *aac (3)-IV, aph (3'')-Ib* and *aph (6)-Id*, *sul2*, and *dfrA5* genes, responsible for gentamycin, streptomycin, sulfisoxazole, and trimethoprim/sulfamethoxazole resistance, were observed in one isolate each. By contrast, one tetracycline-resistant isolate harboured no associated resistance genes.

Table 12. Agreement between antimicrobial resistance phenotypes and resistance gene detection among 33 isolates submitted for WGS

Antimicrobial class	AMR isolates (%)	Resistance gene observed (%)	Agreement (%)
Aminoglycosides	Gentamycin (n=0; 0)	*aac(3)-IV* (n=1; 3.0)	0
	Streptomycin (n=11; 33.3)	*aph(3'')-Ib*, *aph(6)-Id* (n=10; 30.3)	100
		aph(3'')-Ib, *aph(6)-Id*, *aadA1* (n=1; 3.0)	
β-lactam	Ampicillin (n=15; 45.4)	*bla*$_{TEM-1B}$ (n=6;18.2)	100
		bla$_{TEM-1C}$ (n=2; 6.1)	
		bla$_{CTX-M-27}$ (n=2; 6.1)	
		bla$_{CTX-M-15}$ (n=1; 3.0)	
		bla$_{CMY-2}$ (n=1; 3.0)	
		bla$_{CMY-2}$, *bla*$_{TEM-1B}$ (n=1; 3.0)	
		bla$_{CTX-M-15}$, *bla*$_{TEM-1B}$ (n=2; 6.1)	
	Amoxicillin/clavulanic acid (n=2; 6.1)	*bla*$_{CMY-2}$ (n=2; 6.1)	100
	Cefoxitin (n=2; 6.1)	*bla*$_{CMY-2}$ (n=2; 6.1)	100
	Ceftiofur (n=7; 21.2)	*bla*$_{CTX-M-15}$ (=3; 9.1)	100
		bla$_{CMY-2}$ (n=2; 6.1)	
		bla$_{CTX-M-27}$ (n=2; 6.1)	
	Ceftriaxone (n=7; 21.2)	*bla*$_{CTX-M-15}$ (=3; 9.1)	100
		bla$_{CMY-2}$ (n=2; 6.1)	
		bla$_{CTX-M-27}$ (n=2; 6.1)	
Folate pathway inhibitor/antagonists	Sulfisoxazole (n=9; 27.3)	*sul2* (n=8; 24.2)	100
		sul1, *sul3* (n=1; 3.0)	
	Trimethoprim/sulfamethoxazole (n=3; 9.1)	*dfrA5* (n=1; 3.0)	100
		dfrA12 (n=1; 3.0)	
		dfrA14 (n=1; 3.0)	
Macrolides	Azithromycin (n=3; 9.1)	*mph(A)* (n=2; 6.1)	100
		mph(A), *msr(E)* (n=1; 3.0)	
Phenicols	Chloramphenicol (n=1; 3.0)	*cmlA1* (n=1; 3.0)	100
Fluoroquinolones	Ciprofloxacin (n=0; 0)	*qnrS1* (n=1; 3.0)	0
Tetracycline	Tetracycline (n=29; 87.9)	*tet(A)* (n=12; 36.4)	96.5
		tet(B) (n=16; 48.5)	

3.2. *Salmonella*

The frequency of *Salmonella* sp. isolation from the target and hospital pen samples was 2.4% and 16.7% of samples, respectively (Table 13). *Salmonella* was cultured from 8 (5.5%) and 6 (4.1%) of the subiliac and prescapular lymph nodes. While a range of *Salmonella* serotypes were isolated from the target and hospital pens, only *S.* Infantis was identified in lymph node samples. *Salmonella* was not detected in rectal samples collected from the feedlot entry and slaughterhouse (exit) animals. All *Salmonella* isolates were sensitive to all antimicrobials tested.

Table 13. The occurrence of *Salmonella* serotypes isolated from the faecal pat and lymph node samples.

Sample (n)	Number of isolates (%)	Salmonella species
Target pen (42)	1 (2.4)	*S. bovismorbificans*
Hospital pen (42)	4 (9.5)	*S.infantis*
	2 (4.8)	*S.bovismorbificans*
	1 (2.4)	*S.anatum*
Neighboring pen (42)	2 (4.8)	*S. infantis*
Prescapular lymph node (146)	6 (4.1)	*S.infantis*
Subiliac lymph node (144)	8 (5.5)	*S.infantis*

3.3. *Enterococcus*

Overall, 696 *Enterococcus* spp. isolates were obtained. From these, 35.6% of isolates were from rectal faeces samples obtained at entry and exit. The recovery rate of *Enterococcus* spp. from rectal faecal samples at entry and exit was 69.3% and 96%, respectively. However, there was no difference in isolation from prescapular and subiliac lymph nodes (Table 14).

Table 14. The number of *Enterococcus* spp. Isolates obtained from different sample sources.

Sample source	Type of sample	Number of samples	*Enterococcus* isolates (%)
Entry	Rectal faeces	150	104 (69.3)
Feedlot	Target pen faecal pat	42	39 (92.9)
	Hospital pen faecal pat	42	30 (71.4)
	Neighboring pens faecal pat	42	41 (97.6)
	Target pen water	5	4 (80.0)
	Hospital pen water	5	2 (40.0)
	Neighboring pens water	6	5 (83.3)
	Target pen air	12	1 (8.3)
	Hospital pen air	12	2 (16.7)
Exit	Rectal faeces	150	144 (96.0)
	Hip/round swab	150	45 (30.0)
	flank and brisket swab	149	25 (16.8)
	Prescapular lymph node	146	128 (87.7)
	Subiliac lymph node	144	126 (87.5)

Overall, 12 *Enterococcus* spp. were identified and their relative abundance is shown in Table 15. *E. faecium* (373), *E. hirae* (238), *E. gilvus* (25), *E. faecalis* (17) and *E. mundtti* (16) were the most commonly isolated species. There were obvious differences in the relative abundance of enterococci among sample types, with some species clearly predominant in certain environments and sampling points. *E. hirae* was the most prevalent species from samples collected during the feeding program and *E. faecium* from the slaughter house.

Table 15. Type of *Enterococcus species* isolated from different sample sources

		***Enterococcus* species**										
Sample source	**Type of sample**	***E. casseliflavus* (3)**	***E. divriesei* (9)**	***E. durans* (11)**	***E. faecalis* (17)**	***E. faecium* (373)**	***E. gallinarum* (1)**	***E. gilvus* (23)**	***E. hermanniensis* (2)**	***E. hirae* (238)**	***E. malodoratus* (2)**	***E. mundtii* (16)**	***E. sulfureus* (1)**
Entry	Rectal faeces	1		1		9				90		3	
Feedlot	Target pen faecal pat	1		2		4				31		1	
	Hospital pen faecal pat	1		2		7				18		2	
	Neighbour pens faecal pat			4		3				33		1	
	Target pen water						1			2		1	
	Hospital pen water								1			1	
	Neighbour pens water					2				2		1	
	Target pen air									1			
	Hospital pen air								1	1			
Exit	Rectal faeces			1		117				25		1	
	Hip swab				4	16		21		4			
	Flank and brisket swab		1	1		10				13			
	Prescapular lymph node		2		8	103		1		9	1	3	1
	Subiliac lymph node		6		5	102		1		9	1	2	

3.3.1. Phenotypic determination of antimicrobial resistance

The frequency of AMR in *E. faecalis*, *E .faecium*, *E. hirae* and other species isolates as group is shown in Table 16. Lincomycin resistance was most commonly observed in all species, in addition resistance to quinupristin/dalfopristin was observed in 8.6%, 2.1% and 8.8% of *E. faecium, E. hirae* and other *Enterococcus* spp, respectively. All *E. faecalis* isolates were resistant to lincomycin and quinupristin/dalfopristin. Furthermore, daptomycin resistance was observed in 8.3% and 23.9% of *E. faecium* and *E. hirae* isolates, respectively. However, ciprofloxacin resistance was identified only in *E. faecium* isolates. Overall, all species were sensitive to chloramphenicol, gentamycin, linezolid, penicillin and vancomycin.

Table 16. Prevalence of AMR in *Enterococcus* spp. isolated from different samples

Antimicrobial class	Antimicrobial agent	Resistance (%)			
		***Enterococcus faecalis* (17)**	***E. faecium* (373)**	***E. hirae* (238)**	**Other species (68)**
Aminoglycosides	Gentamycin	0	0	0	0
	Kanamycin	0	0.3	0	0
	Streptomycin	0	0.3	0	0
β-lactam	Pencillin	0	0	0	0
Fluoroquinolones	Ciprofloxacin	0	6.4	0	0
Glycopeptides	Vancomycin	0	0	0	0
Glycylcyclines	Tigecycline	5.9	3.7	2.1	4.4
Lincosamide	Lincomycin	100	89.8	68.5	50.0
Lipopeptides	Daptomycin	0	8.3	23.9	0
Macrolides	Erythromycin	0	2.1	3.4	1.5
	Tylosin tartrate	0	1.1	4.2	2.9
Nitrofurantoin	Nitrofurantoin	0	38.6	5.0	8.8
Oxazolidinones	Linezolid	0	0	0	0
Phenicols	Chloramphenicol	0	0	0	0
Streptogramins	Quinupristin/dalfopristin	100	8.6	2.1	8.8
Tetracycline	Tetracycline	0	3.5	9.2	7.3

3.3.1.1. Rectal faeces

The overall recovery of *Enterococcus* spp. in faecal samples at entry was 69.3%. *E. hirae* was the predominant species recovered with a frequency of 86.5%, followed by *E. faecium* (8.7%), *E. mundtii* (*2.9%), E. durans,* and *E. casseliflavus* (1.0% each). The highest frequency of resistance was observed to lincomycin (60.6%), followed by daptomycin (25.0%), nitrofurantoin (8.7%), ciprofloxacin (6.7%), tetracycline (4.8%), tigecycline (3.9%), and quinupristin/dalfopristin (2.9%). All isolates were sensitive to chloramphenicol, gentamycin, kanamycin, linezolid, penicillin, streptomycin, and vancomycin. Similarly, enterococci were recovered from 96.0% of faecal samples collected at exit. However, the most frequently isolated species were *E. faecium* (81.3%), *E. hirae* (17.4%), *E. durans* (0.7%) and *E. mundtii* (0.7%). The highest frequency of resistance was observed to lincomycin (84.0%), followed by nitrofurantoin (53.5%), daptomycin (22.9%), quinupristin/dalfopristin (18.1%), ciprofloxacin (7.6%), and tetracycline (6.9%). All isolates were sensitive to chloramphenicol, gentamycin, linezolid, penicillin, and vancomycin (Table 17).

Table 17. Results of the antimicrobial susceptibility testing of *Enterococcus* spp. isolated from rectal faeces at the entry (n=104) and exit (n=144) of the feedlot. For the origin of the cut-off value see Table 1.

Antimicrobial class	Antimicrobial agent	Sample (150 each)	% resistant	95 % CI	Proportion of isolates per MIC value (µg/mL)*															
					0.02	0.03	0.1	0.1	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Aminoglycosides	Gentamycin	Entry	0.0	0.0														100.0			
		Exit	0.0	0.0														100.0			
	Kanamycin	Entry	0.0	0.0														98.1	1.0	1.0	
		Exit	0.7	0.10-4.76														70.1	25.7	3.5	0.7
	Streptomycin	Entry	0.0	0.0																100.0	
		Exit	0.7	0.10-4.76																99.3	0.7
β-lactam	Pencillin	Entry	0.0	0.0					23.1	20.2	31.7	21.2	3.9								
		Exit	0.0	0.0					8.3	12.5	11.1	23.6	43.8	0.7							
Fluoroquinolones	Ciprofloxacin	Entry	6.7	3.24-13.45					2.9	76.9	10.6	2.9	6.7								
		Exit	7.6	4.28-13.27					1.4	13.2	38.2	39.6	7.6								
Glycopeptides	Vancomycin	Entry	0.0	0.0					1.0	50.0	48.1		1.0								
		Exit	0.0	0.0						60.4	34.0	4.2)	1.4								
Glycylcyclines	Tigecycline	Entry	4.8	2.02-11.03		14.4	43.3	29.8	7.7	4.8											
		Exit	1.4	0.35-5.38	0.7	3.5	52.1	38.2	4.2		1.4										
Lincosamide	Lincomycin	Entry	60.6	50.91-69.48							36.5	1.0	1.9	5.8	54.8						
		Exit	84.0	77.11-89.15							13.2	2.8		1.4	82.6						
Lipopeptides	Daptomycin	Entry	25.0	17.62-34.19					1.0		4.8	19.2	50.0	23.1	1.9						
		Exit	22.9	16.77-30.48							1.4	12.5	63.2	22.2	0.7						
Macrolides	Erythromycin	Entry	1.0	0.14-6.51					90.4	2.9	1.0	3.9	1.0		1.0						
		Exit	4.2	1.88-8.96					60.4	1.4	3.5	22.2	8.3	1.4	2.8						
	Tylosin tartrate	Entry	1.0	0.14-6.51					1.0	3.9	24.1	57.7	11.5	1.0			1.0				
		Exit	3.5	1.45-8.07							0.7	25.7	24.3	45.8			3.5				
Nitrofurantoins	Nitrofurantoin	Entry	8.7	4.56-15.80											2.9	40.4	48.1	8.7			
		Exit	53.5	45.30-61.46												3.5	43.1	53.5			
Oxazolidinones	Linezolid	Entry	0.0	0.0						1.0	3.9	94.2	1.0								
		Exit	0.0	0.0							0.7	97.2	2.1								
Phenicols	Chloramphenicol	Entry	0.0	0.0								1.0	92.3	6.7							
		Exit	0.0	0.0									10.4	89.6							
Streptogramins	Quinupristin/	Entry	2.9	0.93-8.56						38.5	17.3	41.4	1.9		1.0						

	dalfopristin	Exit	18.1	12.59-25.20		13.2	4.2	64.6	17.4	0.7			
Tetracycline		Entry	3.9	1.45-9.80			96.2				1.0	2.9	
	Tetracycline	Exit	6.9	3.78-12.43			93.1				0.7	6.3	

* The range of MICs tested are indicated in white boxes and the vertical line indicates the resistance breakpoint cut off value

Overall, 75% of isolates detected in rectal faeces were resistant to at least one of the tested antimicrobials. Of the 104 isolates at entry, 50 (48.1%) were resistant to one antimicrobial, 18 (17.3%) to two antimicrobials, 8 (7.7%) to three antimicrobials, and 2 (1.9%) to four antimicrobials (Table 18). At exit, 42 isolates (29.2%) were MDR, while just four isolates (2.8%) were sensitive to all tested antimicrobials.

Table 18. Antimicrobial resistance patterns of *Enterococcus* spp. isolated at entry and exit from the feedlot.

Antimicrobial classes	**Total no. of isolates (%)**		**Resistance pattern (no. of isolates)**	
	Entry (104)	**Exit (144)**	**Induction**	**Exit**
All susceptible	26 (25.00)	4 (2.8)	26	4
1	50 (48.1)	44 (30.6)	LIN (38) DAP (6) CIP (3) TGC (2) TET (1)	LIN (38) NIT (6)
2	18 (17.3)	55 (38.2)	DAP-LIN (11) CIP-NIT (1) CIP-TET (1) DAP-NIT (1) LIN-TIG (3) LIN-Q/D (1)	LIN-NIT (28) LIN-Q/D (9) CIP-LIN (1) DAP-NIT (7) DAP-LIN (7) CIP-NIT (1) LIN-TET(1) ERY-LIN-TYL (1)
3	8 (7.7)	31 (21.5)	DAP-LIN-NIT (6) CIP-LIN-NIT (1) DAP-LIN-TET (1)	LIN-NIT-Q/D (10) CIP-LIN-NIT (3) CIP-DAP-NIT (3) CIP-NIT-TIG (1) DAP-LIN-TET (2) DAP-LIN-Q/D (1) DAP-LIN-NIT (8) NIT-STR-TET (1) LIN-NIT-TET (1) ERY-LIN-TIG-TYL (1)
4	2 (1.9)7	9 (6.2)	CIP-DAP-LIN-Q/D (1) ERY-LIN-Q/D-TET-TYLT (1)	DAP-LIN-NIT-TET (1) KAN-LIN-NIT-Q/D (2) DAP-ERY-LIN-NIT (1) CIP-DAP-LIN-NIT (1) LIN-NIT-Q/D-TET (1) ERY-LIN-Q/D-TET-TYL (2) ERY-LIN-NIT-TET-TYL (1)
5		1 (0.7)		CIP-DAP-LIN-NIT-Q/D (1)
Non-MDR	68 (65.4)	99 (68.8)		
MDR	11 (10.6)	41 (28.5)		
Resistance	79 (76.0)	140 (96.3)		

CIP (Ciprofloxacin), **DAP** (Daptomycin), **ERY** (Erythromycin), **KAN** (Kanamycin), **LIN** (Lincomycin), **NIT**(Nitrofurantoin), **Q/D** (Quinupristin/dalfopristin), **STR** (Streptomycin), **TET** (Tetracycline), **TIG** (Tigecycline), **TYL**(Tylosin tartrate)

The frequency of AMR in *Enterococcus* spp. isolated from treated (92.3%) and non-treated (97.7%) beef cattle did not differ (P=0.288; Table 19).

Table 19. Comparative analysis of antimicrobial resistance in *Enterococcus* spp. isolated from antimicrobial-treated and non-treated cattle.

Beef cattle	No. of *Enterococcus* isolates	Prevalence (%)	OR	P-value
Not treated	131	97.7	0.28	0.288
Treated	13	92.3		

Enterococcus spp. isolated from treated cattle were resistant to one (7.7%), two (15.4%), three (30.8%), or four (38.5%) antimicrobial classes with six isolates (46.2%) identified as MDR. From these, four isolates were from tulathromycin-treated cattle and the remaining two isolates were from ceftiofur-treated cattle. By contrast, 128 isolates from non-treated cattle were resistant to at least one of the tested antimicrobials (97.7%). Of the 131 isolates, 42 (32.1%) were resistant to one antimicrobial, 51 (38.9%) to two, 26 (19.8%) to three, eight (6.1%) to four and one (0.8%) to five antimicrobials. In total, thirty five isolates (26.7%) were MDR, while just three (2.3%) were sensitive to all tested antimicrobials (Table 20).

Table 20. The antimicrobial resistance pattern of *Enterococcus* spp. isolated from antimicrobial treated and non-treated cattle at feedlot exit.

Number of antimicrobial classes	Total no. of isolates (%) Not treated (131)	Treated (13)	Resistance pattern (no. of isolates) Not treated	Treated
All sensitive	3(2.3)	1 (7.7)	3	1
1	42 (32.1)	2 (15.4)	LIN (36) NIT (6)	LIN (2)
2	51 (38.9)	4 (30.8)	LIN-NIT (28), CIP-NIT (1), CIP-LIN(1), LIN-TET(1) LIN-Q/D (7), DAP-LIN(7), DAP-NIT(5), ERY-LIN-TYL (1)	DAP-NIT (1)*,DAP-NIT (1)***, LIN-Q/D (2)*
3	26 (19.8)	5 (38.5)	LIN-NIT-Q/D (9), DAP-LIN-NIT (6), DAP-LIN-Q/D(1), CIP-LIN-NIT (3), CIP-DAP-NIT (3), NIT- STR-TET (1), LIN-NIT-TET (1), DAP-LIN-TET (1) ERY-LIN-TIG-TYL (1)	DAP-LIN-TET (1)**, DAP-LIN-NIT (1)** DAP-LIN-NIT (1)* CIP-NIT-TIG (1)*, LIN-NIT-Q/D (1)*
4	8 (6.1)	1 (7.7)	DAP-LIN-NIT-TET(1), CIP-DAP-LIN-NIT (1) DAP-LIN-NIT-Q/D (1), DAP-ERY-LIN-NIT(1) LIN-NIT-Q/D-TET (1), ERY-LIN-Q/D-TET-TYL (2) ERY-LIN-NIT-TET-TYL(1)	KAN-LIN-NIT-Q/D (1)*
5	1 (0.8)		CIP-DAP-LIN-NIT-Q/D (1)	
Resistance (%) P-value	128 (97.7) 0.288	12(92.3)		

*Draxxin (Tulathromycin); ** Excede (Ceftiofur); ***Bivatop (Oxytetracycline)

CIP (Ciprofloxacin), **DAP** (Daptomycin), **ERY** (Erythromycin), **KAN** (Kanamycin), **LIN** (Lincomycin), **NIT**(Nitrofurantoin), **Q/D** (Quinupristin/dalfopristin), **STR** (Streptomycin), **TET** (Tetracycline), **TIG** (Tigecycline), **TYL**(Tylosin tartrate)

Comparison of resistance among the different *Enterococcus* spp. is shown in Table 21. At entry, the breakdown of species was as follows; *E. hirae* (n=90), *E. faecium* (n=9), and other *Enterococcus* spp. (n=5). *E. faecium* isolates were resistant to more antimicrobial classes compared to *E. hirae,* including exhibiting resistance to ciprofloxacin (77.8%), lincomycin (33.3%), tetracycline (33.0%), quinupristin/dalfopristin, and nitrofurantoin (22.2% each). By contrast, among *Enterococcus* spp. isolated at exit, *E. faecium* (n=117) was the most abundant species followed by *E. hirae* (n=25). The highest resistance was observed to lincomycin (82.9%), followed by nitrofurantoin (61.5%), quinupristin/dalfopristin (21.4%), daptomycin (17.9%) and ciprofloxacin (9.4%).

Table 21. Antimicrobial resistance profile of *E. faecium* and *E. hirae* isolated at entry and exit from the feedlot.

	Sample source and no. (%) of *Enterococcus* isolates					
	Feedlot (Entry)			Slaughterhouse (Exit)		
Antimicrobial	*E. faecium* (n=9)	*E. hirae* (n=90)	Other *Enterococcus* spp (n=5)	*E. faecium* (n=117)	*E. hirae* (n=25)	Other *Enterococcus* spp (n=2)
Chloramphenicol	0	0	0	0	0	0
Ciprofloxacin	77.8	0	0	9.4	0	0
Daptomycin	11.1	27.8	0	17.9	48	0
Erythromycin	11.1	0	0	2.56	8	50
Gentamycin	0	0	0	0	0	0
Kanamycin	0	0	0	0.9	0	0
Lincomycin	33.3	61.1	100	82.9	84	100
Linezolid	0	0	0	0	0	0
Nitrofurantoin	22.2	7.8	0	61.5	16	50
Penicillin	0	0	0	0	0	0
Quinupristin/dalfopristin	22.2	0	20	21.4	0	50
Streptomycin	0	0	0	0.9	0	0
Tetracycline	33	1.1	0	5.1	12	50
Tigecycline	0	4.4	20	0.9	4	0
Tylosin tartrate	11	0	0	1.7	8	50
Vancomycin	0	0	0	0	0	0

All *E. faecium* isolates were resistant to at least one antimicrobial class, with three (33.3%) and 34 (29.0%) identified as MDR at entry and exit, respectively. Additionally, among *E. hirae,* a significant number of isolates were found susceptible to all tested antimicrobials (Table 22).

Table 22. Antimicrobial resistance phenotypes of *Enterococcus* spp. isolated from cattle faecal samples at entry and exit of feedlot

	Entry			Exit		
Antimicrobial classes	*E. faecium* (n=9)	*E. hirae* (n=90)	Other *Enterococcus* spp. (n=5)	*E. faecium* (n=117)	*E. hirae* (n=25)	Other *Enterococcus* spp. (n=2)
Susceptible		26 (28.9)			4 (16)	
CIP	3 (33.3)					
DAP		6 (6.7)				
LIN		35 (38.9)	3 (60)	32 (27.3)	6 (24)	
NIT				6 (5.1)		
TET	1 (11.1)					
TIG		2 (2.2)				
CIP-NIT	1 (11.1)			1 (0.8)		
CIP-TET	1 (11.1)					
CIP-LIN				1 (0.8)		
DAP-LIN		11 (12.2)			7 (28)	
DAP-NIT		1 (1.1)		7 (6.0)		
LIN-NIT				26 (22.2)	1 (4)	1 (50)
LIN-Q/D			1 (20)	9 (7.7)		
LIN-TET				1 (0.8)		
LIN-TIG		2(2.2)	1 (20)			
CIP-DAP-NIT				3 (2.6)		
CIP-LIN-NIT	1 (11.1)			3 (2.6)		
CIP-NIT-TIG				1 (0.8)		
DAP-LIN-NIT		6 (6.7)		6 (5.1)	2 (8)	
DAP-LIN-QD				1 (0.8)		
DAP-LIN-TET		1 (2.2)			2 (8)	
ERY-LIN-TYL					1 (4)	
LIN-NIT-Q/D				10 (8.5)		
LIN-NIT-TET				1 (0.8)		
NIT-STR-TET				1 (0.8)		
CIP-DAP-LIN-NIT				1 (0.8)		
CIP-DAP-LIN-Q/D	1 (11.1)					
DAP-LIN-NIT-Q/D				1 (0.8)		
DAP-LIN-NIT-TET					1 (4)	
DAP-ERY-LIN-NIT				1 (0.8)		

ERY-LIN-TIG-TYL		1 (4)	
KAN-LIN-NIT-Q/D		1 (0.8)	
LIN-NIT-Q/D-TET		1 (0.8)	
ERY-LIN-Q/D-TET-TYL	1 (11.1)	1 (0.8)	1 (50)
ERY-LIN-NIT-TET-TYL		1 (0.8)	
CIP-DAP-LIN-NIT-Q/D		1 (0.8)	

CIP (Ciprofloxacin), **DAP** (Daptomycin), **ERY** (Erythromycin), **KAN** (Kanamycin), **LIN** (Lincomycin), **NIT**(Nitrofurantoin), **Q/D** (Quinupristin/dalfopristin), **STR** (Streptomycin), **TET** (Tetracycline), **TIG** (Tigecycline), **TYL** (Tylosin tartrate)

3.3.1.2. Fresh faecal pats

From the target pen, a high-level of daptomycin (28.6 % of isolates) and tetracycline (33.3%) resistance was observed in isolates at 12 and 2 week sample time points, respectively. The lowest frequency of resistance (16.7%) was observed to ciprofloxacin, erythromycin, nitrofurantoin, quinupritin/dalfopristin, tigecycline and tylosin tartrate. The diversity of antimicrobial-resistant isolates was higher at early sampling point compared to the late samples. Ciprofloxacin (16.7%), erythromycin (50.0%), quinupristin/dalfopristin (16.7%), tetracycline (33.3%) and tigecycline (16.7%) resistance were observed among *Enterococcus* spp. isolated at the first sampling timepoint (2 weeks), whereas only daptomycin and lincomycin resistance was observed among *Enterococcus* spp. isolated at 12 weeks post-entry (Figure 3). No significant differences were observed between target and neighbouring pen with respect to resistance levels to individual antimicrobials (data not shown). However, a significantly higher proportion of antimicrobial-resistant *Enterococcus* spp. were observed in samples collected from the hospital pen. Resistance to lincomycin and quinupristin/dalfopristin was 100% in *Enterococcus* spp. isolated at the first sampling. The prevalence of tetracycline resistance was 50.0%, 33.3%. 33.3%, 14.3% and 66.7% in isolates obtained at 2, 4, 6, 8, and 10 weeks post-entry (Figure 4).

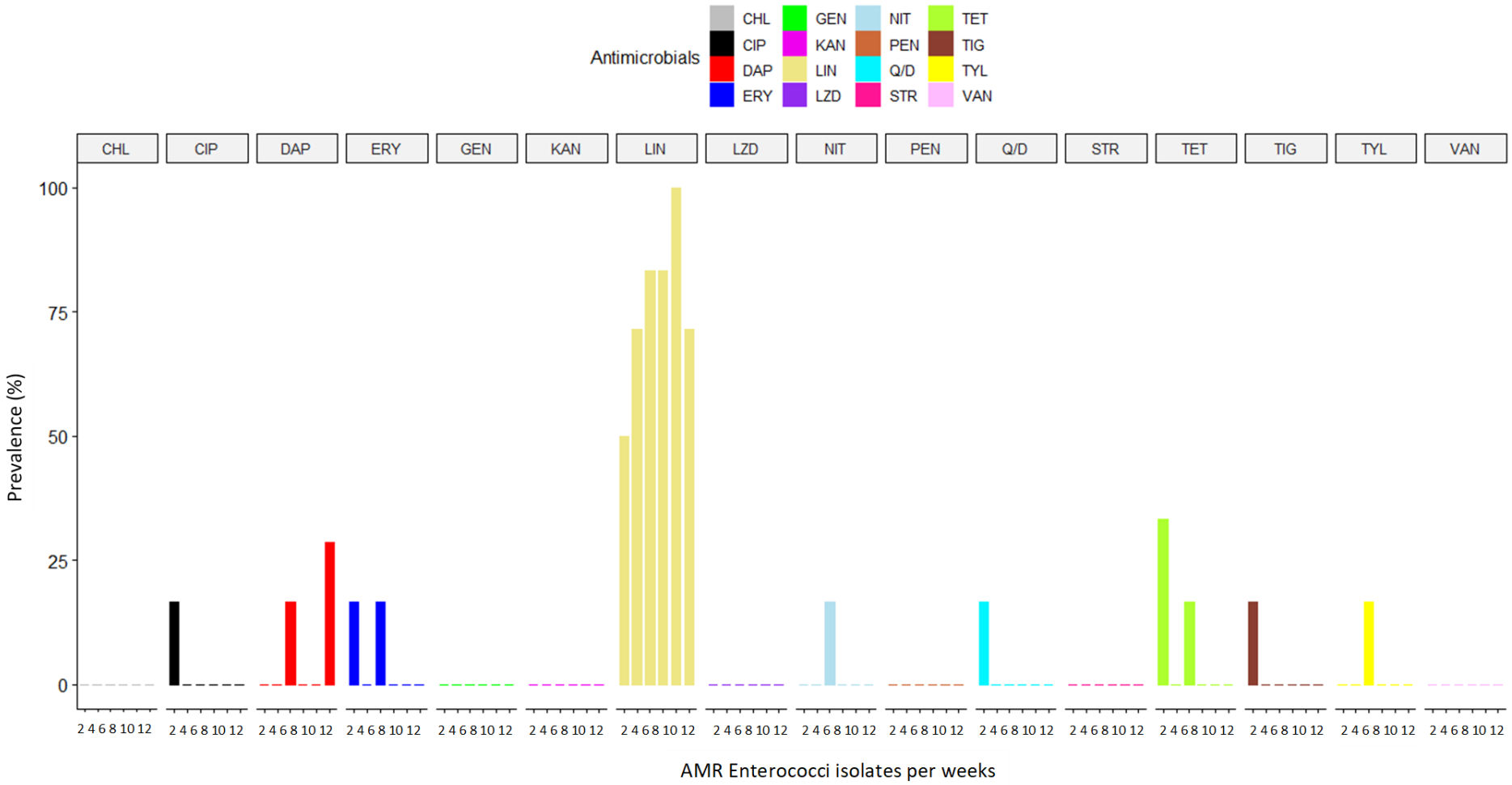

Figure 3. Longitudinal analysis of the antimicrobial resistance among *Enterococcus* spp. isolated from fresh faecal pats collected from the target cattle pen (n=42) collected from non-treated cattle. **CHL** (Chloramphenicol), **CIP** (Ciprofloxacin)**, DAP** (Daptomycin), **ERY** (Erythromycin), **GEN** (Gentamycin), **KAN** (Kanamycin), **LIN** (Lincomycin), **LZD** (Linezolid), **NIT**(Nitrofurantoin), **PEN** (Penicillin), **Q/D** (Quinupristin/dalfopristin**),** **STR** (Streptomycin), **TET** (Tetracycline), **TIG** (Tigecycline), **TYL** (Tylosin tartrate**), VAN** (Vancomycin)

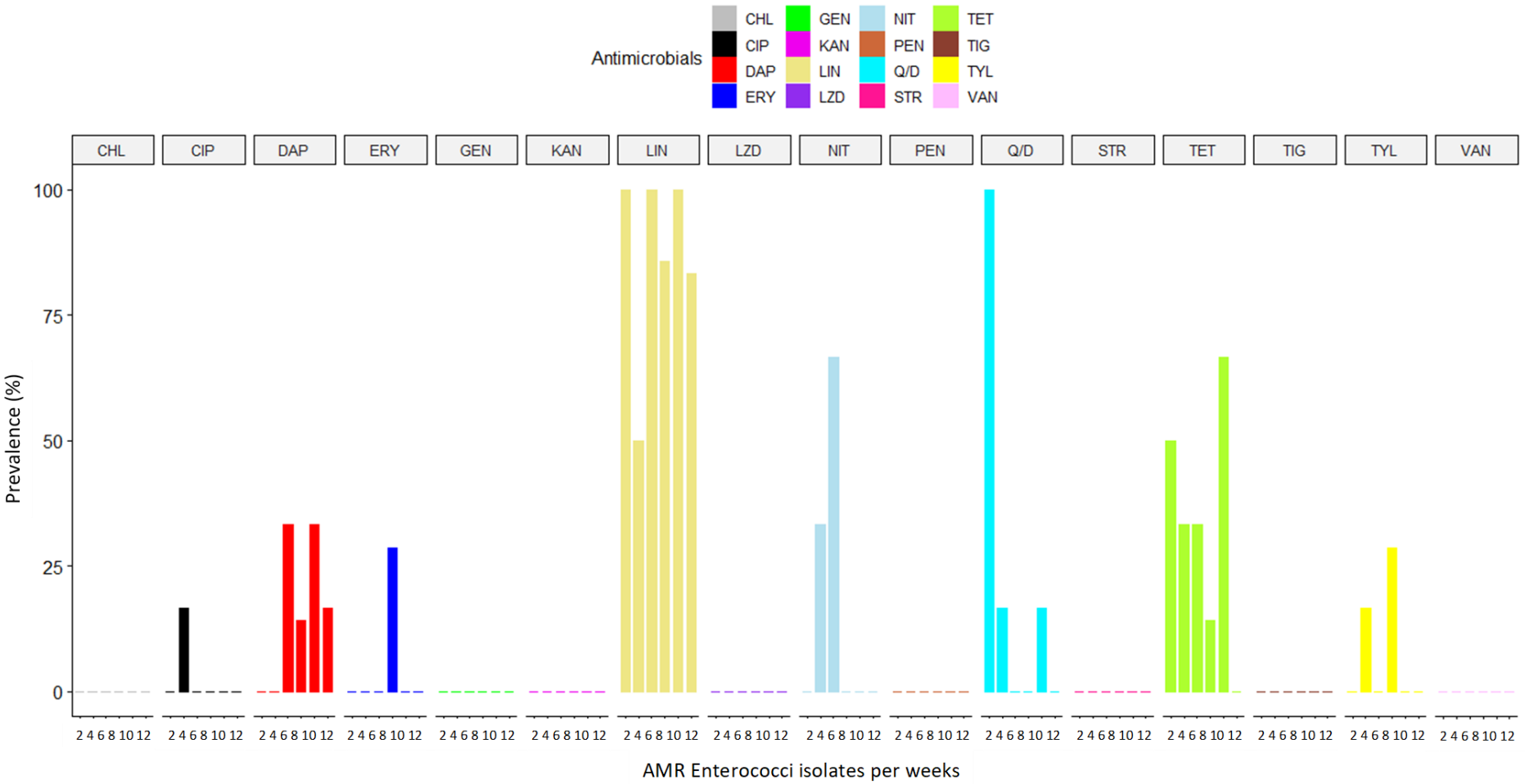

Figure 4. Longitudinal analysis of antimicrobial resistance among *Enterococcus* spp. isolated from fresh faecal pats collected from the sick cattle pen (n=42). **CHL** (Chloramphenicol), **CIP** (Ciprofloxacin)**, DAP** (Daptomycin), **ERY** (Erythromycin), **GEN** (Gentamycin), **KAN** (Kanamycin), **LIN** (Lincomycin), **LZD** (Linezolid), **NIT**(Nitrofurantoin), **PEN** (Penicillin), **Q/D** (Quinupristin/dalfopristin**), STR** (Streptomycin), **TET** (Tetracycline), **TIG** (Tigecycline), **TYL** (Tylosin tartrate**), VAN** (Vancomycin)

A total of 42 faecal pat samples were collected and 92.8% (n=39) yielded *Enterococcus* spp. The highest frequency of resistance was observed to lincomycin (76.9%), tetracycline and daptomycin (7.7% each), erythromycin (5.1%) in the target pen (Table 23). Similarly, 30 *Enterococcus* spp. were recovered from the hospital pen faecal pat samples. The highest frequency of resistance was observed to lincomycin (83.3%), followed by daptomycin (16.7%), nitrofurantoin (13.3%), tylosin (10%), and erythromycin (6.7%).

Table 23. Antimicrobial susceptibility of *Enterococcus* spp. isolated from target (n=39) and hospital (n=30) pen faecal pat samples

Antimicrobial class	Antimicrobial agent	Sample (42 each)**	Resistant (%)	CI (95 %)	Proportion of isolates per MIC value (µg/mL)*															
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Aminoglycosides	Gentamycin	TP	0.00	0.0														100			
		HP	0.00	0.0														100			
	Kanamycin	TP	0.00	0.0														94.9	2.6	2.6	
		HP	0.00	0.0														93.3	6.7		
	Streptomycin	TP	0.00	0.0																100	
		HP	0.00	0.0																100	
β-lactam	Pencillin	TP	0.00	0.0					7.7	43.6	33.3	10.3	5.1								
		HP	0.00	0.0					10	30	36.7	16.7	6.7								
Fluoroquinolones	Ciprofloxacin	TP	2.56	0.36-16.09					17.9	53.8	20.5	5.1	2.6								
		HP	3.33	0.47-20.20				3.3	10	53.3	16.7	13.3	3.3								
Glycopeptides	Vancomycin	TP	0.00	0.0						61.5	35.9		2.6								
		HP	0.00	0.0						60	33.3	3.3	3.3								
Glycylcyclines	Tigecycline	TP	2.56	0.36-16.09		30.8	38.5	20.5	7.7	2.6											
		HP	0.00	0.0		20	40	40													
Lincosamide	Lincomycin	TP	76.92	61.28-87.53							20.5		2.6	5.1	71.8						
		HP	83.33	65.68-92.89							16.7				83.3						
Lipopeptides	Daptomycin	TP	7.69	2.50-21.30					2.6		10.3	38.5	41	7.7							
		HP	16.67	7.11-34.32					3.3	3.3	3.3	6.7	66.7	16.7							
Macrolides	Erythromycin	TP	5.13	1.29-18.32					94.9					2.6	2.6						
		HP	6.67	1.67-23.07					70	3.3	10	6.7	3.3		6.7						
	Tylosin tartrate	TP	2.56	0.36-16.09						5.1	20.5	59	10.3	2.6			2.6				
		HP	10.00	3.26-26.81								63.3	23.3	3.3			10				
Nitrofurantoins	Nitrofurantoin	TP	2.56	0.36-16.09										2.6		30.8	64.1	2.6			
		HP	13.33	5.09-30.60										3.3	3.3	13.3	66.7	13.3			
Oxazolidinones	Linezolid	TP	0.00	0.0							7.7	89.7	2.6								
		HP	0.00	0.0							13.3	80	6.7								
Phenicols	Chloramphenicol	TP	0.00	0.0									74.4	25.6							
		HP	0.00	0.0									56.7	43.3							
Streptogramins	Quinupristin/dalfopristin	TP	2.56	0.36-16.09						20.5	20.5	56.4			2.6						
		HP	13.33	5.09-30.60						16.7	6.7	63.3	3.3			10					
Tetracycline	Tetracycline	TP	7.69	2.50-21.30							92.3						7.7				

HP	30.00	16.41-48.34			66.7	3.3	6.7	23.3

***** The range of MICs tested are indicated in white boxes and the vertical line indicates the resistance breakpoint cut off value

**TP, target pen; HP, hospital pen

3.3.1.3. Water

From a total of five samples collected from the target pen water trough, *Enterococcus* spp. were recovered from four, including two *E.hirae*, one *E. gallinarum* and one *E.mundtii* isolates. Only lincomycin resistance was observed in one *E. hirae* and one *E. gallinarum,* the remaining two isolates were susceptible to all antimicrobials. Only two *Enterococcus* spp., namely *E. hermanniensis* and *E. mundtii*, were recovered from the samples collected from the hospital pen. The *E. hermanniensis* isolate was resistant to tetracycline while the *E. mundtii* was resistant to lincomycin.

3.3.1.4. Air

A total of 12 air samples were collected from 20m and 200m distance from the target and hospital pens. Only one *E. hirae* isolate was obtained from 200m distance from the target pen and it was resistant to nitrofurantoin. By contrast, two *Enterococcus* spp., *E. hermanniensis* and *E. hirae*, were identified from air samples obtained 200m from the hospital pen. The *E. hermanniensis* was resistant to both quinupristin/dalfopristin and tetracycline, whereas the *E. hirae* was susceptible to all antimicrobials.

3.3.1.5. Carcase

Overall, 25 (16.8%) and 45 (30.0%) *Enterococcus* spp. isolates were cultured from the flank and brisket, and hip/round, respectively. The most prevalent species identified in flank and brisket samples were *E. hirae* (52.0%) and *E. faecium* (40.0%). The most prevalent species identified hip/round sample swab were *E. gilvus* (46.7%) and *E. faecium* (35.6%). The highest frequency of AMR was observed to lincomycin (80.0%) and tetracycline (24.0%) in *Enterococcus* spp. isolated from flank and brisket swab samples. Similarly, a high frequency of resistance to lincomycin (46.7%), and quinupristin/dalfopristin (8.9%) was observed in isolates from hip/round carcase swab isolates (Figure 5).

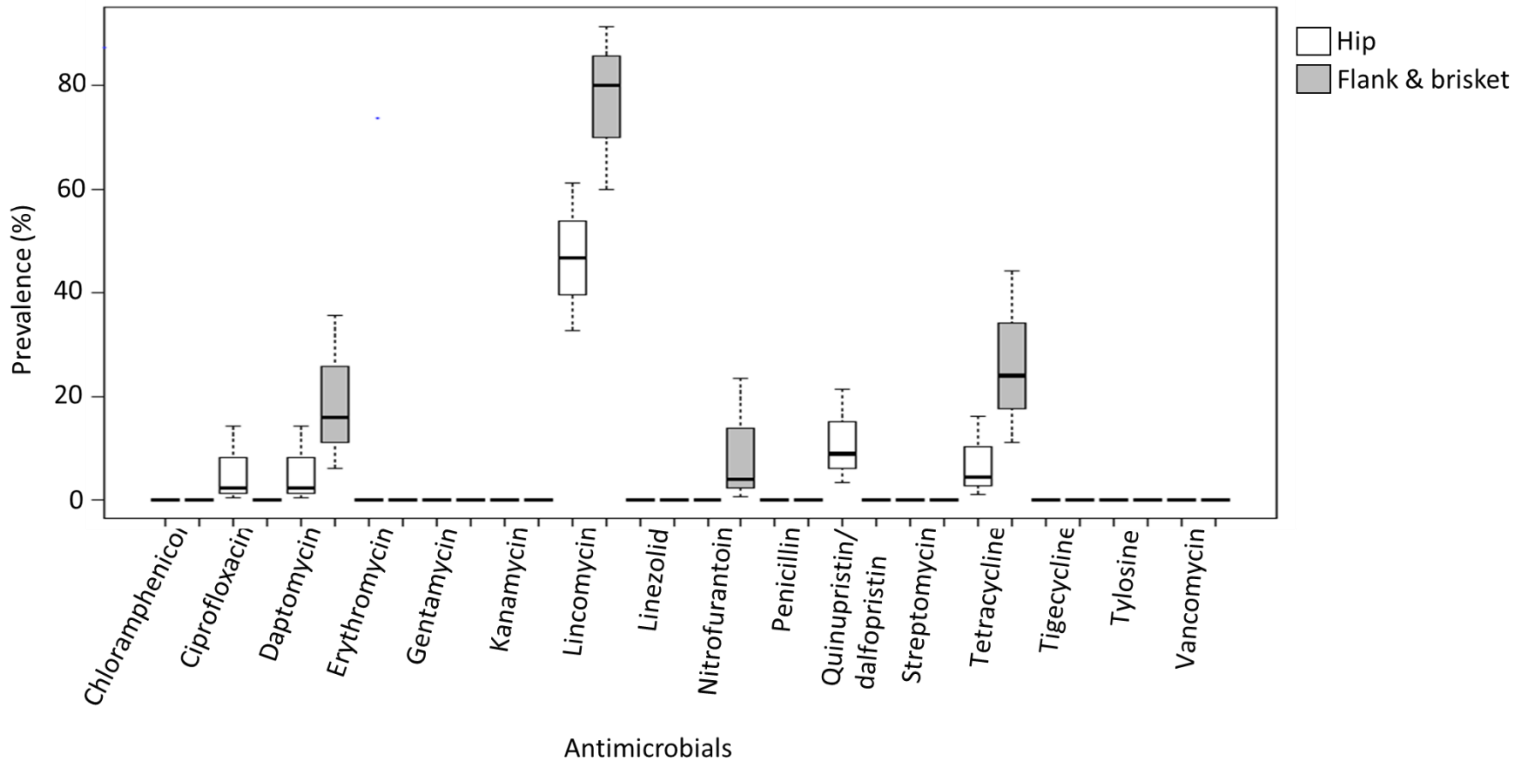

Figure 5. The prevalence of antimicrobial resistance (% of isolates) in *Enterococcus* spp. isolated from flank and brisket, and hip swabs

The proportion of pan-susceptible *Enterococcus* spp. isolated from hip and flank and brisket was 51.1% and 16.0%, respectively. Four isolates from the flank and brisket were resistant to three antimicrobial classes. The overall prevalence of resistance was 48.9% and 84.0 % in hip, and flank and brisket samples, respectively (Table 24).

Table 24. The AMR pattern of *Enterococcus* isolated from carcase

Antimicrobial classes	**Total no. of isolates (%)**		**Resistance pattern (no. of isolates)**	
	Hip	**Flank and Brisket**	**Hip**	**Flank and Brisket**
All susceptible	23 (51.11)	4 (16.00)	23	4
1	16 (31.37)	15 (60.00)	LIN (15) TET (1)	LIN (14) TET (1)
2	5 (11.11)	2 (8.00)	LIN-Q/D (4) CIP-LIN (1)	LIN-TET (2)
3	1 (2.22)	4 (16.00)	DAP-LIN-TET (1)	DAP-LIN-TET (3) DAP-LIN-NIT (1)
Total	45	25		

CIP (Ciprofloxacin), **DAP** (Daptomycin), **LIN** (Lincomycin), **NIT** (Nitrofurantoin), **Q/D** (Quinupristin/dalfopristin), **TET** (Tetracycline)

3.3.1.6. Lymph nodes

The frequency of *Enterococcus* spp. Isolation in prescapular and subiliac lymph node samples was 87.7% and 87.5%, respectively. Species-level identification revealed that *E. faecium* (81.0%) was predominant followed by *E. hirae* (7.1%), *E. divriesei* (4.8%), *E. faecalis* (4.0%), *E. mundtii* (*1.6%)*, *E. gilvus* (0.8%), and *E. malodoratus* (0.8%)

in the subiliac lymph nodes. The distribution of MICs and the levels of resistance to different antimicrobials in *Enterococcus* spp isolated from lymph node are presented in Appendix 2. In the subiliac lymph nodes, the highest prevalence of resistance was observed to lincomycin (89.7%), followed by nitrofurantoin (27.0%), quinupristin/dalfopristin (6.3%), tigecycline (5.6%), and daptomycin (3.2%) (Figure 6). Among these isolates, 6.4% were MDR. In the prescapular lymph nodes, the most common species were *E. faecium* (80.5%), *E. hirae* (7.1%), *E. faecalis* (6.3%), *E. mundtii* (2.3%), and *E. divriesei* (1.6%) with 3.9% MDR (Table 25). None of the isolates were resistant to chloramphenicol, gentamycin, kanamycin, linezolid, penicillin, streptomycin, tetracycline, tylosin and vancomycin.

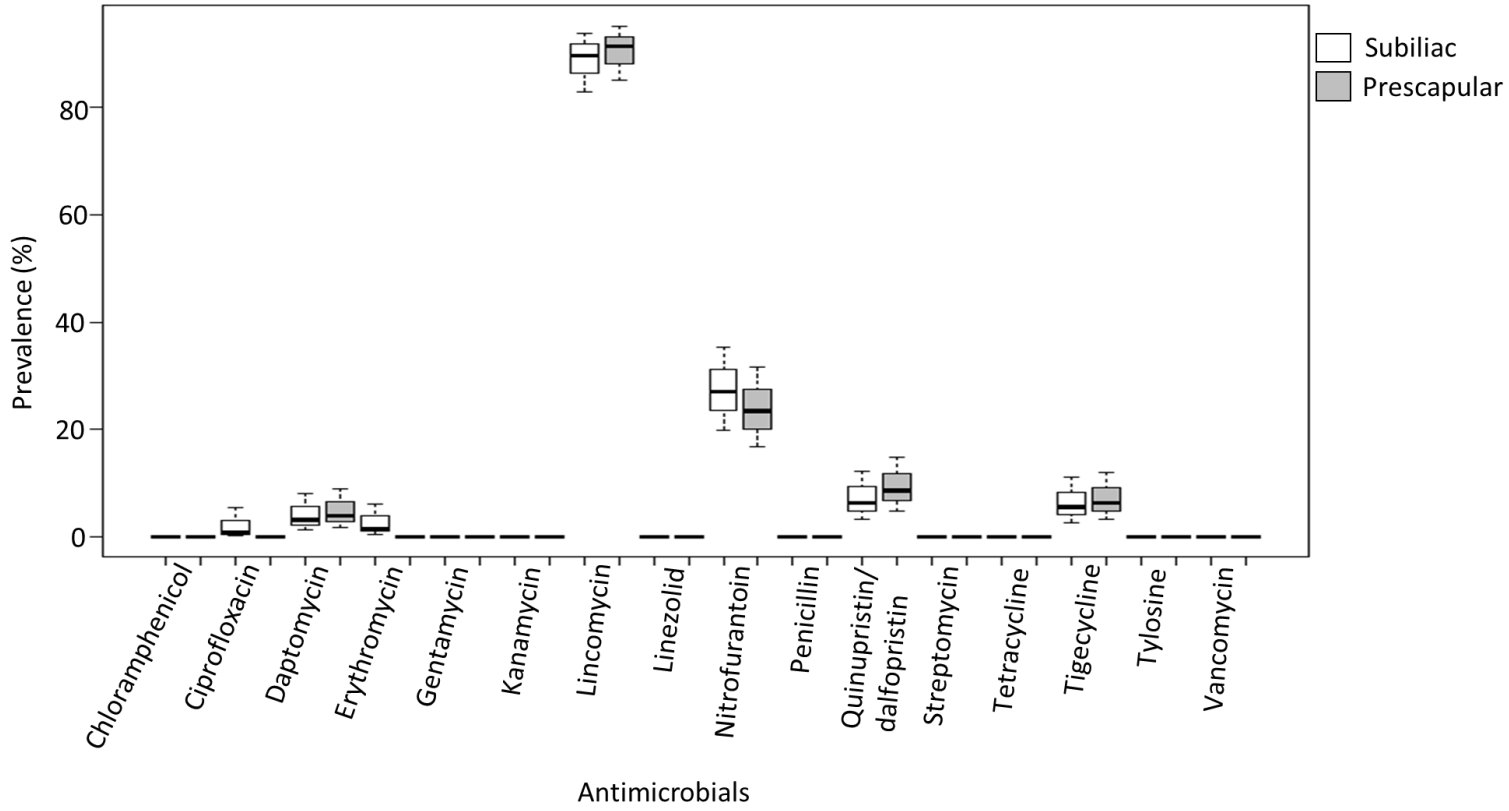

Figure 6. The prevalence of antimicrobial resistance (% of isolates) in *Enterococcus* spp. isolated from subiliac and prescapular lymph nodes

Table 25. The AMR pattern of *Enterococcus* spp. isolated from lymph nodes (LN)

Number of antimicrobial classes	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	Subiliac LN (126)	Prescapular LN (128)	Subiliac LN	Prescapular LN
Susceptible	11 (8.73)	9 (7.03)	11	9
1	69 (54.76)	72 (56.25)	LIN (67) DAP (1) NIT (1)	LIN (70) TIG (1) NIT (1)
2	38 (30.16)	42 (32.81)	LIN-Q/D (5) LIN-NIT (27) LIN-TIG (3) DAP-LIN (3)	LIN-NIT (25) LIN-Q/D (9) LIN-TIG (3) DAP-LIN (5)
3	8 (6.35)	5 (3.91)	LIN-NIT-Q/D (3) CIP-LIN-NIT (1) ERY-LIN-TIG (2) LIN-NIT-TIG (2)	LIN-NIT-TIG (3) LIN-NIT-Q/D (1) LIN-Q/D-TIG (1)
Total	126	128		

CIP (Ciprofloxacin), **DAP** (Daptomycin), **ERY** (Erythromycin), **LIN** (Lincomycin), **NIT** (Nitrofurantoin), **Q/D** (Quinupristin/dalfopristin), **TIG** (Tigecycline)

3.3.2. Genotypic determination of antimicrobial resistance

3.3.2.1. Enterococcus faecium

Ciprofloxacin, daptomycin, erythromycin, quinupristin/dalfopristin and tigecycline-resistant *E. faecium* (62*)* isolates from entry and exit were selected for further analysis. All *E. faecium* were resistant to at least one antimicrobial, so only lincomycin-resistant isolates (n=4) were chosen as a control group. The antimicrobial-resistant *Enterococcus* spp. genomes were screened against the CARD and ResFinder database for AMR genes (ARGs) and 14 genes (*aac(6')-Ii*, *aac(6')-Iid*, *ant(6)-Ia*, *pbp5*, *eatAv*, *lnu(G)*, *vat(E)*, *msr(C)*, *erm(B)*, *efmA*, *tet(45), tet(L)*, *tet(M),* and *tet(S),* associated with multi-drug efflux pumps and other transporters were observed (Table 26). In addition, almost half of the isolates (46.7%) had a point mutation in the penicillin-binding protein (pbp5) gene that is responsible for resistance to ampicillin. The chromosomal-encoded ABC-F subfamily protein *msrC* gene which confers resistance to erythromycin and other macrolide and streptogramin B antimicrobials was observed in 95.0% of isolates. ABC-F ATP-binding cassette ribosomal protection protein *eatAv* resistance gene; which confers resistance to lincosamides, streptogramin A and pleuromutilins was observed in 75.8% isolates. A major facilitator superfamily (MFS) antimicrobial efflux pump *efmA* gene; which is important for removal of macrolide and fluoroquinolone antimicrobials from the intracellular environment of bacterial cells was observed in 33.9% of the isolates. Macrolide, lincosamide, and streptogramin (MLS) resistance may also result, usually due to a ribosomal alteration mediated by a ribosomal methylase encoded by the *erm (B)* gene. In this study, *erm (B)* gene was found in three isolates and it is responsible for cross-resistance to all macrolides, lincosamides, and streptogramin B antimicrobials. A transposon-mediated lincosamide nucleotidyl transferase resistance gene *lnuG;* which inactivates lincosamide antimicrobials was also observed in two isolates. The *vat* (E) gene a virginiamycin acetyltransferase streptogramin resistance gene encoding resistance to quinupristin/dalfopristin was observed in one isolate. The resistance gene which encodes an enzyme that confers resistance to aminoglycosides; *aac (6')-Ii* was found in 59 (95.2%) isolates followed by aac *(6')-Iid 2* (3.2%) and *ant (6)-Ia* 1 (1.6%). Similarly, a number of genes encoding tetracycline resistance were observed in these isolates; *tet(M)*, which encodes for ribosomal protection protein, was the most frequently observed gene (n=3), followed by *tet(L)* (n=2), which encodes for a tetracycline efflux, *tet(45)* (n=2) gene which is closely related to the *tet(L)* efflux pump gene was found in two isolates and *tet(S)* in two isolates.

Table 26. The frequency of antimicrobial resistance genes in *E. faecium* selected for whole genome sequencing

Resistance gene	Antimicrobial class	Resistance phenotype	Number of isolates (n=62)
aac(6')-Ii	Aminoglycosides	GEN	59 (95.2)
aac(6')-Iid	Aminoglycosides	AMK	2 (3.2)
ant(6)-Ia	Aminoglycosides	STR	1 (1.6)
pbp5	β-lactam	AMP	29 (46.8)
eatAv	LsaP (lincosamides, streptogramin As and pleuromutilins)	Q/D,LIN	47 (75.8)
lnu(G)	Lincosamide	LIN	2 (3.2)
vat -E	Streptogramin	VIR, Q/D	1 (1.6)
msr(C)	Macrolide, streptogramin	ERY, Q/D, VIR	59 (95.2)
erm(B)	MLS (macrolide, lincosamide, streptogramin)	ERY, LIN, Q/D	3 (4.8)
efmA	Macrolides, fluoroquinolones	CIP	21 (33.9)
tet(M)	Tetracyclines	TET	3 (4.8)
tet(L)	Tetracyclines	TET	2(3.2)
tet(45)	Tetracyclines	TET	2(3.2)
tet(S)	Tetracyclines	TET	2(3.2)

Among the 62 isolates, the number of resistance genes in individual isolates ranged from one to eight, with 96.8% of isolates carrying at least three resistant genes. For instance, a resistance gene pattern consisting of *aac (6')-Ii, eatAv, msr(C)* was identified in 25 isolates.

Table 27. The antimicrobial resistance phenotype and genotype of 62 *E. faecium* isolates obtained at entry and exit from feedlot cattle faecal samples and subjected to whole genome sequencing analysis

Antimicrobial classes pattern	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	Phenotypic	Genotypic	Phenotypic (MIC)	Genotypic(resistance gene)
1	20 (32.2)	2 (3.2)	CIP (3) DAP (13) LIN (4)	*aac(6')-Iid* (2)
2	13(21.0)	0	CIP-LIN (1) CIP-NIT (2) CIP-TET (1) Q/D-LIN (9)	
3	19 (30.6)	28 (45.2)	CIP-LIN-NIT (4) CIP-DAP-NIT (3) CIP-TIG-NIT (1) DAP-Q/D-LIN (1) Q/D-LIN-NIT (10)	*aac(6')-Ii ,eatAv, msr(C) (25)* *aac(6')-Ii, pbp5,msr(C)* (1) *aac(6')-Ii, efmA, pbp5* (1) *eatAv, efmA, msr(C)* (1)
4	9 (14.5)	23 (37.1)	CIP-DAP-LIN-NIT (1) CIP-DAP-Q/D-LIN (1) DAP-ERY-LIN-NIT (1) DAP-Q/D-LIN-NIT (1) Q/D-KAN-LIN-NIT (1) Q/D-LIN-NIT-TET (1) ERY-Q/D-TYL-LIN-TET (2) ERY-TYL-LIN-NIT-TET (1)	*aac(6')-Ii , eatAv, pbp5, msr(C),* (9) *aac(6')-Ii , efmA, pbp5, msr(C)* (11) *aac(6')-Ii , eatAv, lnu(G), msr(C)* (2) *aac(6')-Ii , eatAv, msr(C), tet(S) (1)*
5	1 (1.6)	5 (8.1)	CIP-DAP-Q/D-LIN-NIT (1)	*aac(6')-Ii , eatAv, efmA, pbp5, msr(C)* (5)
6		4 (6.4)		*aac(6')-Ii , eatAv, efmA, pbp5, msr(C), tet(M), (1)* *aac(6')-Ii , eatAv, efmA, erm(B), msr(C), tet(L),tet(M), tet(45) (1)* *aac(6')-Ii ,ant(6)-Ia, eatAv, erm(B), pbp5, msr(C), tet(S), vat(E) (1)* *aac(6')-Ii, eatAv, efmA, erm(B),msr(C),tet(L),tet(M),tet(45) (1)*
Non-MDR	33 (53.2)	2 (3.2)		
MDR	29 (46.7)	60 (96.8)		
Resistance	62(100)	62(100)		

CIP, Ciprofloxacin; **DAP**, Daptomycin; **ERY**, Erythromycin; **KAN**, Kanamycin; **LIN**, Lincomycin; **NIT**, Nitrofurantoin; **Q/D**, Quinupristin/dalfopristin; **TET**, Tetracycline; **TIG**, Tigecycline; **TYL**, Tylosin tartrate

Erythromycin, tetracycline and tylosin-resistant isolates possessed genes responsible for their resistance. The resistance genes *eatAv* and *msr(C)* and *vat(E)* were found in 96.3% of quinupristin/dalfopristin-resistant enterococci. The resistance gene *efmA* accounted for approximately 66.7% of ciprofloxacin-resistant enterococci isolates. However, 9/44 (20.4%) of the ciprofloxacin-susceptible isolates also harboured the *efmA* resistance gene. In this study, daptomycin, tigecycline and nitrofurantoin resistance genes were not observed.

Table 28. Agreement between phenotypic and genotypic resistance among the 62 *E. faecium* isolates subjected to whole genome sequencing

Antimicrobial class	AMR isolates (%)	Resistance gene observed (%)	Agreement (%)
Aminoglycosides	Kanamycin (n=1;1.6)	*aac(6')-Ii* (n=1;1.6)	100
	Streptomycin(n=0)	*ant(6)-Ia* (n=1; 1.6)	0
Fluoroquinolones	Ciprofloxacin (n=18;29.0)	*efmA* (n=12; 19.3)	66.7
Glycylcyclines	Tigecycline (n=1; 1.6)		0
Lipopeptides	Daptomycin (n=22; 35.5)		0
Lincosamide	Lincomycin (n=39; 62.9)	*eatAv* (n=38; 61.3)	97.4
		erm(B) (n=3; 4.8)	
		lnu(G) (n=2; 3.2)	
Macrolides	Erythromycin(n=4; 6.4)	*msr(C)* (n=4; 6.4)	100
		erm(B) (n=3; 4.8)	
	Tylosin tartrate(n=3; 4.8)	*erm(B)* (n=3; 4.8)	100
Nitrofurantoin	Nitrofurantoin (n=27; 43.5)		0
Streptogramins	Quinupristin/dalfopristin (n=27; 43.5)	*eatAv* (n=26; 41.9)	96.3
		msr(C) (n=26; 41.9)	
		Vat(E) (n=1; 1.6)	
Tetracycline	Tetracycline (n=5; 8.1)	*tet(M)* (n=3; 4.8)	100
		tet(L) (n=2; 3.2)	
		tet(S) (n=2; 3.2)	
		tet(45) (n=2; 3.2)	

3.3.2.2. Enterococcus hirae

All daptomycin, erythromycin, and tigecycline-resistant *E. hirae* (43) and 4 antimicrobial-sensitive isolates were selected for further analysis. Aminoglycoside, tetracycline, macrolide and streptogramin resistance genes were observed in these isolates. The aminoglycoside resistance genes *aac (6')-Ii* and *aac (6')-Iid* were found in 57.4% and 42.5% of the isolates. The tetracycline resistance genes *tet (L)*, *tet (M)* and *tet (45)* were observed in 6.4%, 8.5% and 6.4% of the isolates.

Table 29. The frequency of antimicrobial resistance gene in *E. hirae*

Resistance gene	Antimicrobial class	Resistance phenotype	Number of isolates (n=47)
aac(6')-Ii	Aminoglycosides	GEN	27 (57.4)
aac(6')-Iid	Aminoglycosides	AMK	20 (42.5)
erm(B)	MLS (macrolide, lincosamide, streptogramin)	ERY, LIN, Q/D	2 (4.2)
tet(L)	Tetracyclines	TET	3 (6.4)
tet(M)	Tetracyclines	TET	4 (8.5)
tet(45)	Tetracyclines	TET	3 (6.4)

Enterococcus hirae harboured fewer resistance genes compared to *E. faecium*. Among the isolates 87.2% were resistant to a single antimicrobial class. All isolate harboured at least one ARG (Table 30).

Table 30. The antimicrobial resistance phenotype and genotype of 47 *E. hirae* isolates obtained at entry and exit from feedlot cattle faecal samples and subjected to whole genome sequencing analysis

Antimicrobial classes pattern	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	Phenotypic	Genotypic	Phenotypic (MIC)	Genotypic(resistance gene)
Sensitive	4 (8.5)		4	
1	20 (42.5)	41 (87.2)	DAP (18) TIG (2)	*aac(6')-Ii* (27) *aac(6')-Iid (14)*
2	15(31.9)	6 (12.7)	DAP-LIN (10) DAP-NIT (2) TIG-LIN (2) ERY-TYL-LIN (1)	*aac(6')-Iid,erm(B)* (2) *aac(6')-Iid,tet(M)(1)* *aac(6')-Iid*, *tet(L),tet(M),tet(45)* (3)
3	8 (17.0)	0	DAP-LIN-NIT (6) DAP-LIN-TET (1) ERY-TIG-TYL-LIN (1)	
Non-MDR	35 (74.5)	47 (100)		
MDR	8 (17.0)	0		
Resistance	43(91.5)	47(100)		

DAP, Daptomycin; **ERY**, Erythromycin; **LIN**, Lincomycin; **NIT**, Nitrofurantoin; **TET**, Tetracycline; **TIG**, Tigecycline; **TYL**, Tylosin tartrate

One of the most widespread mechanisms of resistance to macrolides is mediated by methylation of a specific adenine residue in 23S rRNA and is associated with the *erm(B)* gene. In this study, all of the enterococci isolates that were phenotypically resistant to macrolides i.e., erythromycin and tylosin, harboured *erm(B)* resistance gene (Table 31). However, of the daptomycin (n=37) and tigecycline (n=4)-resistant isolates, none harboured AMR genes that could explain the phenotype.

Table 31. Agreement between phenotypic and genotypic resistance among the 47 *E. hirae* isolates subjected to whole genome sequencing

Antimicrobial class	AMR isolates (%)	Resistance gene observed (%)	Agreement (%)
Glycylcyclines	Tigecycline (n=4; 8.5)	0	0
Lipopeptides	Daptomycin (n=37; 78.7)	0	0
Lincosamides	Lincomycin (n=21; 44.7)	*erm(B)* (n=2; 4.2)	9.5
Macrolides	Erythromycin(n=2; 4.2)	*erm(B)*(n=2; 4.2)	100
	Tylosin tartrate(n=2; 4.2)	*erm(B*)(n=2; 4.2)	100
Nitrofurantoin	Nitrofurantoin (n=8; 17.0)	0	0
Tetracyclines	Tetracycline (n=1; 2.1)	*tet(M)* (n=1; 2.1)	100

5. Discussion

This study described the prevalence of AMR in *E. coli*, *Salmonella* and *Enterococcus* spp. isolated from Australian cattle from entry into the beef feedlot to the end of the chain within the slaughter house. In general, cattle arrived at the feedlot with a low frequency of antimicrobial-resistant bacteria. Among *E.coli* isolated at entry, very low levels of resistance to amoxicillin-clavulanate, ceftiofur, streptomycin, and tetracycline were observed. The whole genome sequence of the resistance isolate revealed *aph (3'')-Ib, bla*$_{TEM-1B,}$ *bla*$_{CMY-2,}$ and *tet(A)* resistance genes which are responsible for streptomycin, β-lactams (both aminopenicillins and third-generation cephalosporins) and tetracycline resistance phenotypes. This result showed that cattle entering feedlots may already be colonised with commensal *E. coli* resistant to critically important antimicrobials (i.e. ceftiofur), albeit at extremely low frequency and abundance (Table 5). At entry, *E. hirae* (86.5%) was the most predominant *Enterococcus* spp recovered followed by *E. faecium* (8.7%). However, *E. faecium* is the species of greater clinical importance in human medicine and entry isolates showed a high level of resistance to ciprofloxacin, tetracycline and quinupristin/dalfopristin. A corresponding resistance gene *efmA* (50%), *eatAv, msr(C) or Vat(E) (100%)* and *tet(M)* or *tet(S)* (100%) was detected in ciprofloxacin-, quinupristin/dalfopristin- and tetracycline-resistant isolates (Appendix 2). As for *E. coli*, it appears that cattle arriving at the feedlot may already harbour *E. faecium* isolates resistant to critically important antimicrobials. The following antimicrobials: daptomycin, ciprofloxacin, nitrofurantoin, quinupristin/dalfopristin and tigecycline are not registered for use in animals in Australia (ASTAG, 2018) but some level of resistance was detected. However, these antimicrobials are undoubtedly used in human medicine. Hence, we hypothesised that there may be a potential for a spread of resistant strains or genetic determinants from humans to animals. Antimicrobial resistance can spread from humans to animals by transfer of the resistant bacteria through direct contact (Argudín et al., 2017).

Fresh faecal pat samples were also collected from target and hospital pens in order to identify AMR trends during the entire cattle feeding program. Higher levels of resistance were observed in samples collected from the hospital pen compared to the target pen. Of the isolates obtained (n=42), 76.2% (n=32) were ESBL-producing *E. coli*. Resistance to ampicillin (78.6%), ceftiofur (76.2%), and tetracycline (88.1%) were observed. High levels of resistance to ampicillin (86%), ceftiofur (70.2%), and tetracycline (87.7%), were recently reported among *E. coli* isolated from cattle deposited faeces in Western Canadian feedlots (Adator et al., 2020). In this study, azithromycin resistance was observed in 47.6% of the isolates for faecal pats from the hospital pen but none in the target pen. We hypothesise that the reason for the high proportion of azithromycin resistance in the hospital faecal pats was the use of macrolides for treatment of treated sick animals. The azithromycin resistance was likely of only transient character, as no resistance was found at slaughter. The risk for the development of azithromycin resistance and its longevity post-treatment are an area that requires further research.

Our results on faecal pat samples were similar to studies in humans where higher rates of resistance to clinically used antimicrobials, in particular resistance to third-generation cephalosporins, are observed in *E. coli* isolated from hospitalised patients compared to outpatients (AURA, 2016). Unlike the situation in the target pen, *E. coli* resistant to β-lactams

could be isolated from the hospital pen water trough and air/dust samples collected at a distance of 200m from the hospital pen. These results confirmed that the hospital pen environment is the most significant source of antimicrobial-resistant bacteria at the feedlot. However, the results are based on a limited number of isolates. Hence, further research to confirm these results, including larger sample size of cattle with a high rate of treatment is required.

Among *E. coli* isolated from the same cohort of cattle, resistance to antimicrobials increased from entry to exit sampling points. At exit, the non-ESBL producing *E. coli* isolates were resistant to tetracycline (17.8%), ampicillin (5.4%), streptomycin (4.6%), and sulfisoxazole (3.9%). Similar proportions of resistance were recently observed in the Australia-wide cattle AMR surveillance study focused on gastrointestinal samples collected at the abattoir, e.g., tetracycline (15.9%), ampicillin (4.7%) and streptomycin (4.5%) (Barlow et al., 2020). The recovery of ESBL-producing *E.coli* (albeit at extremely low abundance on the ESBL plate) increased from one sample (0.7%) at entry to six samples (4.4%) at exit and ceftiofur resistance were observed in all isolates. The whole genome sequence analysis of the resistance isolates revealed one or more resistance gene responsible for the observed resistance phenotypes. For instance, either *tet (A*) or *tet (B)* resistance genes were identified in almost all tetracycline-resistant isolates. Likewise, bla_{TEM}, bla_{CTX}, and bla_{CMY} resistance genes were also identified in isolates resistant to both aminopenicillins (bla_{TEM}) and third generation cephalosporins (bla_{CTX}, and bla_{CMY}).

At slaughter attention to handling practices of the stock and carcases resulted in a high level of carcase hygiene. As a result, no *E. coli* was detected from the carcase swab samples. In addition, only a few Salmonella serovars namely *S. anatum*, *S. bovismorbificans* and *S. infantis* were detected in faecal pat and lymph node samples. Currently, MDR *S. Infantis* has increasingly been reported from food-producing animals and humans (EFSA, 2018). In this study, all salmonella serovars were sensitive to all the tested antimicrobials. Most of the large lymph nodes on the carcase are incorporated in the ground beef production. Hence, if Salmonella contamination does occur, it is likely to finish in the ground beef. All care should be taken to avoid the possible incorporation of infected lymph node into the ground beef. The importance of ground beef in the risk to human health from Salmonella food-poisoning and spread of resistance determinants is yet to be investigated.

Unlike at entry, *E. faecium* (81.2%) was the most predominant *Enterococcus* spp. identified at exit. Diet is the possible reason for the observed change in species diversity, although age may have shifted the faecal microbial community (Devriese L.A. , 1992). The isolated *E. faecium* were resistant to lincomycin, quinupristin/dalfopristin, nitrofurantoin, and daptomycin. The levels of resistance to daptomycin, erythromycin, lincomycin, and tetracycline in this study were consistent with other Australian studies (2013 and 2019 surveys) among beef cattle (Barlow et al., 2020; Barlow et al., 2017). Interestingly, in this study we also found resistance to nitrofurantoin that has not been used in food producing animals in Australia since 1992. However, it is used to treat urinary tract infection in humans. We hypothesise that nitrofurantoin resistance in this study may be yet to be elapsed or more likely a transfer from human isolates has occurred to cattle. High level of nitrofurantoin has been reported also elsewhere (e.g., in Canada 45% (Zaheer et al., 2020b)).

Enterococcus spp. are naturally resistant to many classes of antimicrobials such as aminoglycosides and β-lactams, and can also acquire resistance to other classes, including

glycopeptides, quinolones and tetracyclines (Faron et al., 2016). In this study, the *E. faecium* isolates were shown to carry *aac (6')-Ii, msr(C), eatAv*, and *efmA* resistance genes imparting resistance to aminoglycosides, lincosamides and fluoroquinolones, respectively. Similarly, aminoglycoside, lincosamide, nitrofurantoin, macrolide, oxazolidinone, streptogramin A, and tetracycline ARGs have been reported in *E. faecium* isolated from beef cattle in Canada (Zaheer et al., 2020b). The observed AMR phenotypes present in faecal samples obtained from beef feedlots likely reflect differences in the types of antimicrobials used on the different enterprises but this is unlikely to be the only reason. In this study, the mutated form of the wildtype *eatA* ABC-F subfamily protein *eatAv* gene; which confers resistant to lincosamides, streptogramin A and pleuromutilins was observed in 75.8 % of the *E. faecium* isolates. The antimicrobial efflux pump *efmA* gene; which is important for removal of macrolide and fluoroquinolone antimicrobials from the intracellular environment of bacterial cells was observed in 66.7% of ciprofloxacin-resistant *E. faecium* isolates. However, interestingly, the prevalence of ciprofloxacin resistance among *E. faecium* isolates was higher in the much smaller number of isolates obtained at entry compared to exit samples. Ciprofloxacin resistance occurs either by chromosomal mutation of DNA gyrase (*gyrA*) and topoisomerase IV (*ParC)* genes, active efflux pump (*efmA*) or target protection (*Qnr*-like determinants) (Arsène and Leclercq, 2007; Jonas et al., 2001; KIM et al., 2018). In this study, only the efflux pump gene *efmA* was observed, but the *gyrA* and *parC* genes were found without known mutation. Surprisingly, resistant isolates were found despite the lack of use of fluoroquinolones in food producing animals in Australia for decades. Resistance to a banned antimicrobials may have occurred due to the acquisition form the environment that may play a great role in the acquisition and selection of antimicrobial resistance. As a result, AMR can spread from the variety of environmental bacteria to pathogenic ones (von Wintersdorff et al., 2016). However, it should be noted that phenotypic resistance to daptomycin, nitrofurantoin and tigecycline may be over-estimated as no recognised resistance genes were identified by whole genome sequencing that would confirm the phenotype.

Daptomycin resistance is reported to be linked with mutations of genes encoding the cell envelope stress response (*LiaFSR* and *YycFGHIJ*) and the genes responsible in the metabolism of phospholipids (*gdpD* and *cls*) (Arias et al., 2011; Bender et al., 2018). In this study, the WGS analysis showed no mutation on the target genes. It is likely that this indicated the observed phenotypic resistance could occur due to unprecedented reasons. In fact, the molecular mechanism of daptomycin resistance in enterococci is yet to be fully elicited. The same is true with nitrofurantoin and tigecycline resistance isolates. Furthermore, resistance outcomes for one antimicrobial can be linked with resistance to other antimicrobials due to the possibility of co-selection (Benedict et al., 2015). It is believed that the development and spread of AMR bacteria from beef feedlot cattle might be influenced by factors other than antimicrobial treatment including feed, environment, farm type, management, and other factors. Further research is required to determine the genetic mechanism of those antimicrobials in *Enterococcus* spp.

6. Conclusions

Results of this study indicated key differences in the AMR status of normal faecal microbiota (*E. coli, Enterococcus* spp) and foodborne pathogens (*Salmonella* sp) at entry and exit from the feedlot. These differences may have been associated with the exposure of cattle to individuals admitted to the hospital pen for antimicrobial treatment where the highest prevalence of AMR was detected but may also be due to changes in the environment and feeding cycle within the feedlot itself. The most evident changes in the microflora were detected for enterococci, where *E. faecium* (including AMR strains) were isolated from only a few of the study cattle at feed lot entry, where this organism was the predominate *Enterococcus* spp at exit. Overall, we can conclude AMR was low at entry (although *E. coli* and *E. faecium* resistant to critically important animals could be isolated from a very low number of animals), increased in the early feeding period and then decreased thereafter. It is also important to note that the design of this study overemphasises the prevalence of ESBL-producing *E. coli* compared to the most dominant *E. coli* likely to be selected from MCA plates. However, even under these circumstances and apart from the hospital pen isolates, ESBL-producing *E.coli* were only detected in small proportion of animals at extremely low abundance. Resistance among enterococci was similar to less than has been reported in international studies and were similar to previously reported slaughter based surveys for Australian cattle. AMR phenotype and possession of corresponding ARGs were in agreement in the majority of cases but not always. Patterns of resistance of significant medical importance were also rare for most of the pathogen/antimicrobial combinations, except for *E. faecium* susceptibility to daptomycin, nitrofurantoin and quinpristin/dalfopristin.

The results from this study would indicate that further research areas or recommendations to the industry

1. Continuous surveillance of feedlot indicator bacteria is essential
2. Larger sample size and number of pens should be included in the future research
3. Most newly-acquired antimicrobial resistance seems to be associated with exposure to the hospital pen and this needs further investigation, particularly in cattle exposed to high rates of treatment
4. Understanding of backgrounding antimicrobial resistance patterns may be important for feedlot operations and needs to be investigated
5. The origin and basis for daptomycin and nitrofurantoin resistance in *E. faecium* need to be investigated
6. No Salmonella resistance was detected from samples collected. A potential source for humans may be ground beef where large carcase lymph nodes are usually incorporated and testing of ground beef is recommended.
7. Changes in resistance associated with the use of macrolides in hospitalised cattle needs to be examined further (e.g. the risk of changing the azithromycin resistance), including the longevity of that resistance following treatment
8. To prevent further development of Quinpristin/Daflopristin resistance in *E. faecium,* the industry should continue focussing on the antimicrobial stewardship, particularly with streptogramin antimicrobials

7. References

Abreham, S., Teklu, A., Cox, E., and Sisay Tessema, T. (2019). Escherichia coli O157:H7: distribution, molecular characterization, antimicrobial resistance patterns and source of contamination of sheep and goat carcasses at an export abattoir, Mojdo, Ethiopia. *BMC Microbiol* **19**, 215.

Adator, E. H., Narvaez-Bravo, C., Zaheer, R., Cook, S. R., Tymensen, L., Hannon, S. J., Booker, C. W., Church, D., Read, R. R., and McAllister, T. A. (2020). A One Health Comparative Assessment of Antimicrobial Resistance in Generic and Extended-Spectrum Cephalosporin-Resistant Escherichia coli from Beef Production, Sewage and Clinical Settings. *Microorganisms* **8**, 885.

Agga, G. E., Cook, K. L., Netthisinghe, A. M. P., Gilfillen, R. A., Woosley, P. B., and Sistani, K. R. (2019). Persistence of antibiotic resistance genes in beef cattle backgrounding environment over two years after cessation of operation. *PLoS One* **14**, e0212510.

Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., Faltyn, M., Hernandez-Koutoucheva, A., Sharma, A. N., Bordeleau, E., Pawlowski, A. C., Zubyk, H. L., Dooley, D., Griffiths, E., Maguire, F., Winsor, G. L., Beiko, R. G., Brinkman, F. S. L., Hsiao, W. W. L., Domselaar, G. V., and McArthur, A. G. (2020). CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res* **48**, D517-d525.

Alvseike, O., Røssvoll, E., Røtterud, O.-J., Nesbakken, T., Skjerve, E., Prieto, M., Sandberg, M., Johannessen, G., Økland, M., and Urdahl, A. M. (2019). Slaughter hygiene in European cattle and sheep abattoirs assessed by microbiological testing and Hygiene Performance Rating. *Food Control* **101**, 233-240.

Argudín, M. A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Denis, O., Nonhoff, C., and Roisin, S. (2017). Bacteria from Animals as a Pool of Antimicrobial Resistance Genes. *Antibiotics (Basel, Switzerland)* **6**, 12.

Arias, C. A., Panesso, D., McGrath, D. M., Qin, X., Mojica, M. F., Miller, C., Diaz, L., Tran, T. T., Rincon, S., Barbu, E. M., Reyes, J., Roh, J. H., Lobos, E., Sodergren, E., Pasqualini, R., Arap, W., Quinn, J. P., Shamoo, Y., Murray, B. E., and Weinstock, G. M. (2011). Genetic Basis for In Vivo Daptomycin Resistance in Enterococci. *New England Journal of Medicine* **365**, 892-900.

Arsène, S., and Leclercq, R. (2007). Role of a qnr-like gene in the intrinsic resistance of Enterococcus faecalis to fluoroquinolones. *Antimicrob Agents Chemother* **51**, 3254-8.

ASTAG (2018). Importance Ratings and Summary of Antibacterial Uses in Human and Animal Health in Australia. Australian Strategic and Technical Advisory Group on AMR (ASTAG). **Version 1.0**

AURA (2016). Australian Commission on Safety and Quality in Health Care (ACSQHC), First Australian report on antimicrobial use and resistance in human health, Sydney.

Barlow, R., McMillan, K., and Abraham, S. (2020). Antimicrobial resistance in commensal bacteria in bovine faeces at slaughter. *Meat and Livestock Australia Limited*.

Barlow, R. S., McMillan, K. E., Duffy, L. L., Fegan, N., Jordan, D., and Mellor, G. E. (2017). Antimicrobial resistance status of Enterococcus from Australian cattle populations at slaughter. *PLoS One* **12**, e0177728.

Beauvais, W., Gart, E. V., Bean, M., Blanco, A., Wilsey, J., McWhinney, K., Bryan, L., Krath, M., Yang, C. Y., Manriquez Alvarez, D., Paudyal, S., Bryan, K., Stewart, S., Cook, P. W., Lahodny, G., Jr., Baumgarten, K., Gautam, R., Nightingale, K., Lawhon, S. D., Pinedo, P., and Ivanek, R. (2018). The prevalence of Escherichia coli O157:H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: A randomized controlled trial. *PLoS One* **13**, e0192149.

Bender, J. K., Cattoir, V., Hegstad, K., Sadowy, E., Coque, T. M., Westh, H., Hammerum, A. M., Schaffer, K., Burns, K., Murchan, S., Novais, C., Freitas, A. R., Peixe, L., Del Grosso, M., Pantosti, A., and Werner, G. (2018). Update on prevalence and mechanisms of resistance to linezolid,

tigecycline and daptomycin in enterococci in Europe: Towards a common nomenclature. *Drug Resist Updat* **40**, 25-39.

Benedict, K. M., Gow, S. P., McAllister, T. A., Booker, C. W., Hannon, S. J., Checkley, S. L., Noyes, N. R., and Morley, P. S. (2015). Antimicrobial Resistance in Escherichia coli Recovered from Feedlot Cattle and Associations with Antimicrobial Use. *PLOS ONE* **10**, e0143995.

Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R. L., Rebelo, A. R., Florensa, A. F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J. K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B. B., Malhotra-Kumar, S., Westh, H., Pinholt, M., Anjum, M. F., Duggett, N. A., Kempf, I., Nykäsenoja, S., Olkkola, S., Wieczorek, K., Amaro, A., Clemente, L., Mossong, J., Losch, S., Ragimbeau, C., Lund, O., and Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* **75**, 3491-3500.

Bourafa, N., Loucif, L., Boutefnouchet, N., and Rolain, J.-M. (2015). Enterococcus hirae, an unusual pathogen in humans causing urinary tract infection in a patient with benign prostatic hyperplasia: first case report in Algeria. *New microbes and new infections* **8**, 7-9.

Brichta-Harhay, D. M., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Schmidt, J. W., Wang, R., Shackelford, S. D., Loneragan, G. H., and Wheeler, T. L. (2012). Microbiological analysis of bovine lymph nodes for the detection of Salmonella enterica. *J Food Prot* **75**, 854-8.

CLSI (2020). Performance Standards for Antimicrobial Susceptibility Testing. *In* "Clinical and Laboratory Standards Institute", Vol. 30th ed.

de Jong, A., Simjee, S., Garch, F. E., Moyaert, H., Rose, M., Youala, M., and Dry, M. (2018). Antimicrobial susceptibility of enterococci recovered from healthy cattle, pigs and chickens in nine EU countries (EASSA Study) to critically important antibiotics. *Veterinary Microbiology* **216**, 168-175.

Devriese L.A. , L. L., De Herdt P. and Haesebrouck F. (1992). Enterococcal and streptococcal species isolated from faeces of calves, young cattle and dairy cows. *Journal of Applied Bacteriology* **72**, 29-31.

Economou, V., and Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infect Drug Resist* **8**, 49-61.

EFSA (2017). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA J* **15**, e04694.

EFSA (2018). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. *EFSA Journal* **16**, e05182.

Faron, M. L., Ledeboer, N. A., and Buchan, B. W. (2016). Resistance Mechanisms, Epidemiology, and Approaches to Screening for Vancomycin-Resistant Enterococcus in the Health Care Setting. *Journal of Clinical Microbiology* **54**, 2436.

Hammerum, A. M. (2012). Enterococci of animal origin and their significance for public health. *Clinical Microbiology and Infection* **18**, 619-625.

Hermanovská, L., Bardoň, J., and Čermák, P. (2016). Vancomycin-resistant enterococci-the nature of resistance and risk of transmission from animals to humans. *Klinicka mikrobiologie a infekcni lekarstvi* **22**, 54-60.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., and Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* **387**, 176-87.

Johnson, C. M., and Grossman, A. D. (2015). Integrative and Conjugative Elements (ICEs): What They Do and How They Work. *Annu Rev Genet* **49**, 577-601.

Jonas, B. M., Murray, B. E., and Weinstock, G. M. (2001). Characterization of emeA, a NorA homolog and multidrug resistance efflux pump, in Enterococcus faecalis. *Antimicrob Agents Chemother* **45**, 3574-9.

Kidsley, A. K., Abraham, S., Bell, J. M., O'Dea, M., Laird, T. J., Jordan, D., Mitchell, P., McDevitt, C. A., and Trott, D. J. (2018). Antimicrobial Susceptibility of Escherichia coli and Salmonella spp. Isolates From Healthy Pigs in Australia: Results of a Pilot National Survey. *Front Microbiol* **9**, 1207.

KIM, Y. B., SEO, H. J., SEO, K. W., JEON, H. Y., KIM, D. K., KIM, S. W., LIM, S.-K., and LEE, Y. J. (2018). Characteristics of High-Level Ciprofloxacin-Resistant Enterococcus faecalis and Enterococcus faecium from Retail Chicken Meat in Korea. *Journal of Food Protection* **81**, 1357-1363.

Kos, V. N., Desjardins, C. A., Griggs, A., Cerqueira, G., Van Tonder, A., Holden, M. T., Godfrey, P., Palmer, K. L., Bodi, K., Mongodin, E. F., Wortman, J., Feldgarden, M., Lawley, T., Gill, S. R., Haas, B. J., Birren, B., and Gilmore, M. S. (2012). Comparative genomics of vancomycin-resistant Staphylococcus aureus strains and their positions within the clade most commonly associated with Methicillin-resistant S. aureus hospital-acquired infection in the United States. *mBio* **3**.

Moudgil, P., Bedi, J. S., Moudgil, A. D., Gill, J. P. S., and Aulakh, R. S. (2018). Emerging issue of antibiotic resistance from food producing animals in India: Perspective and legal framework. *Food Reviews International* **34**, 447-462.

NARMS (2011). Retail Meat Annual Report. *FDA*.

O'Dea, M., Sahibzada, S., Jordan, D., Laird, T., Lee, T., Hewson, K., Pang, S., Abraham, R., Coombs, G. W., Harris, T., Pavic, A., and Abraham, S. (2019). Genomic, Antimicrobial Resistance, and Public Health Insights into Enterococcus spp. from Australian Chickens. *Journal of Clinical Microbiology* **57**, e00319-19.

Sharma, C., Rokana, N., Chandra, M., Singh, B. P., Gulhane, R. D., Gill, J. P. S., Ray, P., Puniya, A. K., and Panwar, H. (2017). Antimicrobial Resistance: Its Surveillance, Impact, and Alternative Management Strategies in Dairy Animals. *Front Vet Sci* **4**, 237.

Tenzin, S., Ogunniyi, A. D., Khazandi, M., Ferro, S., Bartsch, J., Crabb, S., Abraham, S., Deo, P., and Trott, D. J. (2019). Decontamination of aerosolised bacteria from a pig farm environment using a pH neutral electrochemically activated solution (Ecas4 anolyte). *PLOS ONE* **14**, e0222765.

van Hoek, A. H. A. M., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P., and Aarts, H. J. M. (2011). Acquired antibiotic resistance genes: an overview. *Frontiers in microbiology* **2**, 203-203.

Vignaroli, C., Zandri, G., Aquilanti, L., Pasquaroli, S., and Biavasco, F. (2011). Multidrug-resistant enterococci in animal meat and faeces and co-transfer of resistance from an Enterococcus durans to a human Enterococcus faecium. *Curr Microbiol* **62**, 1438-47.

von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., Savelkoul, P. H. M., and Wolffs, P. F. G. (2016). Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Frontiers in microbiology* **7**, 173-173.

Wiedenbeck, J., and Cohan, F. M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiology Reviews* **35**, 957-976.

Yılmaz, E. Ş., Aslantaş, Ö., Önen, S. P., Türkyılmaz, S., and Kürekci, C. (2016). Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. *LWT - Food Science and Technology* **66**, 20-26.

Zaheer, R., Cook, S. R., Barbieri, R., Goji, N., Cameron, A., Petkau, A., Polo, R. O., Tymensen, L., Stamm, C., Song, J., Hannon, S., Jones, T., Church, D., Booker, C. W., Amoako, K., Van Domselaar, G., Read, R. R., and McAllister, T. A. (2020a). Surveillance of Enterococcus spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. *Scientific Reports* **10**, 3937.

Zaheer, R., Cook, S. R., Barbieri, R., Goji, N., Cameron, A., Petkau, A., Polo, R. O., Tymensen, L., Stamm, C., Song, J., Hannon, S., Jones, T., Church, D., Booker, C. W., Amoako, K., Van Domselaar, G., Read, R. R., and McAllister, T. A. (2020b). Surveillance of Enterococcus spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. *Scientific reports* **10**, 3937-3937.

Zankari, E., Allesøe, R., Joensen, K. G., Cavaco, L. M., Lund, O., and Aarestrup, F. M. (2017). PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J Antimicrob Chemother* **72**, 2764-2768.

Appendix 1

Escherichia coli

Table A1.1. Antimicrobial susceptibility test of *E.coli* isolated from hospital pen water trough

Antimicrobial class	Antimicrobial agent	Resistant (%)	Proportion of isolates per MIC value (µg/mL)*															
			0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Aminoglycosides	Gentamycin	0.0						25	75									
	Streptomycin	0.0									25	75						
β-lactam	Ampicillin	50.0									50				50			
	Amoxicillin-clavulanate	25.0									50	25		25				
	Cefoxitin	25.0									50	25		25				
	Ceftiofur	50.0						50				25	25					
	Ceftriaxone	50.0					50						25		25			
Folate pathway	Sulfisoxazole	0.0											75	25				
inhibitor/antagonists	Trimethoprim/ sulfamethoxazole	0.0				100												
Macrolides	Azithromycin	50.0								25	25			50				
Phenicols	Chloramphenicol	0.0									50	50						
Quinolones	Ciprofloxacin	0.0	50	50														
	Nalidixic acid	0.0								75	25							
Tetracycline	Tetracycline	50.0									50				50			

* The range of distribution per dilution and the vertical line in each raw indicates the level of cut off between sensitivity and resistance

Table A1.2. The comparison of phenotypic and genotypic antimicrobial resistance in each *E.coli* species

Sample ID	Sampling point	ESBL Production	Resistance phenotype	Antimicrobial resistance gene
N001	Entry	No	Susceptible	
N002	Entry	No	Susceptible	
N054	Entry	No	AMP-STR-SXT	*aph(3')-Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B}$, *dfrA5*,*sul2*
N112	Entry	Yes	AUG-AMP-FOX-TET-XNL-AXO	*bla*$_{CMY-2}$,*bla*$_{TEM-1B}$,*dfrA5*,*tet(A),*
N305	Exit	No	TET	*tet(B)*
N307	Exit	Yes	AMP-AZI-STR-FIS-TET-XNL-AXO	*aph(3'')-Ib*, *aph(6)-Id*,*bla*$_{CTX-M-27}$, *mph(A)*, *sul2, tet(A)*
N308	Exit	No	Susceptible	
N309	Exit	No	Susceptible	
N314	Exit	No	TET	*tet(B)*
N316	Exit	No	TET	*tet(B)*
N321	Exit	No	AMP-TET	*bla*$_{TEM-1C}$, *tet(A)*
N324	Exit	No	AMP-STR-TET	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B}$,*tet(B)*,
N325	Exit	Yes	AMP-AZI-TET-XNL-AXO	*bla*$_{CTX-M-15}$, *mph(E)*, *mph(A)*, *msr(E)*, *tet(A)*
N330	Exit	Yes	AUG-AMP-FOX-XNL-AXO	*bla*$_{CMY-2}$
N333	Exit	No	AMP-STR-TET	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B,}$ tet(B)
N338	Exit	No	FIS	*sul2*
N341	Exit	No	AMP-STR-TET	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B}$, *tet(B)*
N344	Exit	No	AMP	*bla*$_{TEM-1B}$
N347	Exit	Yes	AMP-AZI-STR-FIS-TET-XNL-AXO	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{CTX-M-27}$,*sul2*,*mph(A), tet(A)*
N348	Exit	No	TET	*tet(A)*
N351	Exit	No	TET	*tet(B)*
N352	Exit	No	TET	*tet(B)*
N354	Exit	Yes	AMP-CHL-STR-FIS-TET-XNL-AXO-SXT	*aac(3)-IV*, *aph(4)-Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *ant(3'')-Ia*, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *cmlA1*, *dfrA12* , *qnrS1*, *sul1*, sul3, *tet(A)*
N355	Exit	No	TET	*tet(A)*
N358	Exit	No	AMP-TET	*bla*$_{TEM-1C}$,*tet(A)*
N369	Exit	No	STR-FIS-TET	*aph(3'')-Ib*, *aph(6)-Id*, *sul2*, *tet(B)*
N373	Exit	No	STR-FIS-TET	*aph(3'')-Ib*, *aph(6)-Id*, *sul2*, *tet(B)*
N381	Exit	No	AMP-TET	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{TEM-1B}$, *tet(B)*
N385	Exit	No	FIS-TET	*sul2*,*tet(B)*
N396	Exit	No	TET	
N401	Exit	No	TET	*tet(B)*
N413	Exit	No	TET	*tet(B)*
N415	Exit	No	TET	*tet(A*
N423	Exit	Yes	AMP-STR-FIS-TET-XNL-AXO-SXT	*aph(3'')-Ib*, *aph(6)-Id*, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B,}$ *dfrA14*,*sul2*, *tet(A)*
N435	Exit	No	TET	*tet(B)*
N442	Exit	No	TET	*tet(A)*
N443	Exit	No	STR-FIS-TET	*aph(3'')-Ib*, *aph(6)-Id*, *sul2*, *tet(B)*

Appendix 2

Enterococcus

Table A2.1. Antimicrobial susceptiblity test of Enterococcus isolated from Subiliac(126) and Prescapular (128)Lymph node

Antimicrobial class	Antimicrobial agent	Sample (n)**	Resistant (%)	CI (95 %)	Proportion of isolates per MIC value (µg/mL)*															
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Aminoglycosides	Gentamycin	SI (144)	0.00	0.0														100			
		PS (146)	0.00	0.0														100			
	Kanamycin	SI (144)	0.00	0.0														88.9	11.1		
		PS (146)	0.00	0.0														80.5	19.5		
	Streptomycin	SI (144)	0.00	0.0																100	
		PS (146)	0.00	0.0																100	
β-lactam	Pencillin	SI (144)	0.00	0.0					77	4.8	8.7	8.7	0.8								
		PS (146)	0.00	0.0					80.5	3.9	6.2	9.4									
Fluoroquinolones	Ciprofloxacin	SI (144)	0.79	0.11-5.41						18.2	75.4	5.6		0.8							
		PS (146)	0.00	0.0						21.9	75	3.1									
Glycopeptides	Vancomycin	SI (144)	0.00	0.0					1.6	82.5	12.7	3.2									
		PS (146)	0.00	0.0						85.9	12.5	1.6									
Glycylcyclines	Tigecycline	SI (144)	5.56	2.67-11.20	0.8	3.2	23.8	30.9	35.7	3.2	2.4										
		PS (146)	6.25	3.16-12	0.8	3.9	27.3	28.9	32.8	3.9	2.3										
Lincosamide	Lincomycin	SI (144)	89.68	83.04-93.91							8.7		1.6	64.3	25.4						
		PS (146)	91.41	85.15-95.18							4.7	0.8	3.1	64.8	26.6						
Lipopeptides	Daptomycin	SI (144)	3.17	1.20-8.15					6.3	0.8	1.6	11.9	76.2	3.2							
		PS (146)	3.91	1.63-9.04					3.9	2.3	5.5	7	77.3	3.9							
Macrolides	Erythromycin	SI (144)	1.59	0.40-6.12					16.7	3.2	0.8	16.7	61.1	1.6							
		PS (146)	0.00	0.0					17.2	2.3	0.8	18.7	60.9								
	Tylosin tartrate	SI (144)	0.00	0.0							5.6	46	48.4								
		PS (146)	0.00	0.0							5.5	42.2	52.3								
Nitrofurantoins		SI (144)	26.98	19.96-35.39									1.6	0.8	4.8	4.8	61.1	27			
	Nitrofurantoin	PS (146)	23.44	16.9-31.54								0.8	1.6		2.3	7.8	64.1	23.4			
Oxazolidinones	Linezolid	SI (144)	0.00	0.0						3.2	3.2	67.5	26.2								
		PS (146)	0.00	0.0						0.8	3.1	70.3	25.8								
Phenicols	Chloramphenicol	SI (144)	0.00	0.0								3.2	7.9	88.9							
		PS (146)	0.00	0.0								1.6	4.7	93	0.8						
Streptogramins	Quinupristin/dalfopristin	SI (144)	6.35	3.21-12.18						10.3	4	79.4	2.4	4							
		PS (146)	8.59	4.82-14.85						6.2	4.7	80.5	1.6	7							
Tetracycline	Tetracycline	SI (144)	0.00	0.0							100										

PS (146)	0.00	0.0		100	

**PS= prescapular; SU= subiliac

* The range of distribution per dilution and the vertical line in each raw indicates the level of cut off between sensitivity and resistance

Table A2.2. Antimicrobial susceptiblity test of Enterococcus isolated from Hip (45), and Flank and Brisket (25) Carcase

Antimicrobial class	Antimicrobial agent	Sample **	Resistant (%)	CI (95 %)	Proportion of isolates per MIC value (µg/mL)*															
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Aminoglycosides	Gentamycin	Hip(150)	0.00	0.0														100			
		F&B(149)	0.00	0.0														100			
	Kanamycin	Hip(150)	0.00	0.0														91.1	8.9		
		F&B(149)	0.00	0.0														84	16		
	Streptomycin	Hip(150)	0.00	0.0																100	
		F&B(149)	0.00	0.0																100	
β-lactam	Pencillin	Hip(150)	0.00	0.0					40	13.3	31.1	13.3	2.2								
		F&B(149)	0.00	0.0					48	24	12	16									
Fluoroquinolones	Ciprofloxacin	Hip(150)	2.22	0.31-14.16				17.8	26.7	17.8	33.3	2.2		2.2							
		F&B(149)	0.00	0.0					4	68	20	8									
Glycopeptides	Vancomycin	Hip(150)	0.00	0.0					11.1	73.3	11.1	4.4									
		F&B(149)	0.00	0.0					4	44	48	4									
Glycylcyclines	Tigecycline	Hip(150)	0.00	0.0	4.4	6.7	24.4	42.2	22.2												
		F&B(149)	0.00	0.0		12	36	36	16												
Lincosamide	Lincomycin	Hip(150)	46.67	32.76-61.12							51.1		2.2	24.4	22.2						
		F&B(149)	80.00	60.02-91.42							16		4	28	52						
Lipopeptides	Daptomycin	Hip(150)	2.22	0.31-14.16					44.4		8.9	13.3	31.1	2.2							
		F&B(149)	16.00	6.14-35.69					4		4	12	64	16							
Macrolides	Erythromycin	Hip(150)	0.00	0.0					28.9	31.1	4.4	6.7	28.9								
		F&B(149)	0.00	0.0					60		4	4	32								
	Tylosin tartrate	Hip(150)	0.00	0.0							8.9	60	31.1								
		F&B(149)	0.00	0.0							8	64	28								
Nitrofurantoins	Nitrofurantoin	Hip(150)	0.00	0.0								31.1	13.3		11.1	6.7	37.8				
		F&B(149)	4.00	0.56-23.55											4	36	56	4			
Oxazolidinones	Linezolid	Hip(150)	0.00	0.0						11.1	31.1	57.8									
		F&B(149)	0.00	0.0						4		92	4								

Phenicols	Chloramphenicol	Hip(150)	0.00	0.0			17.8	33.3	48.9		
		F&B(149)	0.00	0.0			4	24	72		
Streptogramins	Quinupristin/ dalfopristin	Hip(150)	8.89	3.38-21.41	48.9	4.4	37.8		8.9		
		F&B(149)	0.00	0.0	16	8	76				
Tetracycline		Hip(150)	4.44	1.11-16.11		95.6					4.4
	Tetracycline	F&B(149)	24.00	11.2-44.16		76					24

** F&B=flank and brisket

* The range of distribution per dilution and the vertical line in each raw indicates the level of cut off between sensitivity and resistance

Enterococcus faecium

Table A2.3. The comparison of phenotypic and genotypic antimicrobial resistance in *E.faecium*

Sample ID	Sampling point	Resistance phenotype	Antimicrobial resistance gene
P009	Entry	CIP	*aac(6')-Ii,msr(C), pbp5*
P018	Entry	CIP	*aac(6')-Iid*
P051	Entry	CIP-LIN-NIT	*aac(6')-Ii , eatAv, efmA, msr(C), pbp5*
P075	Entry	CIP-NIT	*aac(6')-Ii , efmA, msr(C), pbp5*
P077	Entry	CIP-DAP-Q/D-LIN	*aac(6')-Ii , eatAv, msr(C), pbp5*
P105	Entry	CIP-TET	*aac(6')-Ii , eatAv, efmA, msr(C), pbp5, tet(M)*
P107	Entry	ERY-Q/D-TYL-LIN-TET	*aac(6')-Ii , ant(6)-Ia, eatAv, erm(B), msr(C), pbp5, tet(S), vat(E)*
P110	Entry	CIP	*aac(6')-Ii , eatAv, efmA, msr(C), pbp5*
P247	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P249	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P250	Exit	LIN	*aac(6')-Ii, eatAv, msr(C)*
P251	Exit	LIN	*aac(6')-Ii, eatAv, msr(C)*
P255	Exit	LIN	aac(6')-Ii ,msr(C),pbp5,eatAv
P257	Exit	LIN	*aac(6')-Ii, eatAv, msr(C)*
P258	Exit	ERY-Q/D-TYL-LIN-TET	*aac(6')-Ii, eatAv, efmA, erm(B), msr(C), tet(L),tet(M), tet(45)*
P264	Exit	DAP	*aac(6')-Ii, efmA, msr(C), pbp5*
P268	Exit	DAP	*aac(6')-Ii, eatAv, msr(C), pbp5*
P272	Exit	DAP	*aac(6')-Ii, efmA, msr(C), pbp5*
P273	Exit	DAP	*aac(6')-Ii, eatAv, msr(C)*
P275	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P278	Exit	DAP	*aac(6')-Ii, eatAv, msr(C)*
P282	Exit	DAP	*aac(6')-Ii, eatAv, msr(C)*
P283	Exit	Q/D-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P284	Exit	Q/D-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P287	Exit	CIP-LIN-NIT	*aac(6')-Ii , eatAv, efmA, msr(C), pbp5*
P293	Exit	DAP	*aac(6')-Ii, efmA, pbp5, msr(C)*
P294	Exit	DAP	*aac(6')-Ii, efmA, pbp5, msr(C)*
P297	Exit	CIP-DAP-LIN-NIT	*aac(6')-Ii, eatAv, msr(C), pbp5*
P300	Exit	DAP-Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P303	Exit	CIP-NIT	*aac(6')-Ii, efmA, msr(C), pbp5*
P305	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P310	Exit	DAP-Q/D-LIN-NIT	*aac(6')-Ii , eatAv, msr(C),pbp5*
P313	Exit	DAP	*aac(6')-Ii, efmA, pbp5*
P316	Exit	CIP-LIN	*aac(6')-Ii , eatAv, msr(C), pbp5*
P320	Exit	Q/D-LIN	*aac(6')-Iid*
P325	Exit	Q/D-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P328	Exit	DAP-ERY-LIN-NIT	*aac(6')-Ii, eatAv, msr(C), pbp5*
P329	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P330	Exit	DAP	*aac(6')-Ii, eatAv, msr(C)*
P331	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, lnu(G), msr(C)*
P336	Exit	Q/D-KAN-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P338	Exit	Q/D-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P345	Exit	CIP-LIN-NIT	*aac(6')-Ii, eatAv, efmA, msr(C), pbp5*
P348	Exit	Q/D-LIN-NIT	*aac(6')-Ii, eatAv, msr(C)*
P357	Exit	CIP-DAP-NIT	*aac(6')-Ii, efmA, msr(C), pbp5*
P363	Exit	Q/D-LIN	*aac(6')-Ii, eatAv, msr(C)*
P364	Exit	CIP-DAP-NIT	*aac(6')-Ii , efmA, msr(C), pbp5*

P365	Exit	ERY-TYL-LIN-NIT-TET	*aac(6')-Ii*, *eatAv, efmA*, *erm(B)*,*msr(C)*,*tet(L)*,*tet(M)*,*tet(45)*
P366	Exit	DAP	*aac(6')-Ii*, *efmA*, *msr(C)*, *pbp5*
P367	Exit	Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *msr(C)*
P369	Exit	DAP	*aac(6')-Ii , eatAv*, *msr(C)*, *pbp5*
P373	Exit	Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *msr(C)*
P374	Exit	DAP	*msr(C)*,*eatAv*, *efmA*
P375	Exit	Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *lnu(G)*, *msr(C)*
P380	Exit	Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *msr(C)*
P382	Exit	CIP-TIG-NIT	*aac(6')-Ii*, *efmA*, *msr(C)*, *pbp5*
P385	Exit	CIP-DAP-NIT	*aac(6')-Ii*, *efmA*, *msr(C)*, *pbp5*
P387	Exit	CIP-LIN-NIT	*aac(6')-Ii*, *eatAv*, *efmA*, *msr(C)*, *pbp5*
P391	Exit	Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *msr(C)*
P392	Exit	Q/D-LIN-NIT-TET	*aac(6')-Ii*, *eatAv*, *msr(C)*,*tet(S)*
P394	Exit	CIP-DAP-Q/D-LIN-NIT	*aac(6')-Ii*, *eatAv*, *msr(C)*, *pbp5*
P396	Exit	Q/D-LIN	*aac(6')-Ii*, *eatAv*, *msr(C)*

Enterococcus hirae

Table A2.4. The comparison of phenotypic and genotypic antimicrobial resistance in *E.hirae*

Sample ID	Sampling	Resistance phenotype	Antimicrobial resistance gene
P005	Entry	Susceptible	*aac(6')-Iid*
P006	Entry	Susceptible	*aac(6')-Iid*
P007	Entry	Susceptible	*aac(6')-Iid*
P013	Entry	Susceptible	*aac(6')-Iid*
P014	Entry	DAP-LIN-TET	*aac(6')-Iid*, *tet(M)*
P017	Entry	DAP	*aac(6')-Iid*
P019	Entry	DAP	*aac(6')-Iid*
P024	Entry	TIG-LIN	*aac(6')-Iid*
P028	Entry	TIG	*aac(6')-Iid*
P030	Entry	DAP-LIN-NIT	*aac(6')-Iid*
P033	Entry	DAP	*aac(6')-Iid*
P044	Entry	DAP-LIN	*aac(6')-Iid*
P053	Entry	DAP-LIN-NIT	*aac(6')-Ii*
P063	Entry	DAP-LIN-NIT	*aac(6')-Ii*
P067	Entry	TIG-LIN	*aac(6')-Ii*
P068	Entry	DAP-LIN	*aac(6')-Ii*
P069	Entry	DAP-LIN	*aac(6')-Ii*
P070	Entry	TIG	*aac(6')-Ii*
P076	Entry	DAP-LIN	*aac(6')-Ii*
P078	Entry	DAP-LIN-NIT	*aac(6')-Ii*
P080	Entry	DAP-LIN-NIT	*aac(6')-Ii*
P082	Entry	DAP	*aac(6')-Ii*
P086	Entry	DAP-LIN-NIT	*aac(6')-Ii*
P087	Entry	DAP-LIN	*aac(6')-Ii*
P089	Entry	DAP-LIN	*aac(6')-Ii*
P092	Entry	DAP-LIN	*aac(6')-Ii*
P093	Entry	DAP-NIT	*aac(6')-Ii*
P096	Entry	DAP-NIT	*aac(6')-Ii*
P097	Entry	DAP	*aac(6')-Ii*
P099	Entry	DAP	*aac(6')-Ii*
P102	Entry	DAP-LIN	*aac(6')-Ii*
P104	Entry	DAP-LIN	*aac(6')-Ii*
P109	Entry	DAP-LIN	*aac(6')-Ii*
P252	Exit	DAP	*aac(6')-Ii*

P253	Exit	DAP	*aac(6')-Ii*
P271	Exit	DAP	*aac(6')-Iid*, *tet(L),tet(M),tet(45)*
P277	Exit	DAP	*aac(6')-Ii*
P280	Exit	DAP	*aac(6')-Ii*
P281	Exit	DAP	*aac(6')-Ii*
P295	Exit	DAP	*aac(6')-Iid*, *tet(L), tet(M),tet(45)*
P337	Exit	DAP	*aac(6')-Iid*, *tet(L), tet(M),tet(45)*
P340	Exit	DAP	*aac(6')-Iid*
P344	Exit	DAP	*aac(6')-Iid*
P346	Exit	ERY-TIG-TYL-LIN	*aac(6')-Iid*, *erm(B)*
P358	Exit	DAP	*aac(6')-Iid*
P379	Exit	ERY-TYL-LIN	*aac(6')-Iid*, *erm(B)*
P384	Exit	DAP	*aac(6')-Iid*