MONASH University

Centre to Impact AMR

Final Report

Antimicrobial Resistance in Australian Supermarket Meats

Contract research for World Animal Protection

WORLD ANIMAL PROTECTION PREFACE

Antimicrobial resistance (AMR) is one of the most significant health challenges of our time. AMR pathogens, also known as 'superbugs', are bacteria resistant to antimicrobials, including antibiotics. This means antibiotics are no longer effective at treating bacteria that cause infections. Currently, it is estimated that superbugs are responsible for 1.3 million human deaths each year.[1] This is projected to rise to 10 million deaths a year by 2050. In a world without effective antibiotics, several lifesaving procedures could be lost or compromised. This includes procedures and treatments like chemotherapy, heart bypass surgery, hip and joint replacements, organ transplants, dialysis, or caesarean delivery.[2] In light of evidence suggesting that approximately 60% of antimicrobials sold in Australia are destined for animal agriculture, World Animal Protection sought to better understand any potential growth in resistance that may occur along the food chain.[3]

World Animal Protection commissioned research from the Centre to Impact AMR, at Monash University. This commissioned research tested the prevalence of antimicrobial resistance in packaged meat from Australian supermarkets. In total, 144 beef and 90 salmon packages were purchased from Coles, Woolworths, and Aldi supermarkets in Melbourne Australia. The purchased meat samples were all Australian produce. The pooled samples were then tested for bacteria and coliforms, antimicrobial resistance, and antimicrobial resistance genes. The research found that bacterial and coliform levels in all beef and most salmon samples were low and met specifications of Food Standards Australia. Bacterial levels were similar in samples collected from the three retailers. However, the proportion of those bacteria that had acquired resistance to antimicrobials, including antibiotics that are routinely used in human medicine, was high. The percentage of antimicrobial-resistant bacteria in the meat samples (53%) was virtually indistinguishable from the proportion of antimicrobial resistant isolates in wastewater (58%). The proportion of resistant bacteria in the meat samples was twofold higher than samples taken from a range of soil, water, and industrial environments. The bacteria in the beef and salmon samples were often able to resist antibiotics, including medically important first- and second-line agents from the beta-lactam, tetracycline, and fluoroquinolone classes.

[1] Christopher J.L. Murray et al, 'Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis (2022) 399 *The Lancet* 629, 629.
[2] See, e.g., World Health Organisation, 'Antimicrobial resistance' (webpage 2021) < https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
[3] Laura Higham, *Antimicrobial use governance in the Australian food animal sector* (Report by FAI for World Animal Protection, October 2021) 24.

Further research would be required to identify all the drivers behind the acquired resistance detected in this study. However, given the high proportion of resistant bacteria detected, it is potentially driven by overuse of antimicrobials, including off-label usage, on industrial farms. While a lack of transparent public reporting from the agriculture industry means that we do not have a comprehensive picture of antimicrobial usage patterns across different agricultural sectors, it appears clear that some antibiotics are being used routinely on a preventative basis.[4] Doses of antibiotics can be administered in feed and water supplies to entire herds or flocks of animals that are not sick.[5] Commonly, routine group prophylactic use occurs in situations where animals are kept in low welfare conditions.[6] This may include situations where breeds with low welfare outcomes are used (such as fast-growing broilers), or where animals are fed poor diets high in grain or kept in overcrowded conditions.[7] Routine group prophylactic use can increase overall volumes of use and foster resistance to antibiotics, giving rise to superbugs. Similarly, Australian law still permits the use of antibiotics for growth promotion purposes.[8] This occurs when sub-therapeutic doses of antibiotics are administered to herds of animals to encourage more rapid growth, so the animals reach their slaughter weight at a faster rate. Using low dose, sub-therapeutic levels of antibiotics for growth promotion purposes can also foster resistance.[9]

In addition to revealing that bacteria in the beef and salmon samples are often able to resist antibiotics, this research revealed that antimicrobial resistant genes (ARGs) were prevalent in supermarket beef and salmon samples. ARGs are the building blocks for superbugs; they are fragments of genetic material that can be easily transferred between bacteria and can even be passed from dead to living cells. ARGs can be quickly transmitted throughout a whole population of bacteria, and even between different species of bacteria. Using these fast horizontal transmission mechanisms, ARGs create antimicrobial resistance, the resistance of common bacteria to antibiotics – including those that are critically important for human medicine. Furthermore, antibiotics facilitate the horizontal gene transfer of ARGs

[4] Ibid, 20-21.
[5] Ibid.
6 World Animal Protection, Fuelling the Pandemic Crisis (Report 2020) 6.
[7] See, e.g., Compassion in World Farming, Dutch slower growing broilers require less antibiotics than fast growing chickens (Report 2019), summary available at https://www.ciwf.org.uk/media/7438137/dutch-slower-growing-broilers-require-less-antibiotics-than-fast-growing-chickens.pdf.
[8] Ibid 22-23.
[9] Ibid.

among different bacteria. Antibiotics change bacterial communities by decreasing susceptible bacterial groups while increasing resistant bacterial ones.[10]

Considering the concerning findings in the Monash report, World Animal Protection would encourage the Federal and State Governments to engage in more routine monitoring of antimicrobial resistance on farms and along the food chain, from slaughter and processing through to the supermarkets. This could help provide greater certainty on the drivers leading to the high proportion of bacteria with acquired resistance found in the beef and salmon samples. Furthermore, we would encourage the Federal Government to introduce mandatory public reporting of antibiotic use across the agriculture sector, as is common in many other countries. This transparency will enable greater oversight of the volume of antibiotics used, and the purpose for which they are administered. As antibiotic use in intensive farming is one potential driver of antimicrobial resistance, monitoring use and providing public transparency is critical.

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Country Director

[10]Wenguang Xiong et al, 'Selective Pressure of Antibiotics on ARGs and Bacterial Communities in Manure-Polluted Freshwater-Sediment Microcosms' (2015) 6 *Frontiers in Microbiology* 1, 2.

1. EXECUTIVE SUMMARY

In contract research commissioned by World Animal Protection, the Centre to Impact AMR tested the prevalence of antimicrobial resistance in packaged meats from Australian supermarkets. In total, 144 beef and 90 salmon packages were purchased from Coles, Woolworths, and ALDI supermarkets in Melbourne, Australia. Pooled samples were tested for: (i) pathogen and coliform levels by isolating and identifying bacteria present using selective media, (ii) antimicrobial resistance phenotypes by measuring antibiotic susceptibilities (by MIC assays) of bacterial isolates, and (iii) antimicrobial resistance genotypes by quantitative PCR of DNA extracted from whole meats.

Bacterial and coliform levels in all beef and most salmon samples were low and met specifications of Food Standards Australia. However, a wide range of potentially opportunistic human and animal pathogens were isolated from the samples, including *Enterococcus*, *Yersinia*, *Acinetobacter*, *Proteus*, *Aeromonas*, *Serratia*, *Pseudomonas*, and *Hafnia* species. Further experiments, however, would be required to determine whether these isolates are capable of pathogenesis and thus whether the sampled meats are relevant pathogen reservoirs. Several foodborne pathogens, namely *E. coli*, *Campylobacter* spp., and *Salmonella* spp., were not detected.

There was strong evidence that bacteria inhabiting the samples have acquired resistance to antimicrobials used in human medicine. There was phenotypic evidence for acquired resistance to specific first- and second-line beta-lactams, tetracyclines, aminoglycosides, and fluoroquinolones, as well as multidrug resistance in certain isolates. Of the Gram-negative bacteria subject to antimicrobial susceptibility testing, at least 55% of beef isolates and 39% of salmon isolates exhibited potentially acquired resistance to at least one antibiotic. Further studies would be required to understand the basis, pathways, and drivers of resistance in these bacteria.

The genotypic analysis showed antimicrobial resistant genes were prevalent in the beef and salmon samples. Eight antimicrobial resistance genes were detected, including those known to confer resistance to aminoglycoside, tetracycline, beta-lactam, and macrolide antibiotics. Given concordant findings from the phenotypic and genotypic analysis, there is particularly strong evidence of acquired resistance to beta-lactam and tetracycline antibiotics. Altogether, these results suggest a significant burden of antibiotic-resistance bacteria and potential pathogens in commercial beef and salmon samples.

2. SAMPLE COLLECTION AND PROCESSING

144 beef packages and 90 salmon packages were purchased in batches from Coles, Woolworths, and Aldi supermarkets in Melbourne, Australia between 26/10/2021 and 2/11/2021. Full details on the products sampled, supermarket locations, and sampling dates are provided in **Table A1**. Samples were transported on ice packs to One Health Microbiology Laboratory, Department of Microbiology, Monash University and immediately processed. 5 g from each package were cut aseptically in a Class II Biosafety Cabinet, diced, and combined into a pooled sample. Pooling resulted in 16 pooled beef samples and ten pooled salmon samples (each containing meat from nine packages (detailed in **Table A1**). These pooled samples were subject to three comprehensive analyses:

(i) measurement of pathogen levels by isolating, characterising, and identifying bacteria using four different types of selective media **(Section 3)**;
(ii) determination of antimicrobial resistance phenotypes of isolates by measuring minimum inhibitory concentrations of a panel of antimicrobials **(Section 4)**;

(iii) identification of antimicrobial resistance genes by quantitative PCR of DNA extracted from pooled meat samples **(Section 5)**.

3. PATHOGEN LEVELS AND IDENTITIES

PROCEDURES

Selective and differential media were used to measure the load of four major pathogens in the meats, namely *Escherichia coli*, *Enterococcus* spp., *Campylobacter* spp., and *Salmonella* spp. **(Table 1)**. For *Salmonella* spp. isolation, 5 g of the pooled samples were incubated in 5 ml of Selenite Broth (Lactose) SS at 37°C with 200 rpm agitation for 24 hours. 100 µl of the overnight cultures with appropriate dilution factor was plated onto Hektoen Enteric agar plates and incubated at 37°C for 24 hours. For isolation of *Campylobacter* spp., *E. coli*, and *Enterococcus* spp., 20 g of the pooled samples were resuspended in 180 ml of sterile Phosphate-Saline Diluent (PSD) solution. The bottles were shaken vigorously for 30 s twice and diluted to appropriate dilution factor. 100 µl of the diluted solution were plated on to Campylobacter Agar, Chromogenic E. coli/Coliform Medium, and Slanetz and Bartley Agar, and incubated according to **Table 1**.

Table 1. Selective media and growth conditions used.

Selective media	Targeted strains	Expected appearance	Growth conditions
Campylobacter Agar	*Campylobacter* spp.	Small grey colonies	37°C, 24 – 48 h
Chromogenic E. coli/ Coliform Medium	*Escherichia coli* *Salmonella enteritidis*	Blue colonies Colourless colonies	37°C, 24 h
Slanetz and Bartley Medium	*Enterococcus* spp.	Deep red/maroon/pink colonies	37°C, 48 h
Kanamycin Aesculin Azide Agar	*Enterococcus* spp.	White/grey colonies surrounded by black zones	37°C, 24 – 48 h
Hektoen Enteric Agar	*Salmonella* spp. *Escherichia coli*	Blue-green colonies Orange colonies	37°C, 24 h

Four isolates of interest from each selective plate were selected based on their morphologies and biochemistry reactions, and streaked on to the same type of selective plates to further isolate the strains. For *Enterococcus* spp. isolation, the isolates from Slanetz and Bartley Agar were streaked onto Kanamycin Aesculin Azide (KAA) Agar to further distinguish strains. When the isolates appeared pure on the selective agar plates, they were streaked onto LB agar and incubated at 37°C overnight for storage and further analysis. For long-term storage, isolate stocks were prepared by culturing in 5 ml LB broth at 37°C with 200 rpm agitation for 24 hours and stored in 25% v/v sterile glycerol at -80°C.

Bacterial isolates were tentatively identified based on colony morphologies and biochemical reactions on the selective agar plates, then genotyped by Sanger sequencing of their 16S rRNA gene. Specifically, isolates on the LB agar plates were picked for colony PCR by resuspending the cells in 50 µl of UltraPure DNase/RNase-Free Distilled Water and boiled at 100°C for 10 minutes. The tubes were cooled on ice for 10 minutes before centrifuged at 16,000 × *g* for 10 minutes. The supernatant was used as a DNA template in the PCR. The 16S rRNA gene was amplified using universal 16S rRNA primers, i.e. 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), with an expected amplicon size of ~1400 base pairs. Individual reactions contain 1 × Taq MasterMix (New

England Biolabs), 400 µM of each primer, and 2 µl of the DNA template mixed to a final volume of 50 µl with UltraPure DNase/RNase-Free Distilled Water. Cycling conditions were as follows: 95°C for 2 min followed by 35 cycles of 95°C for 30 sec, 56°C for 30 sec, and 68°C for 1 min followed by the last cycle at 68°C for 5 min before cooling down to 4°C. The PCR products were loaded onto 1% w/v agarose gel at 90 V for 35 min. The gel was visualised against UV lamp and the band with the correct size (~1400 bp) was cut and extracted using the ISOLATE II PCR and Gel Kit before sending for 16s Sanger sequencing at Micromon Genomics. In case of failed colony PCR, genomic DNA was extracted using standard protocols before performing 16S rRNA gene PCR. The sequences were compared against the database from Standard Nucleotide BLAST (NCBI).

RESULTS

Bacterial levels were low to moderate in the packaged samples. Most pooled beef samples yielded 10^2 to 10^4 colonies per millilitre (CFU mL^{-1}) on the four selective plates. In contrast, salmon levels were variable: whereas the smoked salmon samples yielded no colonies on any of the four media used, bacteria load was considerable in many of the fresh salmon samples (10^3 to 10^7 CFU mL^{-1}). Plate counts for coliforms were within acceptable ranges for Food Standards Australia (<10^4 CFU mL^{-1}) with exception of one pooled fresh salmon sample. **Table A2** describes pathogen levels for each of the 26 pooled samples on each media. Bacterial levels were similar in samples collected from the three retailers **(Table A2)**.

Bacterial colonies were further analysed if they shared similar morphology and biochemical characteristics to the four pathogens targeted **(Table 1)**. In total, 218 axenic bacterial isolates were obtained from the 26 pooled samples, of which 164 were genotyped by 16S rRNA gene sequencing and 104 were subject to antimicrobial susceptibility profiling **(Section 4)**. **Table A3** describes the identities and morphologies of the isolates. While many of the isolates exhibited the expected phenotypic characteristics of the targeted species, based on 16S rRNA gene sequencing, none genotypically matched those of *Campylobacter* spp., *Salmonella* spp., or *E. coli*. Only two *Enterococcus* spp. were identified. However, the sequencing reveal numerous other potential opportunistic pathogens were present in the samples as summarised in **Table 1**. *Pseudomonas*, *Hafnia*, *Yersinia*, *Buttiauxella*, and *Serratia* spp. accounted for the false positives, i.e. bacterial isolates that were phenotypically similar to the four targets, but did not genotypically match them. In addition, there were multiple isolates of the major hospital-acquired pathogens *Acinetobacter* and *Proteus*.

Table 2. Selective media and growth conditions used.

Selective media	No. isolates	No. genotyped	Pathogenic genera detected (listed by abundance)
Campylobacter	64	55	*Pseudomonas*, *Myroides*, *Brevundimonas*, *Serratia*
Coliform	32	30	*Yersinia*, *Buttiauxella*, *Serratia*, *Aeromonas*, *Rahnella*, *Acinetobacter*, *Myroides*, *Erwingella*, *Stenotrophomonas*
Slanetz and Bartley	54	18	*Enterococcus* (other non-pathogenic *Carnobacterium*)
Hektoen Enteric	68	61	*Hafnia*, *Proteus*, *Acinetobacter*, *Buttiauxella*, *Aeromonas*, *Providencia*, *Moellerella*

CONCLUSIONS

Bacterial levels in the packaged beef and salmon samples from all three retailers were low to moderate. No isolates were obtained from the major foodborne pathogens *Campylobacter*, *Salmonella*, or *E. coli*. However, diverse potential opportunistic pathogens

were nevertheless isolated from these samples. It cannot yet be concluded whether they can cause human or animal disease or instead are more related to non-commensal environmental / commensal strains.

4. ANTIMICROBIAL RESISTANCE PHENOTYPES

PROCEDURES

104 bacterial strains were selected for antimicrobial susceptibility testing using minimum inhibitory concentration (MIC) assays. 78 Gram-negative strains were tested using Sensititre Gram Negative GN6F plates and 26 Gram-positive strains were tested with Sensititre Gram Positive GPALL3F plates, according to the Sensititre Plate Guide Booklet. The cultures were grown from glycerol stocks on either LB agar plates or Mueller-Hinton agar plates and incubated overnight at 37°C. Several colonies were selected from the plates and resuspended in phosphate-buffered saline (PBS) solution (13.7 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). Turbidity was adjusted equivalent to 0.5 McFarland standard (0.24 mM $BaCl_2 \cdot 2H_2O$ in 1% v/v H_2SO_4), i.e. approximately $OD_{600} \approx 0.15 – 0.18$, and inoculated into Mueller-Hinton broth (Oxoid, Thermo Fisher). 50 µl of the derived cultures were dispensed into each well of the Sensititre plate before closing with perforated sealing. Cultures were incubated at 37°C for 18 – 24 hours and the turbidity of each well was recorded.

RESULTS

As expected, each of the bacterial isolates were susceptible to most antimicrobials tested and intrinsic resistance accounted for most observed resistance. However, 54 of the 78 Gram-negative bacterial isolates tested (i.e. 69%) displayed unexplained and potentially acquired resistance to at least one antimicrobial compound, specifically 31 (73%) of beef and 23 (64%) of salmon isolates. This decreased to 37 of the 78 isolates (47%) when the high susceptibility to cefazolin among Enterobacteriaceae isolates is disregarded, i.e. 23 (55%) of beef and 14 (39%) of salmon isolates**.**

As summarised in **Table 3**, potentially acquired resistance was observed to a wide range of antibiotics. These include beta-lactams (e.g. cefazolin, ceftazidime, ceftriaxone), tetracyclines (e.g. tetracycline, tigecycline), and for an *Enterococcus* isolate, the fluoroquinolone ciprofloxacin that are all widely used as first- or second-line agents to treat human bacterial infections. There was also much variability within individual genera as to which antibiotics were resistant; for example, whereas the *Yersinia* isolates from beef were highly antibiotic-sensitive, two *Yersinia* isolates from salmon appeared to be multidrug-resistant. **Table A4** and **Table A5** detail the observations and interpretations of antimicrobial resistance among the isolates.

The percentage of antimicrobial-resistant isolates in these meat samples was compared with those from eight types of environmental samples, collected around Melbourne by the Centre to Impact AMR using equivalent methods. The percentage of antimicrobial-resistant isolates in the salmon and beef samples (53%; including both Gram-negative and Gram-positive isolates) was at least twofold higher than in seven sample types (beach sand, park soil, park water, constructed wetland, river, and agricultural runoff; 0% to 25% isolates resistant) and comparable to wastewater (58% isolates resistant). The proportion of antimicrobial resistant isolates was higher in samples from Aldi (61%) and Coles (58%) compared to Woolworths (46%) supermarkets, though further testing would be required to determine if these differences are statistically significant **(Table A5)**.

Table 3. List of antibiotics resisted by bacterial isolates (intrinsic resistance not shown).

Selective media	Antibiotics resisted by one or more isolates
Acinetobacter spp.	Piperacillin / tazobactam, cefepime
Aeromonas spp.	No resistance detected
Buttiauxella spp.	Cefazolin, ampicillin
Enterococcus spp.	Ciprofloxacin, erythromycin, rifampin
Hafnia spp.	Cefazolin, ceftazidime, ceftolozane / tazobactam, ceftriaxone, trimethoprim / sulfamethoxazole, ampicillin, ampicillin / sulbactam
Myroides spp.	Trimethoprim / sulfamethoxazole, amikacin, aztreonam, ceftazidime, gentamicin, tobramycin
Proteus spp.	Cefazolin, tetracycline, tigecycline, ampicillin, minocycline, nitrofurantoin
Providencia spp.	Cefazolin
Pseudomonas spp.	Ceftazidime / avibactam, ceftolozane / tazobactam, piperacillin / tazobactam, trimethoprim / sulfamethoxazole, aztreonam, ceftriaxone, doripenem, meropenem, ceftazidime
Rahnella spp.	Cefazolin, ceftriaxone, ampicillin
Serratia spp.	Cefazolin, ceftazidime / avibactam, ceftolozane / tazobactam, piperacillin / tazobactam, ampicillin
Yersinia spp.	Aztreonam, ceftazidime, ceftazidime / avibactam, ceftriaxone, trimethoprim / sulfamethoxazole

CONCLUSIONS

Bacteria in beef and salmon samples from all three retailers are often able to resist antibiotics, including medically important first- and second-line agents from the beta-lactam, tetracycline, and fluoroquinolone classes. With the available data, conclusions cannot be drawn as to the basis or drivers of this resistance. Genomic data would be needed to resolve the genetic basis of this resistance and determine whether the identified isolates are related to strains that cause human infections, while comprehensive surveillance at animal rearing and food production facilities would be required to infer transmission pathways.

5. ANTIMICROBIAL RESISTANCE GENOTYPES

PROCEDURES

Total community DNA was extracted from 10 g of each of the 26 pooled samples. The pooled samples were first resuspended with 10 ml of phosphate-saline solution and the supernatant was filtered through a 0.22 µm membrane filter (Millipore, Merck), then frozen prior to extraction. Total community DNA was extracted from the filtered paper using the DNeasy PowerSoil DNA extraction kit (Qiagen). Total DNA yield was confirmed using a Qubit fluorometer 3.0 with the dsDNA BR Assay Kit (Thermo Fisher Scientific). Bacterial DNA

concentration was quantified by quantitative PCR of the 16S rRNA gene using bacterial-specific primers.

97 antimicrobial resistance genes from the extracted community DNA were quantified using Microbial DNA qPCR Arrays Plate E (Qiagen). Inside a Class II Biosafety Cabinet, 510 µl of Microbial qPCR Mastermix was mixed with 20 µl of the extracted DNA and adjusted to a 1020 µl volume with PCR-grade water. 10 µl of the reaction mix was dispensed into each well of the array card according to the supplier's protocol. The plate was sealed with optical adhesive film and centrifuged at 2000 rpm for 2 min. The PCR array plate was processed with QuantStudio 7 Flex Real-Time PCR System (The Applied Biosystems, Thermo Fisher Scientific) by starting with a cycle of initial PCR activation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 sec and annealing and extension at 60°C for 2 min. Analysis was performed according to the Antibiotic Resistance + MRSA Microbial Identification Data Analysis workflow.

RESULTS

Antimicrobial resistance genes (ARGs) were at sufficiently high levels to be detectable in the quantitative PCR array in 8 of the 16 pooled beef samples and 4 of the 10 salmon samples. ARGs were also likely present in four other beef samples and three other salmon samples, though amplification was too low to definitively confirm this and more sensitive methods would be required to prove that these samples harbour these genes. Of the 87 ARGs analysed, eight genes were detected in the pooled samples and four other genes were ambiguously detected as summarised in **Table 4** and detailed in **Table A6**. These include genes that confer resistance to aminoglycoside, tetracycline, beta-lactam, and macrolide antibiotics. The most prevalent genes were the aminoglycoside resistance gene *aadA1* and tetracycline efflux pump pair *tetAB* **(Table 4)**. These results support phenotypic observations of potentially acquired resistance to tetracycline and beta-lactam antibiotics by certain potentially pathogenic isolates **(Table 3)**.

Table 4. Antimicrobial resistance genes found in pooled samples.

Gene	Function	Beef prevalence	Salmon prevalence
AacC1	Aminoglycoside resistance	1 positive, 2 ambiguous	Not detected
AadA1	Aminoglycoside resistance	4 positive, 1 ambiguous	2 positive, 2 ambiguous
ACC-1 group	Class C beta-lactamase	1 ambiguous	1 positive
ACC-3	Class C beta-lactamase	1 positive, 2 ambiguous	3 ambiguous
MOX	Class C beta-lactamase	Not detected	1 ambiguous
OXA-51 group	Class D beta-lactamase	1 ambiguous	Not detected
QnrD	Fluoroquinolone resistance	Not detected	1 ambiguous
QnrS	Fluoroquinolone resistance	Not detected	3 ambiguous
ErmB	Macrolide / lincosamide / streptogramin B resistance	2 positive, 1 ambiguous	1 ambiguous

MefA	Macrolide / lincosamide / streptogramin B resistance	1 positive	Not detected
TetA	Tetracycline efflux pump	1 positive, 1 ambiguous	3 positive, 3 ambiguous
TetB	Tetracycline efflux pump	2 positive, 3 ambiguous	2 positive

CONCLUSIONS

Antimicrobial resistance genes are prevalent in supermarket beef and salmon samples. Genes for aminoglycoside, tetracycline, beta-lactam, and macrolide resistance were among those present.

6. APPENDICES

All supporting tables are provided as excel spreadsheets (xlsx format).

Table A1. Details of meat products purchased and pooled.

Table A2. Pathogen load of each pooled sample on each media type.

Table A3. Identity and morphology of the bacterial isolates.

Table A4. Antibiotic susceptibility test data for bacterial isolates.

Table A5. Interpretation of antimicrobial resistance for bacterial isolates.

Table A6. Quantitative PCR data for 87 antimicrobial resistance genes.

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