FOOD STANDARDS
Australia • New Zealand
Te Mana Kounga Kai - Ahitereiria me Aotearoa

National Surveillance of Antimicrobial Resistant Bacteria in Raw Retail Beef, Chicken and Pork Meat

Australia 2022–2023

Acknowledgment of country

Food Standards Australia New Zealand acknowledges the Traditional Owners and Custodians of Country throughout Australia and the Māori as tangata whenua of Aotearoa New Zealand, and their continuing connection to land, sea and community. We pay our respects to them and their cultures.

Copyright statement

Attribution

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For more information, email information@foodstandards.gov.au.

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Guide to report structure

This document is structured to support both expert and non-expert readers from diverse stakeholder groups. Key elements include:

1. **Summary**

An accessible summary is provided at the beginning of the report. It is written for a broad audience and combines text and visual elements.

2. **Technical Report**

A comprehensive technical report structured for both expert and non-expert readers. Key concepts and methods are introduced at the outset to aid understanding, with full methodological details at the end of the report. The report aims to balance scientific rigor with accessibility and clarity. Several intentional structural choices were made to achieve these goals:

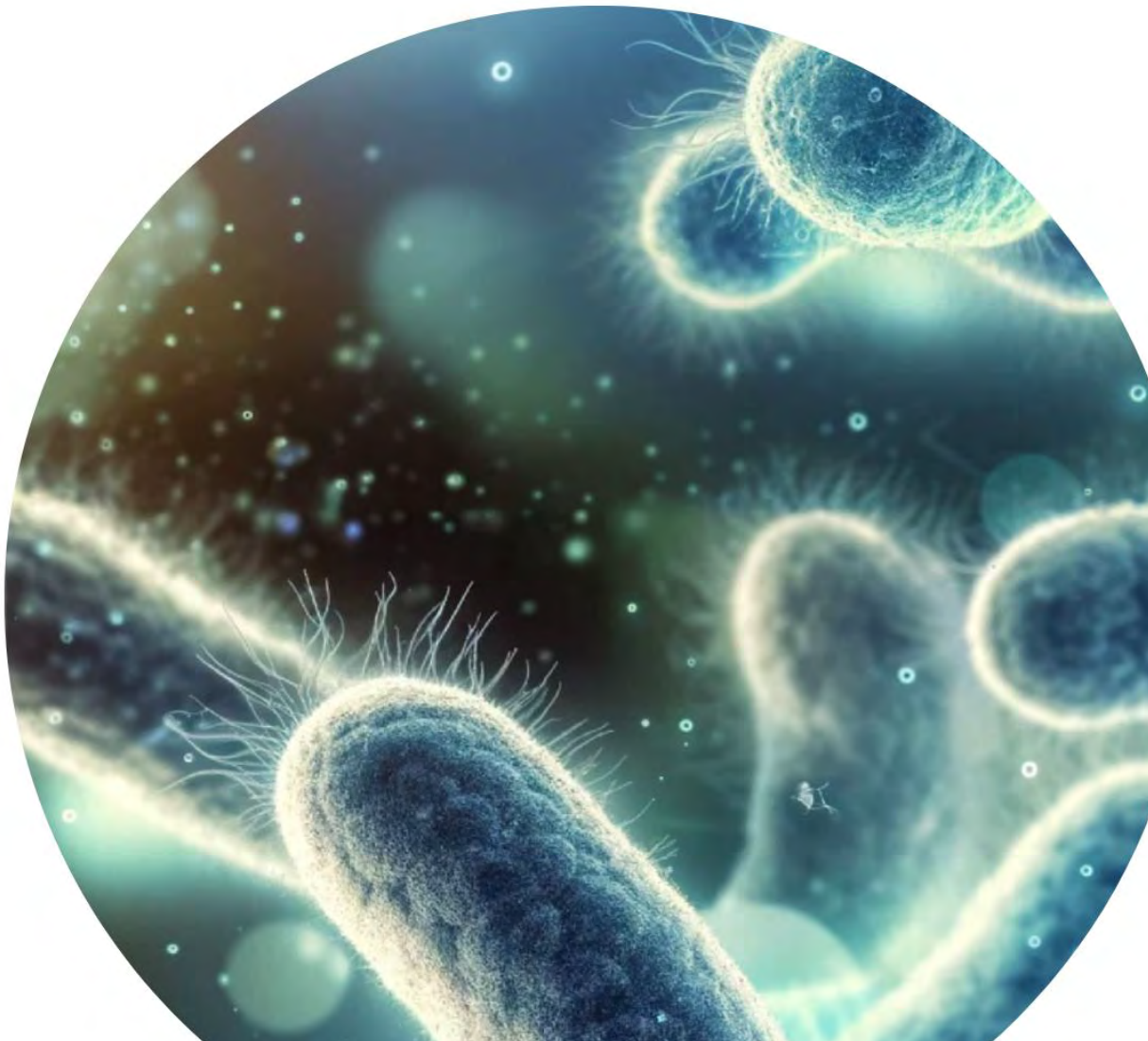
- Intentional repetition: Key concepts are repeated where necessary to support accurate understanding, even when sections are read in isolation. We recognise that many readers may only consult selected sections of the report, and this repetition helps ensure that essential context and interpretation accompany key findings wherever they appear.
- Combined results and discussion: Results are presented alongside interpretive context to ensure that findings are understood in context, not in isolation. This integrated approach promotes clearer interpretation and reduces the risk of misinterpretation.
- Key results grouped by commodity: Key results are grouped and repeated by commodity to help readers quickly locate information most relevant to their interests, improving the usability of the report for diverse stakeholder groups.
- Tables and graphs consolidated: Data tables and visualisations for each bacteria are grouped at the end of their respective sections to make it easier to find, view and compare information.
- Key messages and unified conclusion: Each bacteria section includes key messages that summarise the most important findings. A single, unified Conclusion section highlights overarching results and implications to support consistent interpretation across the full report.
- Emphasis on contextual understanding: Throughout the report, care was taken to present findings within the appropriate scientific and practical context. This ensures that results are not overgeneralised or misunderstood, and that their relevance and limitations are clearly communicated.

3. **Supplementary tables**

Supplementary Tables are provided to present more detailed technical information.

Contents

Summary



AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

SURVEY HIGHLIGHTS

FSANZ DEVELOPED AND COORDINATED
A RETAIL FOOD SURVEY PLAN
TO LOOK FOR
ANTIMICROBIAL RESISTANT BACTERIA

SUPPORTED BY AN
EXPERT PANEL

OVER 100 PEOPLE
FROM STATE AND TERRITORY DEPARTMENTS INVOLVED

4,151 RAW MEAT
SAMPLES COLLECTED
FROM ALL CAPITAL CITIES

SAMPLING CONDUCTED FROM
2022 - 2023
581 BEEF, 2,005 CHICKEN, 1,565 PORK MEAT SAMPLES

2,452 BACTERIA
TESTED FOR AMR
AT MURDOCH UNIVERSITY

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

BACKGROUND – ANTIMICROBIAL RESISTANCE

ANTIMICROBIAL RESISTANCE (AMR)

- Antimicrobials, such as antibiotics, are medicines used to prevent and treat infections caused by microorganisms in humans, animals and plants.
- They work by killing bacteria, slowing their growth or stopping them from causing infection.
- AMR occurs when bacteria, viruses, fungi and parasites evolve and become resistant to antimicrobial treatments.
- This makes infections harder to treat and increases the risk of severe illness, disease spread and death.

AMR IS A WORLDWIDE PROBLEM

- AMR affects countries in all regions and at all income levels. It is one of the top global public health and development threats.
- The global misuse and overuse of antimicrobials in humans, animals and plants are the main drivers in the development of drug-resistant pathogens.
- In 2015, countries adopted the Global Action Plan on AMR and committed to creating national action plans using a 'One Health' approach.
- Four key organizations – FAO, UNEP, WOAH, and WHO – work togther to combat AMR. They developed the One Health Joint Plan of Action (2022–2026) to address AMR globally.

TACKLING AMR NEEDS A ONE HEALTH APPROACH

- A One Health approach is important because antibiotic-resistant bacteria can potentially spread between and within the sectors of public health, agriculture, environment and food.
- One Health links humans, animals and the environment to tackle all aspects of AMR – prevention, detection and management – to support global health security.
- It works at all levels – local to global – through shared governance, communication, collaboration and coordination.
- One Health helps find balanced solutions to AMR while promoting responsible use of antimicrobials across all sectors.
- Although AMR is complex, a One Health response could save millions of lives and ensure antimicrobials remain effective for future generations.

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

BACKGROUND – AMR RESPONSE IN AUSTRALIA

AUSTRALIA'S RESPONSE TO AMR

- Australia's response recognises that AMR affects human and animal health, agriculture, food and the environment.
- *Australia's National Antimicrobial Resistance Strategy – 2020 and Beyond* outlines a 20-year plan to control AMR and ensure effective antimicrobials remain available.
- Like other countries, it uses a holistic, multi-sectoral One Health approach.
- The strategy is led by the Australian Government Department of Health, Disability and Ageing and the Department of Agriculture, Fisheries and Forestry.

For more information on AMR, the Australian Government National Strategy 2020 and Beyond, and what you can do to help reduce AMR please visit https://www.amr.gov.au

For more information on AMR and food safety please visit https://www.foodstandards.gov.au/consumer/safety/antimicrobial-resistance

THE FSANZ ROLE IN MONITORING AMR

- Other agencies including FSANZ are involved in the national strategy to ensure a whole-of-government approach.
- Food sits at the interface between humans, animals and the environment. It is considered an important link because it can spread resistant bacteria to humans.
- By focusing on retail food surveillance, Australia can monitor resistant bacteria that have potential to spread to and from different sectors.
- However, monitoring retail food is just one part of Australia's broader strategy and integrated effort to combat AMR.
- Alongside food surveillance, Australia's strategy includes work in healthcare, agriculture and animal husbandry, as well as public health initiatives to reduce unnecessary and inappropriate antibiotic use.
- Tackling AMR from multiple angles helps prevent resistance from spreading across different sectors, with the ultimate goal of protecting public health.

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

PLANNING AND OBJECTIVES

HOW FSANZ DID THIS WORK

- The Australian Government Department of Health, Disability and Ageing funded FSANZ to do the survey.
- FSANZ led the development and coordination of the AMR surveillance plan, managing laboratory services, food sampling plans, contract requirements and communication materials.
- FSANZ undertook extensive planning, with advice from an Expert Scientific Advisory Group and a working group of jurisdictional members, ensuring a high-standard surveillance plan.
- FSANZ coordinated with state and territory departments who funded and provided personnel to collect food samples.
- FSANZ contracted Murdoch University to undertake the isolation of bacteria, conduct antimicrobial susceptibility testing and complete whole genome sequencing.

WHAT WAS ACHIEVED

Collected contemporary nationally representative, phenotypic antimicrobial resistance data for *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococcus* in prioritised retail meat commodities.

Collected data to identify the emergence of AMR to high-importance rated antimicrobials in these bacteria.

Undertook whole genome sequencing of bacteria displaying AMR phenotypes of interest (e.g. multidrug resistance or resistance to high-importance rated antimicrobials) and identified known resistance determinants.

Ensured data are scientifically robust, reliable, defensible and comparable to international data and standards.

Provided a foundational design based on international best practices for ongoing surveillance of resistant bacteria in food, enabling data comparison with integrated human, animal and environmental datasets under Australia's One Health approach.

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY METHODS AND CONCEPTS

HOW RAW RETAIL MEATS AND BACTERIA WERE COLLECTED

The food sampling strategy

- The sampling plan adhered to international guidelines, considering factors like sampling frequency, statistical power, sample size, strata selection and storage/transport procedures.

Prioritised food samples and bacteria

- Prioritised food commodities included beef, chicken meat and pork based on epidemiological/public health factors, production patterns and antimicrobial resistance prevalence.
- Target bacteria considered were *E. coli*, and *Enterococcus* spp., *Salmonella* and *Campylobacter*.

Types of raw meat and retail distribution

- Samples included raw chicken Maryland, beef mince and pork mince, with reserves collected if these were unavailable.
- Raw meat was sourced from large supermarkets (60%), small supermarkets (20%) and independent butchers (20%) to reflect Australian consumer purchasing patterns.

Raw meat sample collection and random allocation

- Samples were collected from metropolitan areas of all major cities across Australian jurisdictions, weighted by population.
- Sampling spanned 40 weeks from September 2022 to July 2023, with random allocation across the areas.

Transport

- Meat samples were transported to multiple laboratories to ensure timely processing despite any pandemic-related constraints.
- Samples with packaging issues or improper temperature conditions were flagged and replaced in subsequent sampling runs.

Bacterial isolation

- Australian Standard methods were used where possible to detect the presence or absence of bacteria in raw meat samples. The bacteria collected were then tested for AMR.

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY METHODS AND CONCEPTS

ANTIBIOTICS INCLUDED IN THE STUDY

- Antibiotics were chosen because they are useful for treating infections and detecting resistance within their class or through specific resistance mechanisms.
- One or two key antibiotics from key antibiotic classes were included. If bacteria are resistant to one antibiotic, they can often be resistant to others in the same class. This helps identify broader resistance patterns.
- Selection was guided by international scientific recommendations and Australia's antibiotic importance list, which supports responsible use of antibiotics.
- Australia's list, developed by the Australian Government, classifies antibiotics as high, medium or low importance. These ratings are based on how important the antibiotic is for treating infections in humans and how serious the consequences would be if resistance increased.
- Antibiotics important for both human and animal health were included to support a One Health approach. This includes some antibiotics rated as low importance for humans but that are critical for veterinary medicine.

HOW BACTERIA WERE TESTED FOR AMR

Broth Dilution Test (phenotypic method)

- Bacteria were exposed to a series of antibiotic concentrations in a liquid medium.
- The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antibiotic that prevents visible growth of the bacteria.
- MIC values were compared to epidemiological cut-off values (ECOFFs) to determine resistance.

Whole genome sequencing (genotypic method)

- Short-read whole genome sequencing (WGS) was used to predict AMR by analysing the bacterial genomes for known resistance genes, mutations and plasmids.

DIFFERENT BREAKPOINTS TO DESCRIBE AMR

EPIDEMIOLOGICAL CUT-OFFs

USED FOR SURVEILLANCE
and indicate when different resistant bacteria are starting to appear

CLINICAL BREAKPOINTS

USED FOR TREATMENT
and indicate which antibiotics are likely to work to treat infection

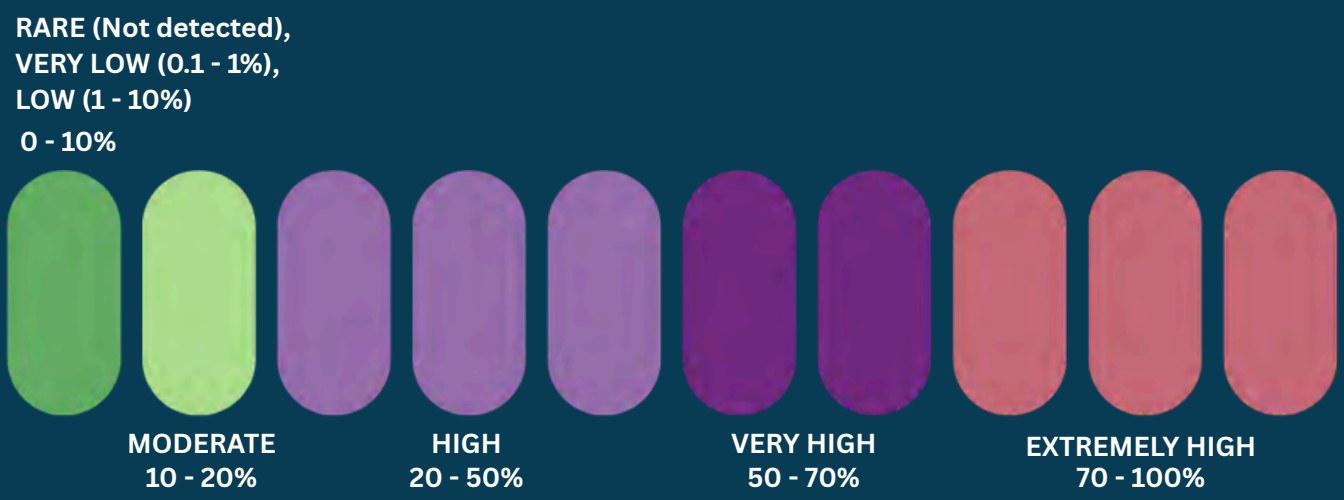
AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY METHODS AND CONCEPTS

HOW AMR IS DESCRIBED IN THIS REPORT

- AMR is assessed using two key frameworks: epidemiological cut-off values (ECOFFs) and clinical breakpoints.
- In this study, AMR levels based on ECOFFs are primarily reported using the following terminology:
  - **AMR:** Occurs when bacteria acquire resistance to antibiotic treatments to which they were previously susceptible.
  - **Microbiological complete susceptibility:** All antibiotics tested were effective at stopping bacteria growing at concentrations at or below the specified cut off (i.e., wild type MIC ≤ ECOFF) and bacteria are not expected to have acquired resistance mechanisms.
  - **Microbiological susceptibility:** Bacteria were not able to grow in the presence of an antibiotic at concentrations at or less than the specified cut off (i.e., wild-type MIC ≤ ECOFF) and are not expected to have acquired resistance mechanisms.
  - **Microbiological resistance:** Bacteria were able to grow in the presence of an antibiotic at concentrations above the specified cut off (i.e., non-wildtype MIC > ECOFF) and may harbour acquired resistance mechanisms, but this does not necessarily mean clinical treatment failure.
  - **Multidrug microbiological resistance (MDmR):** Bacteria were classified as MDmR if they were microbiologically resistant to 3 or more antibiotic classes. As mentioned above, if an isolate showed microbiological resistance to at least one antibiotic in a class it was considered resistant to that class.

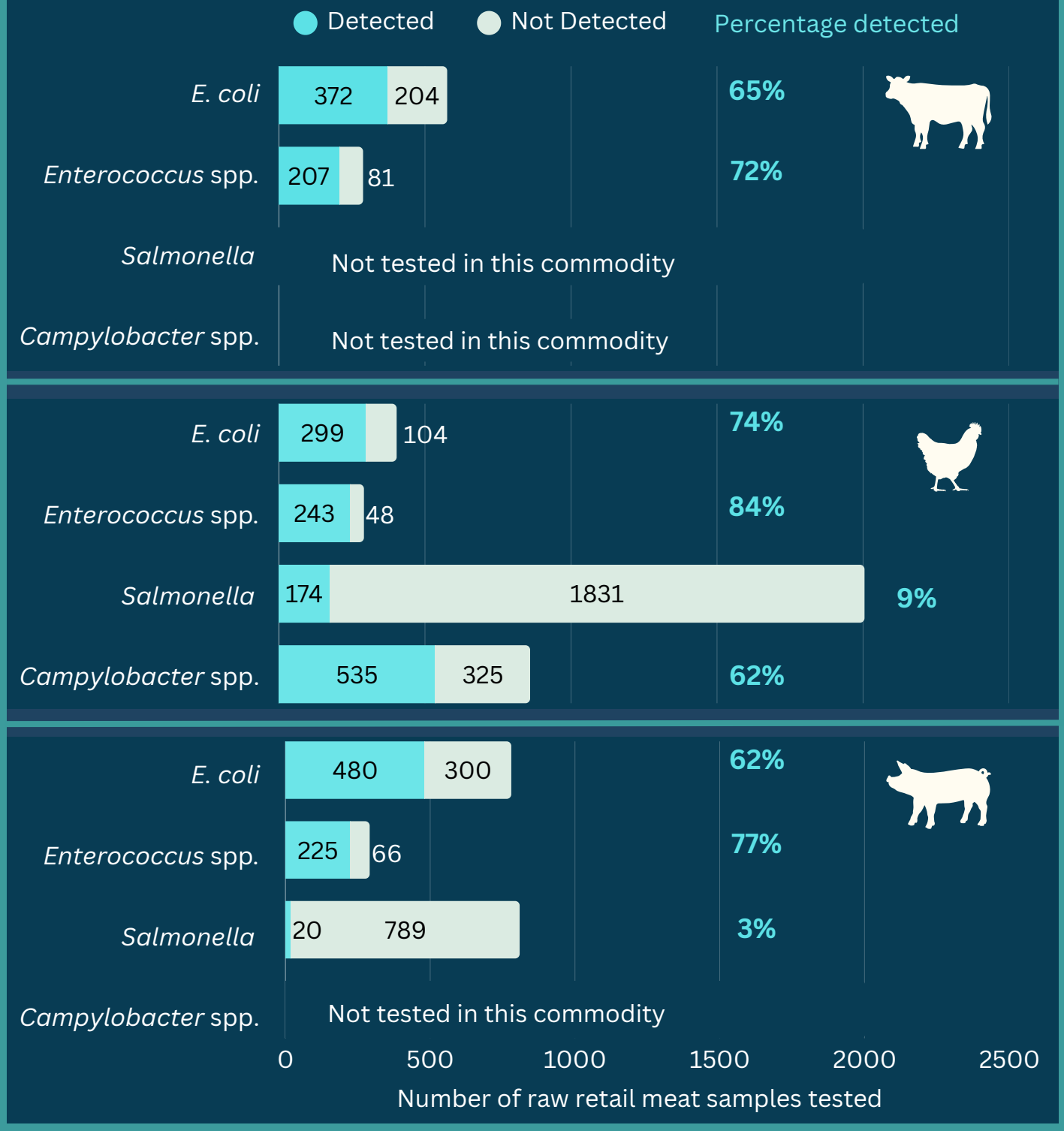
AMR levels have been described using categories based on those developed by the European Food Safety Authority (e.g., rare to extremely high). See below.



AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY RESULTS – DETECTING BACTERIA

This study first checked raw retail meat to see how many target bacteria were present before looking at antibiotic resistance. The graphs below for beef, chicken and pork show how many samples were collected, how many had the bacteria and how many didn't, and the percentage detected. These results show the baseline level of bacteria found before any AMR testing was done (AMR results are on the following pages).



AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY RESULTS – DETECTING AMR BACTERIA

AMR detection in *Escherichia coli* from raw retail beef, chicken and pork meat

	Beef	Chicken	Pork
Not microbiologically resistant to any antibiotic tested	81%	66%	44%
Microbiological resistance to high importance antibiotics for human health	0 – 1% RARE – VERY LOW	0 – 3% RARE – LOW	0 – 3% RARE – LOW
Multidrug microbiological resistance	4% LOW	7% LOW	25% HIGH

AMR detection in *Enterococcus faecalis* from raw retail beef, chicken and pork meat

	Beef	Chicken	Pork
Not microbiologically resistant to any antibiotic tested	78%	47%	65%
Microbiological resistance to high importance antibiotics for human health	0 – 1 % RARE – VERY LOW	0 – 1 % RARE – LOW	0 – 3 % RARE – LOW
Multidrug microbiological resistance	0 % RARE	1% LOW	5% LOW

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

KEY RESULTS - AMR

AMR detection in *Salmonella* from raw retail chicken meat

92%

Not microbiologically resistant to any antibiotic tested

0%

RARE

Microbiological resistance to high importance antibiotics for human health

0%

RARE

Multidrug microbiological resistance

AMR detection in *Campylobacter coli* from raw retail chicken meat

97%

Not microbiologically resistant to any antibiotic tested

1%

LOW

Microbiological resistance to high importance antibiotics for human health

0.5%

VERY LOW

Multidrug microbiological resistance

AMR detection in *Campylobacter jejuni* from raw retail chicken meat

80%

Not microbiologically resistant to any antibiotic tested

16 %

MODERATE

Microbiological resistance to high importance antibiotics for human health

0.4%

VERY LOW

Multidrug microbiological resistance

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

CONCLUSIONS

A nationwide survey of AMR among bacteria isolated from Australian raw retail beef, chicken and pork meat samples was completed between September 2022 and July 2023. The study provides a comprehensive national snapshot of AMR in foodborne and commensal bacteria among raw retail beef, chicken and pork meat.

Escherichia coli and *Enterococcus* were isolated as indicator organisms for AMR because they are common commensal bacteria of the human and animal gut. These bacteria can be indicators of emerging and persistent resistance and can contribute to tracking AMR across sectors. The study also targeted two key foodborne pathogens, *Salmonella* (chicken meat and pork) and *Campylobacter* (chicken meat only).

Overall, the study indicates a low risk of bacteria from these raw retail meats being involved in transmission of bacteria that may become involved in resistant infections or spreading resistance when safe primary production, processing, cooking and food handling is practiced.

Key findings included:

- Rare to low microbiological resistance was detected for high-importance antibiotics critical for treating human infections. The only exception was moderate ciprofloxacin resistance observed among *Campylobacter jejuni*, but resistance to other macrolide antibiotics commonly used to treat human campylobacteriosis was rare to low.
- High rates of complete microbiological susceptibility to all antibiotics tested were common across all bacteria and commodities.
- Low levels of multidrug microbiological resistance (MDmR) were mostly observed. The Majority of MDmR in *E. coli* was linked to low-importance antibiotics, and MDmR involving high-importance antibiotics was low across all commodities.
- Resistance to antibiotics considered low-importance for human medicine but that are often critical in veterinary contexts were consistent with expectations based on the 2007 pilot study.

These findings are broadly consistent with the 2007 survey by Barlow and Gobius (2008) and recent surveillance of Australian livestock showing support for the effectiveness of current antimicrobial stewardship practices in food-producing animals.

Moderate levels of quinolone (ciprofloxacin) microbiological resistance were detected in *Campylobacter jejuni* isolates from raw retail chicken meat. This aligns with global trends and findings from Australian human clinical and livestock chicken samples. Importantly, quinolones have never been registered for use in Australian livestock, highlighting the unique global challenge of quinolone-resistant *Campylobacter*.

AUSTRALIAN NATIONAL SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN RAW RETAIL BEEF, CHICKEN AND PORK MEAT

CONCLUSIONS

Repeated and harmonised surveillance is needed to accurately detect both improvements in the form of reduced resistance levels and the emergence of new resistance risks: While the 2007 study by Barlow and Gobius (2008) reported similar AMR levels, the two studies differ in design and methodology, and their datasets are not directly comparable. Therefore, no definitive trends can be concluded. However, no notable increases in resistance were observed for antibiotics tested in both studies, except for quinolone resistance in *Campylobacter jejuni*, which may have increased.

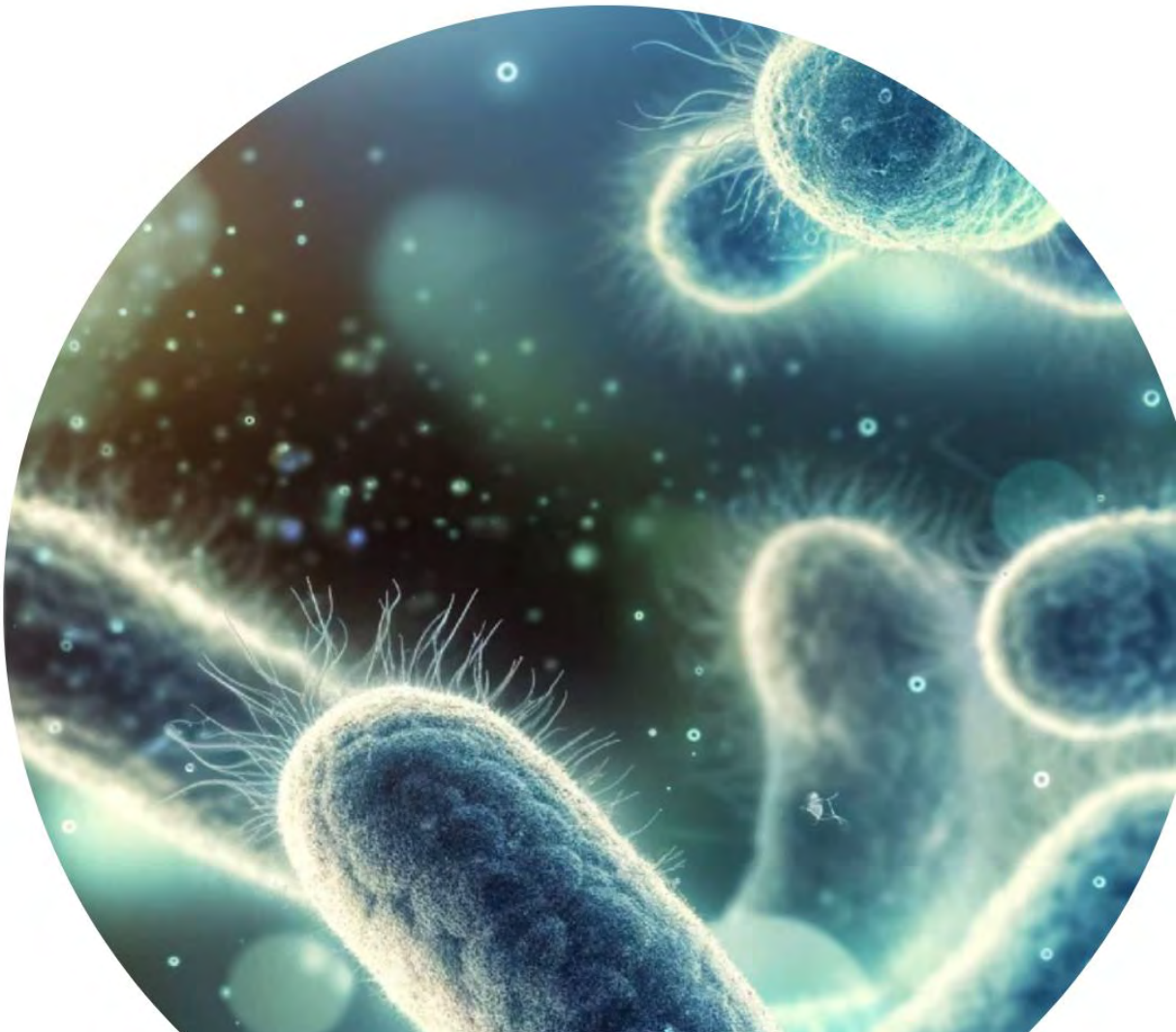
Sustained antimicrobial stewardship and food safety practices from farm to fork are essential to preserve antibiotic effectiveness and protect public health: The study also highlights the interconnectedness of human, animal and environmental health. Low-importance human antibiotics are still common first line treatments but are often critical in veterinary medicine. Differences in resistance profiles across meat types and bacteria for these antibiotics emphasise the importance of coordinated One Health efforts.

More research is needed to trace resistant bacteria. A national genomic database from this study supports ongoing cross-sector collaboration: Although this study was not designed to determine the origin of bacteria on meat products, the genomic database developed provides a valuable resource for future research. Cross-sector collaboration is encouraged to explore transmission pathways and inform holistic AMR management strategies. The database developed in this study provides a valuable resource for Australian research, and organisations are encouraged to contact FSANZ to discuss potential research projects, particularly cross-sector research, which could be of benefit nationally and internationally.

The same food safety basics used to prevent foodborne illness reduces AMR risks. Public awareness is also vital to prevent foodborne illness and limit AMR spread: While AMR bacteria were detected in raw meat the same proper food safety practices used to prevent foodborne illness can effectively mitigate risks associated with AMR bacteria in food. The bacteria found in this study are easily made harmless through effective cooking and cross-contamination is reduced through safe food handling. Public awareness initiatives on safe food production, food handling, proper cooking temperatures and cross-contamination prevention could further reduce the likelihood of both foodborne illness and foodborne AMR transmission.

This study strengthens Australia's One Health AMR surveillance framework and reinforces the need for ongoing monitoring, collaborative action and sustained stewardship to protect human and animal health, food safety and food security into the future.

Technical report



Abbreviations

Abbreviation	Meaning
AMR	Antimicrobial Resistance
AS	Australian Standard
AST	Antimicrobial Susceptibility Testing
ATCC	American Tissue Culture Collection
CI	Confidence Interval
CLSI	Clinical Laboratory Standards Institute
CSBA	Columbian Sheep Blood Agar
DNA	Deoxyribonucleic Acid
ECOFF	EUCAST Epidemiological Cut-off Value
EFSA	European Food Safety Authority
ESAG	Expert Scientific Advisory Group
ESBL	Extended-spectrum β-lactamase
EUCAST	European Committee of Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionisation – Time of Flight Mass Spectrometry
MDmR	Multidrug microbiologically resistant
MIC	Minimum Inhibitory Concentration
MLST	Multilocus Sequence Type
MU	Murdoch University
QC	Quality Control
RASP	Robotic Antimicrobial Susceptibility Platform
ISFR SEAWG	Implementation Subcommittee for Food Regulation Surveillance, Evidence and Analysis Working Group
ST	Multilocus Sequence Type
VCIAA	Veterinary Critically Important Antimicrobial Agents
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health

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Introduction

Background

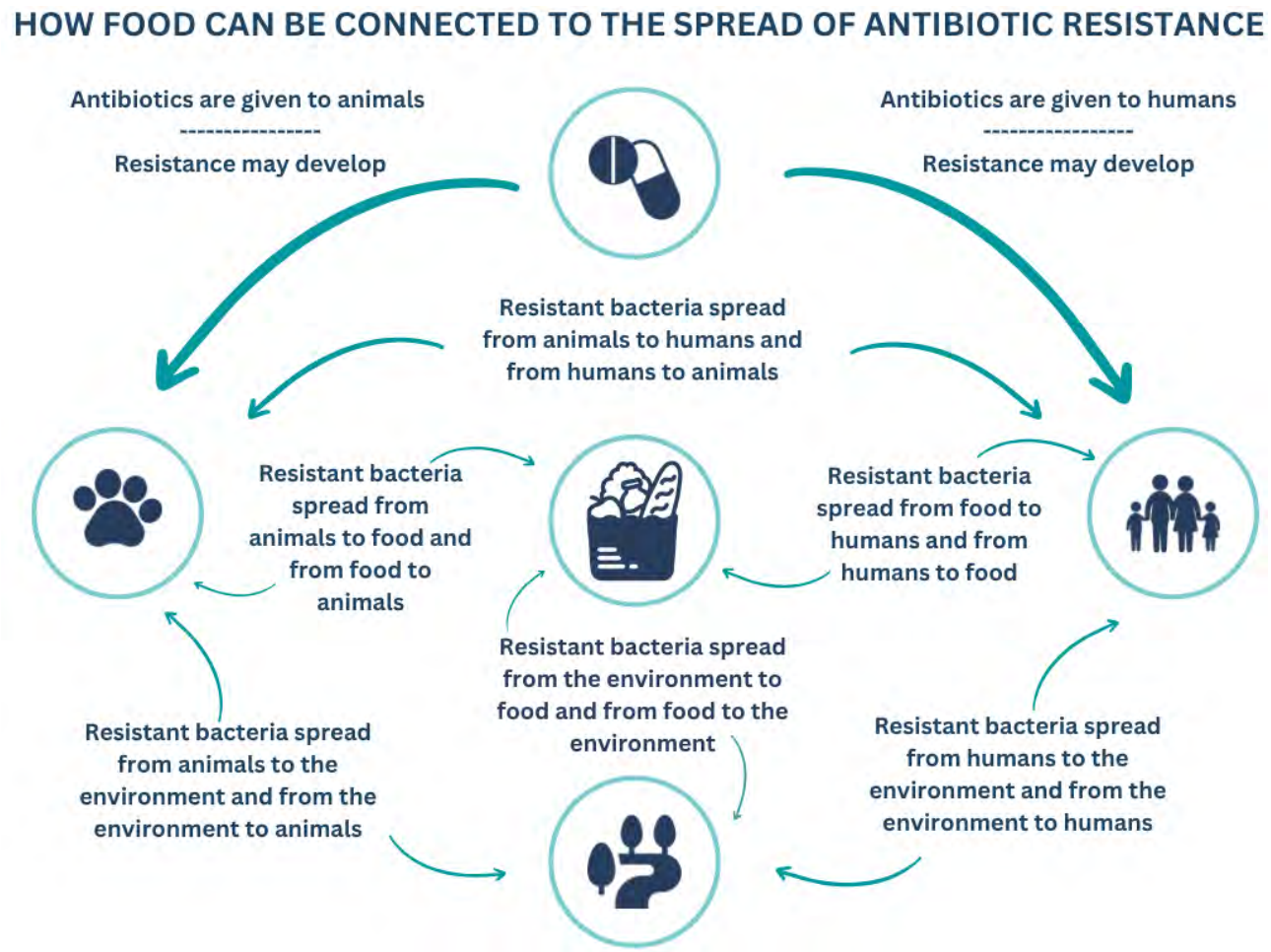
Antimicrobial resistance (AMR) is one of the biggest threats to human and animal health today. AMR occurs when bacteria, viruses, fungi and parasites evolve and become resistant to antibiotic treatments, making infections harder to treat and increasing the risk of severe illness, disease spread and death. The EcoAMR consortium of international partners, led by the World Organisation for Animal Health (WOAH), analysed the latest data from 204 countries and 621 subnational regions to project how AMR will affect mortality, health care costs, food security and the global economy across both human and animal populations (Vollset et al. 2024; McDonnell et al. 2024; Adamie et al. 2024; WOAH 2024a; WOAH 2024b). The reports concluded AMR could be responsible for 38.5 million global deaths between 2025 and 2050, with the annual toll rising by 60%. Economically, AMR costs health systems $66 billion per year today, projected to reach $159 billion by 2050, and could reduce global GDP by $1.7 trillion per year. In agriculture, AMR threatens food security, with livestock production losses by 2050 equivalent to the food needs of up to 2 billion people and cumulative GDP losses in the animal sector alone reaching nearly $1 trillion. This global problem spans human, animal and environmental health, and disproportionately affects low- and middle-income countries, making AMR one of the most urgent and wide-reaching health and economic threats of our time (Vollset et al. 2024; McDonnell et al. 2024; Adamie et al. 2024; WOAH 2024a; WOAH 2024b).

In 2015 the World Health Assembly adopted a global action plan on AMR (WHO 2015) and at the 2024 United Nations General Assembly High-Level Meeting on AMR, member states, including Australia, adopted a political declaration reaffirming their commitment to tackling AMR through a One Health approach (Commonwealth of Australia 2024; WHO 2024a). One Health is defined internationally as an integrated, unifying approach that aims to sustainably balance and optimise the health of people, animals and ecosystems. It recognises that the health of humans, domestic and wild animals, plants and the wider environment (including ecosystems) are closely linked and interdependent. The approach mobilises multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and address threats to health and ecosystems. It also responds to the collective need for clean water, energy and air, safe and nutritious food, climate action and sustainable development (FAO, UNEP, WHO and WOAH 2022).

The One Health approach is essential for addressing AMR due to its complex nature (FAO, UNEP, WHO, and WOAH 2022). The main driver of AMR in bacteria is antibiotic use, and while antibiotics are critical for treating infections in both humans and animals, their misuse and overuse accelerate the development of resistance. Microorganisms and resistance genes can spread globally and can move between people, animals, food and the environment, meaning that practices in one sector can impact all others. The One Health approach promotes global and regional cross-sector collaboration to enable integrated surveillance, consistent stewardship and coordinated public messaging (FAO, UNEP, WHO, and WOAH 2022).

Australia has one of the safest food supplies in the world; however, food can still be a source of human disease. This is primarily due to foodborne zoonotic pathogens like *Campylobacter* and *Salmonella*. For most people, foodborne illness is mild and they do not need to be treated with antibiotics. But people with severe symptoms or more vulnerable groups like the young, old and people with weakened immune systems may need antibiotic treatment to recover. If the bacteria causing foodborne illness are resistant to commonly used antibiotics, infections can be harder to treat, and people can be sick for longer, resulting in increased risk of more severe illness along with higher medical costs. This makes understanding patterns of AMR in these pathogens crucial.

Additionally, food harbours bacteria that naturally live in the bodies of human and food animals without causing harm and can sometimes even be beneficial (for example *Escherichia coli* and *Enterococcus* spp.). However, some of these bacteria (or subspecies of them) can also be specialist pathogens (only infect and cause illness in one or a limited number of host species) or opportunistic pathogens (normally harmless but can cause illness if the host immune system is weakened of transferred to part of the body that is normally sterile).



The greatest current human health risk from AMR is linked to endogenous (self-originating) or nosocomial (hospital-acquired) infections caused by specialist or opportunistic ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. including *E. coli*) (Miller and Arias 2024). While these infections are often associated with health care facilities, human-to-human transmission in the community is being recognised as a substantial and growing contributor to the spread of problematic strains (Miller and Arias 2024). The potential for food to play a role in this transmission is not well understood.

Food sits at the interface between humans, animals and the environment. It is considered an important link because it has potential to transfer resistant bacteria to humans, particularly if food is not cooked properly and other basic food safety is not used during production and preparation. By focusing on retail food surveillance, Australia can monitor resistant bacteria that have potential to spread. This is just one part of Australia's broader strategy to combat AMR.

Australia's strategy to combat AMR acknowledges that a One Health approach is needed. The Australian Government has developed a national strategy: *Australia's National Antimicrobial Resistance Strategy – 2020 and Beyond* (DOH 2019). The 2020 Strategy was endorsed by all state and territory governments in recognition that combating AMR is a matter of national importance and

requires coordinated action by all governments, the private sector, industry, professionals, the research community and the general public. The 2020 Strategy outlines a 20-year plan to control and combat AMR while ensuring the continued availability of effective antibiotics. It also maintains alignment with the World Health Assembly-endorsed Global Action Plan on Antimicrobial Resistance and a commitment to continue to support global and regional efforts to manage the threat of AMR.

This approach is led by the Australian Government's Department of Health, Disability and Ageing and the Department of Agriculture, Fisheries and Forestry, with support from numerous agencies including Food Standards Australia New Zealand (FSANZ), to ensure a comprehensive, government-wide response. Alongside food surveillance, Australia's strategy includes work in the human health, animal and environment sectors, as well as public health initiatives to reduce the risks of AMR development and inappropriate antibiotic use. Reducing AMR is a shared responsibility and no single sector can succeed alone. The strategy also integrates industry's key role in areas such as infection prevention, biosecurity, innovation, education and surveillance, alongside promoting and following best-practice stewardship. Success in tackling AMR depends on broad collaboration and coordinated action across government, professionals, industry, researchers and society. This is essential to understanding how resistance spreads across different sectors and to inform effective, targeted responses.

The Department of Health, Disability and Ageing funded FSANZ to look for AMR bacteria in the Australian food supply to support Objective 5 of *Australia's National AMR Strategy – 2020 and Beyond*: 'Integrated surveillance and response to resistance and usage'. This study provides up-to-date data on antibiotic resistant bacteria in retail beef, pork and chicken meat. This represents the most comprehensive study of retail food in Australia since Barlow and Gobius (2008) undertook a pilot AMR food survey. The pilot survey concluded overall resistance to the majority of antibiotics was low among bacteria isolated from retail meats. In addition, when compared to reports from other countries, Australia had a very low prevalence of bacteria that were resistant to antibiotics, particularly those important for human medicine, on these foods.

For more information on AMR, the *Australian Government National Strategy – 2020 and Beyond*, and what you can do to help reduce AMR please visit

https://www.amr.gov.au

For more information on AMR and food safety please visit

https://www.foodstandards.gov.au/consumer/safety/Antimicrobial-resistance

Objectives

The objectives of this survey were to:

- collect contemporary nationally representative phenotypic AMR data for *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococcus* in prioritised retail meat commodities
- collect data to identify the emergence of AMR to high-importance rated antibiotics in these bacteria
- undertake whole genome sequencing (WGS) of bacteria displaying AMR phenotypes of interest (for example, multidrug resistance or resistance to high-importance rated antibiotics) and identify known resistance determinants

- ensure data are scientifically robust, reliable, defensible and comparable to international data and standards
- provide a foundational design, according to international best practice, for future ongoing surveillance of resistant bacteria in food so that data can be compared alongside integrated human, animal and environmental data collected as part of the Australian One Health approach.

Roles and responsibilities

The Australian Government Department of Health, Disability and Ageing provided the funding for food sample transport, the isolation of bacteria from food samples, antibiotic susceptibility testing (AST) of bacteria, the majority of WGS of bacteria, and costs associated with FSANZ personnel and reporting.

FSANZ was the developer, coordinator and project manager of the surveillance plan. FSANZ undertook procurement for laboratory services, liaised with jurisdictions to develop all state- and territory-based food sampling plans, and liaised with laboratories to ensure delivery of contract requirements. The review of the draft report, redrafting based on the Expert Scientific Advisory Group (ESAG) comments, and clearance of the final report was undertaken by FSANZ. The perspectives, conclusions and recommendations are those of FSANZ. FSANZ developed the communications material.

State and territory authorities purchased the food samples, and provided all personnel and resources on the ground to collect them.

Murdoch University (MU) was contracted to coordinate, undertake and report all analyses of bacteria for AST and WGS. Isolation of bacteria from food samples, and reporting, was the responsibility of subcontractor Symbio Laboratories. MU provided the first draft report including data, analysis and interpretation. MU provided technical clarifications during the review of subsequent draft reports by FSANZ.

A significant amount of planning and coordination was required before the commencement of food sampling in September 2022 to ensure a surveillance plan that met the highest expected standards internationally. To do this the ESAG – consisting of members who are experts in their fields with extensive experience in AMR and AMR surveillance – was formed by FSANZ to advise on all aspects of the project (from planning through to reporting). The Implementation Subcommittee for Food Regulation Surveillance, Evidence and Analysis Working Group (ISFR SEAWG) jurisdictional members were also frequently consulted on practical and implementation aspects of the plan to ensure smooth and efficient collection of food samples on the ground.

FSANZ took into consideration all feedback provided by the ESAG and ISFR SEAWG to develop a supported, scientifically robust and achievable national AMR survey. This expert and practical advice allowed for a surveillance plan to be developed based on accuracy, precision and power for statistical analysis, as well as affordability and practicality.

The ESAG provided advice on the following topics:

- the current state of knowledge on AMR and retail food in Australia
- establishing the monitoring and surveillance objectives
- potential options for surveillance plans
- key considerations for identifying priority food, organisms and antibiotics
- sampling design considerations and methods of AST
- the target number of isolates required for the study
- the expected prevalence of selected microorganisms in specific commodities

- sample collection parameters for jurisdictional sampling officers
- methods of isolation of microorganisms from retail samples
- determination of antibiotic panels for AST
- final report review
- communications material.

Key methods, concepts and terminology

A detailed description of the methods and materials is provided in the Materials and Methods section. Key methods, concepts and terminology that will assist in understanding the results and discussion in this report are briefly explained below.

Overview of the methodology

The food sampling strategy (See section Sampling**):** The sampling plan adhered to international guidelines (see [WHO 2017]). Development of the plan considered factors like random sampling, sample size, population coverage and avoiding sampling bias to ensure reliable and representative results.

Prioritised food samples and bacteria (See section Prioritising food commodities**):** A prioritisation matrix was used to rank eggs, dairy, seafood, horticulture, beef, chicken meat and pork. Based on the ranking, chicken, beef and pork were selected to be included in the first year of surveillance. Target bacteria included were *E. coli*, *Enterococcus* spp., *Salmonella* spp., and *Campylobacter*. Other commodities are intended to be tested if funding is made available in the future.

Types of raw meat and retail distribution (See section Sample types**):** Raw meat samples included chicken Maryland, beef mince and pork mince, with selected alternative cuts collected if these were unavailable. Raw meat was sourced from large supermarkets (60%), small supermarkets (20%), and independent butchers (20%) to reflect Australian consumer purchasing patterns. All raw chicken and pork collected in this survey was Australian, as imports of these products are not permitted for sale in Australia due to biosecurity restrictions. While raw boneless pork may be imported, it must be cured or processed before being released for sale. All packaged raw beef in the survey was Australian. Although raw beef imports are permitted from approved countries, volumes are small and it is unlikely the survey included imported unpackaged beef.

Raw meat sample collection and random allocation (See section Sample collection**):** Samples were collected from the greater metropolitan areas of all major cities of Australia. About two-thirds (≈66 %) of Australians live in the greater metropolitan regions of the capital cities. Allocation of samples was weighted by population and designed to ensure temporal balance and reduce potential biases related to seasonal or periodic variations. The raw meat sample collection areas within a greater metropolitan area were randomly distributed. Sampling spanned 40 weeks from September 2022 to July 2023.

Transport (See section Sample collection and transport**):** Raw meat samples were transported to multiple laboratories to ensure timely processing if any pandemic-related constraints arose. Samples with packaging issues or improper temperature conditions were not analysed and were replaced in subsequent sampling runs.

Bacterial isolation and transportation (See section Sample preparation and bacterial isolation**):** Australian Standard (AS) methods were used where possible to detect the presence

or absence of bacteria in raw meat samples. Target bacteria were identified using matrix-assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF-MS) and retained for testing for AMR. The bacteria were then transported to MU where their identity was re-confirmed by MALDI-TOF-MS and the bacteria tested for AMR using phenotypic and genotypic methods.

AMR phenotypic testing (See section Antimicrobial susceptibility testing**):** Clinical Laboratory Standards Institute (CLSI) and European Committee of Antimicrobial Susceptibility Testing (EUCAST) methods were applied to determine phenotypic AMR to a panel of prioritised antibiotics important for human and animal health. Results based on EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints are provided in this report, but primarily results from ECOFFs are reported as recommended by the World Health Organization (WHO 2017).

AMR genotypic testing (See section Genetic analysis**):** Short-read WGS was used to predict AMR by analysing bacterial genomes for known resistance genes, mutations and plasmids.

Data analysis (See section Statistical analysis**):** Exact binomial confidence intervals (CIs) of proportions were calculated using the Clopper-Pearson method.

This report does not determine the source of bacteria present on the raw retail beef, chicken or pork meat or what caused AMR

AMR surveillance of retail meats provides valuable insights into resistance patterns among foodborne and other medically important bacteria. However, this study alone cannot pinpoint the exact source of the bacteria found among retail meats or the drivers of detected AMR. This is because there are various entry points where bacteria may contaminate meat before it reaches the supermarket shelves. These different transmission pathways mean that the bacteria present may have been exposed to different AMR drivers from humans, animals or the environment, which can influence the presence of antibiotic resistant bacteria. For recent reviews related to AMR surveillance see (Karp et al. 2017; Kahn 2017; Diallo et al. 2020; Bennani et al. 2020)

The bacteria detected in retail meats – such as *E. coli, Salmonella, Campylobacter and Enterococcus* – commonly originate from the live animal, which is why they are included in AMR surveillance programs, but can also originate from humans, or the environment at lower levels. The resistance traits found in these bacteria may not directly reflect recent antibiotic use on the farm. Resistance may persist over time and may be due to past exposure to antibiotics, movement of animals, or transmission of bacterial populations in humans, wildlife or the environment. This means that the resistance observed in foodborne bacteria is shaped by a complex web of influences that may go beyond the immediate farm setting.

To better interpret AMR surveillance data from food, information from the human, animal, plant and environmental sectors is crucial. In the animal sector, understanding antibiotic use patterns, husbandry practices, and biosecurity measures helps contextualise why certain resistance traits are more or less prevalent on food of animal origin. In the human sector, data on AMR in clinical and community settings provide insights into whether resistance traits found in foodborne bacteria mirror those circulating in people. For example, if specific phenotypic patterns of resistance are detected in *E. coli* from retail meat, knowledge of whether a similar pattern of resistance is prevalent in livestock populations, agricultural settings or human clinical isolates might point to a likely origin. However, it is important to note that identifying the same resistance phenotype or AST profile alone is not sufficient to infer the origin. Additional genotype or sequence information from across all sectors – not just food – is necessary to accurately trace the source of the resistance, whether it be from animals, human-associated transmission or environmental contamination. Without this broader

context, AMR surveillance in food alone provides an incomplete picture, as it captures only a snapshot of resistance in bacteria at the point of retail without explaining how or why it emerged. Combining AMR surveillance with comparable data from the human, animal, food and environmental sectors is needed to provide a more accurate understanding of resistance origins, transmission pathways and potential risks.

However, a cooperative surveillance system – with broad representation across governments, industry and the public sector – that coordinates and shares appropriate data and would allow comparison across the human, animal, food, plant and environmental sectors does not yet exist in Australia. Therefore, to provide some Australian context to both the detection (presence or absence) and the levels of resistance (percentage of isolates) in this work, background on antibiotic use and antibiotic resistance in Australian farm animals and humans is provided where available and considered relevant. It is important to note that the referenced reports from the Australian animal and human sectors are not directly comparable to the data in this report. They are included solely to provide contextual background for the reader.

AMR in this report refers to 'acquired resistance'

AMR occurs when a bacterium becomes resistant to an antibiotic that was previously effective at killing it or stopping it from growing. A bacterium can acquire resistance through a new genetic mutation that helps the bacterium survive or by getting deoxyribonucleic acid (DNA) from a bacterium that already is resistant (for example, by acquiring plasmids that carry resistance genes). AMR is not 'intrinsic resistance', which is resistance due to bacterial characteristics that naturally occur (for example, *Escherichia coli* is naturally resistant to macrolides).

Further information is available on the Australian Government's AMR website: https://www.amr.gov.au/about-amr

AMR was detected among bacteria using phenotypic and genotypic methods

In this study, phenotypic methods were used to detect AMR for all bacteria, and genotypic methods for selected bacteria. For a comprehensive review of current methods for AMR detection see Gajic et al. (2022).

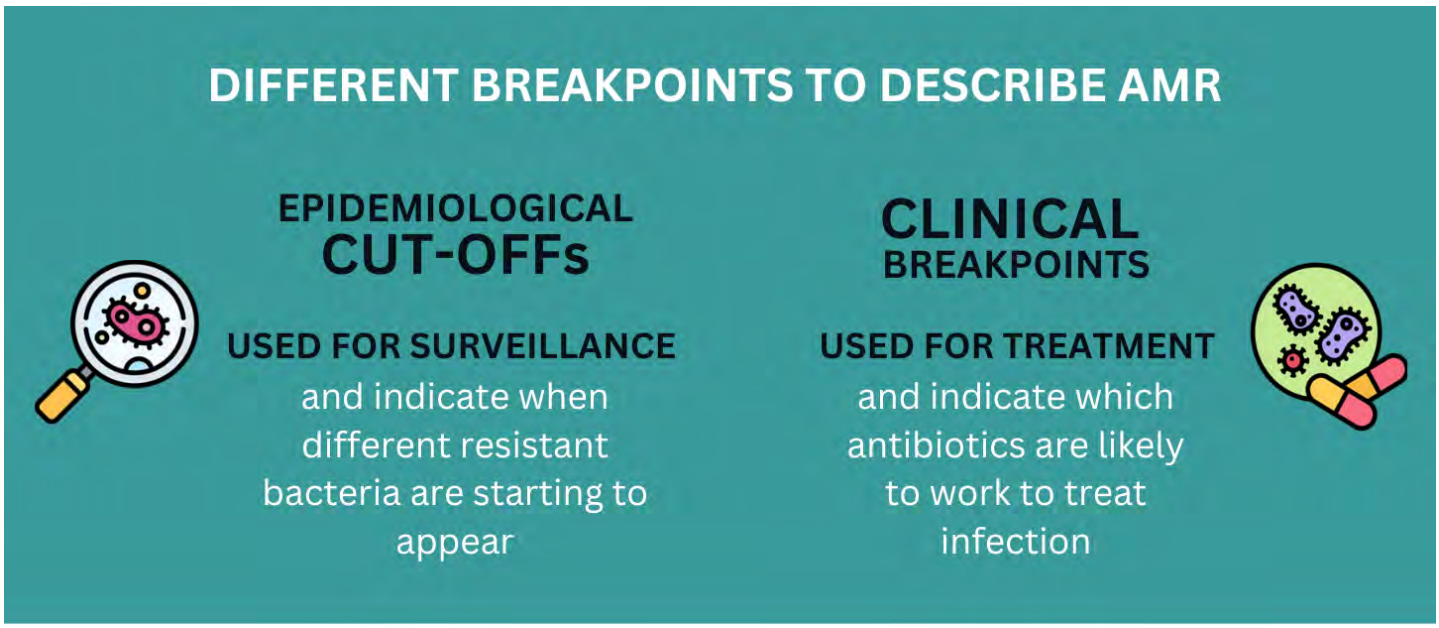
- **Phenotypic method:** An 'AMR phenotype' refers to the observable resistance characteristics of a bacterium. It is determined by measuring a bacterium's actual response to antibiotics by observing its growth inhibition in the presence of the drug. It includes methods like broth dilution and disc diffusion. Because the determination of an AMR phenotype requires bacterial growth, it is a slow method (16 to 24 hours or more), and it may not detect resistance if the gene is not being actively expressed at the time of testing. In this study, the broth-dilution method to determine minimum inhibitory concentrations (MICs) for bacteria was used. The MIC broth-dilution method is a fundamental quantitative tool in AMR testing. It provides data to clinicians and microbiologists for effective infection management and AMR surveillance. The MIC broth-dilution method determines the lowest antibiotic concentration that stops bacterial growth. It uses serial twofold dilutions in a liquid medium, followed by incubation and visual assessment. The bacteria can be classified differently depending on the concentration that stops growth.
- **Genotypic method:** An 'AMR genotype' describes the presence of acquired resistance mechanisms at the DNA level. Genotypic methods detect the presence of resistance genes or mutations using molecular techniques like the polymerase chain reaction or WGS. It is much faster than the phenotypic method (within hours) and is useful for surveillance or early detection of resistance. However, it only *predicts* resistance and does not confirm whether the resistance gene is functional, meaning some bacteria may appear resistant genotypically but

remain susceptible to antibiotics. Furthermore, the prediction of resistance can only be for known resistance mechanisms that are included in databases used to scan the genome. If a bacterium has a new resistance gene it may be phenotypically resistant but not considered genotypically resistant because the new gene has not been identified and entered into the database. In this study, we used short-read WGS to scan for known resistance mechanisms in bacteria. Additionally, if bacteria with AMR of interest are detected, then WGS data can assist in determining where the resistance came from and where else it has been detected. Answering these questions requires source tracking of specific population lineages, which requires the genotype of bacteria.

AMR among bacteria was primarily classified based on microbiological resistance but clinical resistance results have also been presented

Different terms are used to classify resistant bacteria when undertaking surveillance or when treating an infection (Kahlmeter and Turnidge 2022).

- **Microbiological resistance and ECOFFs:**
    - **Definition:** Microbiological resistance (also called 'non-wild type') describes bacteria that have acquired mutations or resistance mechanisms that differentiate them from the normal (or 'wild type') population, which have not acquired resistance. The ECOFF indicates the potential for resistance but does *not* predict whether an antibiotic will be successful for clinical treatment.
    - **Breakpoint used:** ECOFFs, set by EUCAST, separate microbiologically resistant bacteria from wild type populations based on large data sets of MIC distributions.
    - **When is an ECOFF most useful:** For surveillance and resistance mechanism studies.
    - **Interpretation of ECOFFs:**
        - Microbiologically susceptible (wild type, MIC ≤ ECOFF), no acquired resistance, bacteria expected to be susceptible.
        - Microbiologically resistant (non-wild type, MIC > ECOFF), may harbour resistance mechanisms but does *not* necessarily mean clinical treatment failure.
- **Clinical resistance and clinical breakpoints (CLSI and EUCAST):**
    - **Definition:** Clinical resistance means the antibiotic is unlikely to work effectively against the bacteria when treating a patient due to insufficient drug levels at the site of infection.
    - **Breakpoints used:** Clinical breakpoints (S, I, R) are defined by both EUCAST and CLSI, but these can differ slightly due to regional variations in treatment practices, pharmacokinetic/pharmacodynamic data, and clinical outcome interpretations. Clinical breakpoints are based on how the antibiotic behaves in the human body, how much can be given safely, and the MIC of the bacteria.
    - **When are clinical breakpoints most useful:** For guiding treatment decisions.



Antibiotics were included in the study based on their importance in Australia and what they can tell us

Lists ranking the importance of antibiotics have been developed to support antibiotic stewardship and guide responsible antibiotic use in both human and veterinary medicine (ASTAG 2018; WOAH 2024a; WHO 2024b). These classifications help preserve important antibiotics that are critical for treating serious infections.

The Australian Government is advised by the Australian Strategic and Technical Advisory Group on AMR (ASTAG) for classification of antibiotics into three categories – high, medium or low importance. The rating is based on the role of an antibiotic in treating serious infections in humans and the potential consequences if resistance emerges or increases (ASTAG 2018). The following ratings are used to inform regulators, prescribers and users about the significance of each antibiotic (ASTAG 2018):

- **High-importance antibiotics:** These are essential antibiotics for the treatment or prevention of infections in humans where there are few or no treatment alternatives for infections. These have also been termed ‘last-resort’ or ‘last-line’ antibiotics.
- **Medium-importance antibiotics:** These antibiotics have some alternatives available from different classes to treat or prevent human infections, but fewer than those rated as low importance.
- **Low-importance antibiotics:** There are several alternative antibiotics from different classes available to treat or prevent most human infections, even if resistance develops.

ASTAG (2018) state that regardless of rating it is important that all antibacterials are used appropriately regardless of their importance rating because, when resistance emerges to low and medium-importance agents, high-importance agents will be required more often.

The antibiotics included in this study were selected not only for their regional importance, but also for their usefulness in surveillance systems internationally to provide insight into how bacteria may develop resistance to entire classes of antibiotics.

Key definitions:

- **Antibiotic**: A specific drug within a class, with its own spectrum, dosage and clinical use. While antibiotics share similarities within their class, some may be more effective against certain bacteria than others.
- **Antibiotic class**: A group of antibiotics that have a common chemical structure, work in the same way (that is, have the same mechanism of action), target similar bacterial processes and may share cross-resistance mechanisms. Understanding antibiotic classes helps predict cross-resistance (for example, bacteria resistant to ciprofloxacin are often also resistant to other quinolones).
- **Cross-resistance:** When bacteria become resistant to multiple antibiotics within the same class due to shared resistance mechanisms (ASTAG 2018).
- **Co-selection of resistance:** When resistance genes to unrelated antibiotic classes are linked within the same bacterial strain, meaning that the use of one antibiotic can maintain resistance to others (ASTAG 2018).

What was considered:

- Across all four target bacteria, the recommendation of antibiotics for this study was driven by expert consultation, review of international guidance and consideration of Australia's specific context using the Australian importance ratings (ASTAG 2018).
- Priority was given to antibiotics rated as high-importance by ASTAG (2018) because they are essential for the treatment or prevention of infections in humans where there are few or no treatment alternatives for infections. This helps detect early signs of resistance in medicines that matter most.
- Antibiotics rated low-importance for human treatment by ASTAG (2018) were also included to ensures coverage across a range of antibiotic classes but also a One Health context. Many low-importance antibiotics can be common first treatment options for human treatment and also important for veterinary medicine. These are often classified by WOAH as Veterinary Critically Important Antimicrobial Agents (VCIAA). VCIAA refers to antibiotics that are essential for treating specific animal infections, particularly where few or no alternatives exist (WOAH 2024a).
- Some antibiotics were also selected specifically to identify particular resistance mechanisms.
- International guidance and methodologies were considered. Minor adjustments were made to suit Australian conditions, for example, ceftriaxone was used instead of cefotaxime because it is more commonly prescribed in Australia.
- Generally, at least one drug per antibiotic class: In AMR surveillance, it's not practical to test every antibiotic from each class (over 200 in 38 classes are listed by ASTAG). Instead, often at least one representative antibiotic is selected for key classes, based on scientific evidence. This is because resistance to one antibiotic often indicates resistance to others in the same class (cross-resistance). For example, if a bacterium is resistant to ampicillin, it is likely resistant to other penicillins as well. This approach helps identify broader resistance patterns and aligns with international recommendations to use class-representative antibiotics for monitoring (EFSA 2012 & EFSA et al., 2019).
- In this survey, MDmR is defined as microbiological resistance to 3 or more **antibiotic classes**. If bacteria showed microbiological resistance to a tested antibiotic, they were assumed to be resistant to the entire class for the purpose of determining class-based AMR patterns and MDmR.

A more detailed review of the scientific justification for the use of specific antibiotics, resistance mechanisms, potential for cross-resistance and co-selection can be found in the literature (ASTAG 2018; EFSA 2012; EFSA et al., 2019).

Terminology used in this report to describe AMR

This study determined MIC distributions for each antibiotic according to CLSI guidelines (CLSI 2015, 2024), based on the following breakpoints:

- epidemiological cut-off values (ECOFFs) (EUCAST 2020)
- EUCAST clinical breakpoints (EUCAST 2024)
- CLSI clinical breakpoints (CLSI 2016, 2024).

Terminology used in this report includes:

- **AMR:** Occurs when bacteria acquire resistance to antibiotic treatments to which they were previously susceptible.
- **Microbiological complete susceptibility**: All antibiotics tested were effective at stopping bacteria growing at concentrations at or below the specified cut off (that is, wild type MIC ≤ ECOFF) and bacteria are not expected to have acquired resistance mechanisms.
- **Microbiological susceptibility**: Bacteria *were not* able to grow in the presence of an antibiotic at concentrations at or less than the specified cut off (that is, wild type MIC ≤ ECOFF) and are not expected to have acquired resistance mechanisms.
- **Microbiological resistance**: Bacteria *were* able to grow in the presence of an antibiotic at concentrations above the specified cut off (that is, non-wild type MIC > ECOFF) and may harbour acquired resistance mechanisms; this does *not* necessarily mean clinical treatment failure.
- **Multidrug microbiological resistance (MDmR)**: Bacteria were classified as MDmR if they were microbiologically resistant to 3 or more antibiotic classes. As mentioned above, if an isolate showed microbiological resistance to at least one antibiotic in a class, it was considered resistant to that class.
- **Clinical resistance**: The MIC for bacteria were above the clinical breakpoint. Bacteria may harbour acquired resistance mechanisms and standard clinical treatment to treat infection is likely to fail.

Terms used to describe levels of resistance have been reported elsewhere (EFSA 2024) and have been used in this report. This report also refers to ‘rare’ as ‘not detected’:

- not detected/rare, < 0.1%
- very low: 0.1–1.0%
- low: > 1.0–10.0%
- moderate: > 10.0–20.0%
- high: > 20.0–50.0%
- very high: > 50.0–70.0%
- extremely high: > 70.0%.

Results and discussion

Sampling was undertaken as outlined in the Materials and Methods section. Briefly, raw retail meat samples, including beef, chicken and pork, were purchased from retailers across all Australian jurisdictions for analysis. To ensure national representativeness, the number of samples collected and tested in each jurisdiction was weighted by population, based on September 2020 population data (ABS 2020). Sampling was conducted across the greater metropolitan area of the capital city in each jurisdiction. Samples and bacterial testing were allocated evenly over time to ensure temporal balance and reduce potential biases related to seasonal or periodic variations in bacterial prevalence. To ensure sample independence and representativeness, collection areas were randomly allocated based on Local Government Areas or Public Health Regions within the greater metropolitan region of each jurisdiction's capital city (Figure 1).

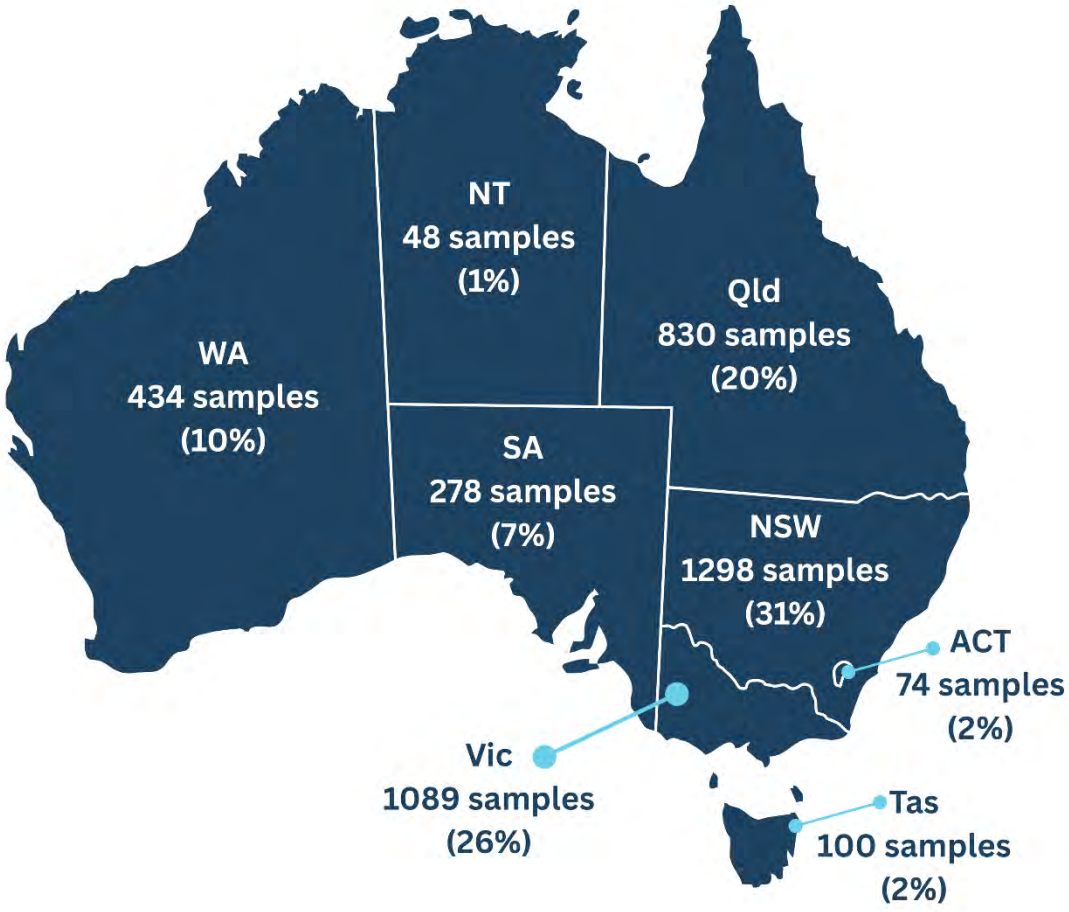


Figure 1: The total number of raw retail meat samples (4,151) collected from the 'greater metropolitan region' of each capital city in Australia.

A total of 4,151 raw retail meat samples were collected across all states and territories in Australia between September 2022 and July 2023 (Figure 1). This included 581 beef, 2,005 chicken meat, and 1,565 pork samples. The samples were collected from large supermarkets, small supermarkets, and independent butchers in proportions representative of Australian purchasing patterns (Table 1).

Figure 2 shows the number of meat samples tested and the number positive for specific bacteria. The number of individual meat samples tested for each bacterium varied depending on its expected prevalence. In cases where a bacterium was not tested for in a particular meat type, this was typically

due to its low expected prevalence. The decision not to test for certain bacteria in the current study was based on evaluation of expected prevalence, associated costs and proportional risk (see Materials and Methods). The prevalence results reflect isolates that were confirmed by MALDI-TOF MS by MU before undergoing AST.

The results for *E. coli*, *Enterococcus, Salmonella* and *Campylobacter* are presented and discussed in the following sections.

Table 1: Number and percentage of raw retail meat samples collected from large supermarkets, small supermarkets and independent butchers for each commodity.

	Beef		Chicken meat		Pork	
	Number of raw meat samples	%	Number of raw meat samples	%	Number of raw meat samples	%
Large supermarkets	325	56%	1205	60%	937	60%
Small supermarkets	121	21%	398	20%	309	20%
Independent butchers	135	23%	402	20%	319	20%
Total	**581**	**100%**	**2005**	**100%**	**1565**	**100%**

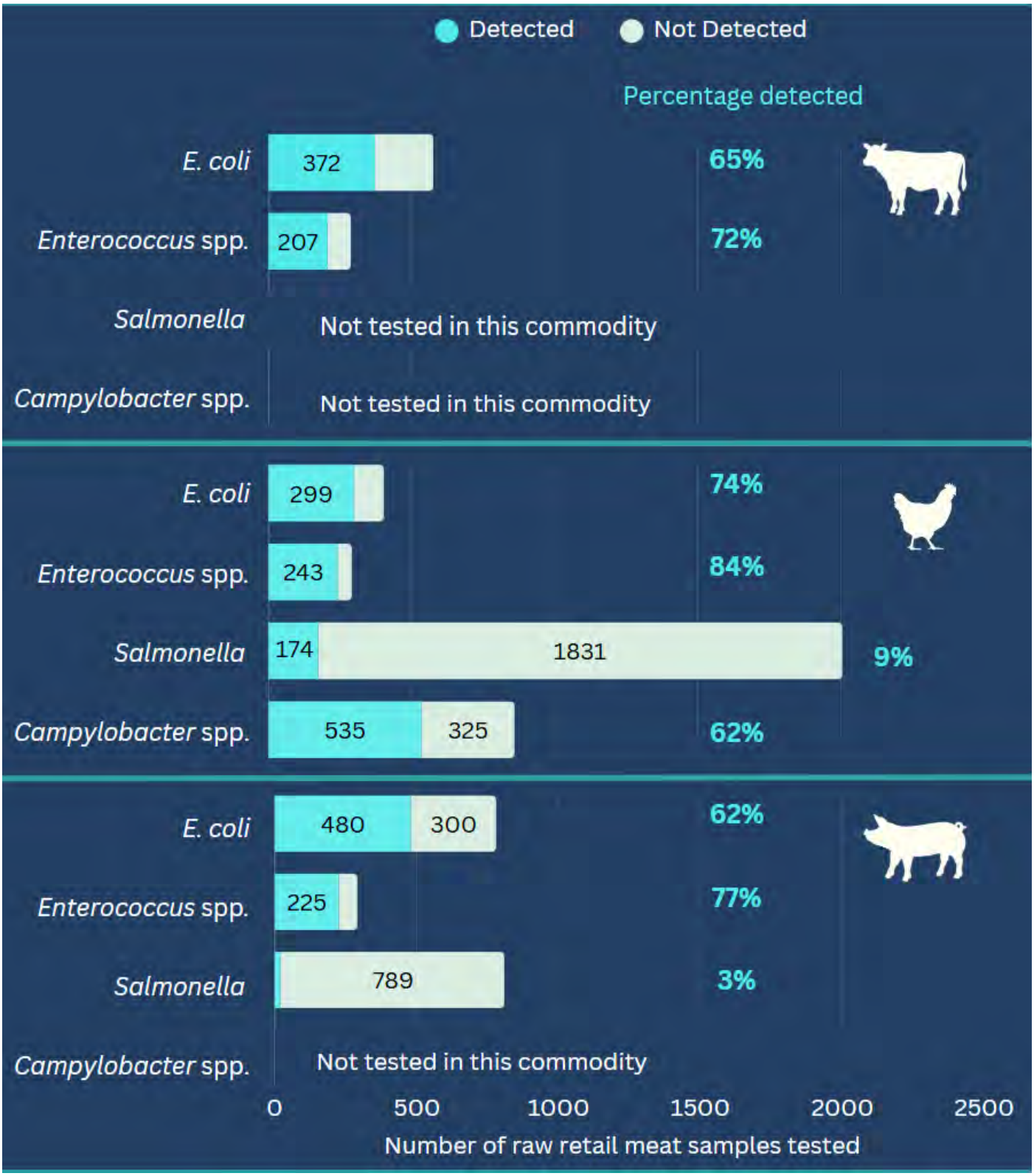


Figure 2: The number of raw retail samples of beef, chicken and pork meat tested for specific bacteria, and the number of positive detections. Bacterial identity was confirmed by MALDI-TOF MS prior to AST. Only *E. faecalis* and *E. faecium* isolates were included for *Enterococcus* spp.

Escherichia coli

Escherichia coli (*E. coli*) was included in AMR surveillance of raw retail beef, chicken meat and pork because:

- *E.coli* was expected to be sufficiently prevalent in raw meat to provide robust estimates of AMR within the population. Raw meat provides a direct snapshot of the bacterial populations, including resistant strains, that have entered the meat production chain (either from food animals, humans, or the environment) before being destroyed by effective cooking.
- *E. coli* are important microorganisms, especially from a One Health perspective, because they are widespread in nature, cause illness in humans and animals, and play a role in horizontal gene transfer that can spread AMR. *E. coli* is a diverse species, with most strains being harmless and natural inhabitants of human and animal guts. However, certain strains can cause various illnesses, primarily affecting the gastrointestinal tract (foodborne illness), urinary tract and other parts of the body. *E. coli* are considered common pathogens for which the impact of resistance is substantial for human health in both hospital and community settings in Australia (ACSQHC 2023).
- *E. coli* are indicators for resistance trends and provide insights into the potential dissemination of AMR genes, which could spread to other bacteria (WHO 2017). Monitoring resistant *E. coli* strains in humans, food, animals and the environment can enable early detection of emergence and spread of resistance (WHO 2021b).

Raw retail meat sampling and detection of target bacteria

Collection and testing for *E. coli* among 576 beef, 403 chicken, and 780 pork raw retail meat samples was undertaken over 40 weeks between 19 September 2022 and 30 July 2023. A total of 1,151 *E. coli* isolates were collected and their identities confirmed with MALDI-TOF MS prior to AST.

The prevalence of *E. coli* in raw retail beef, chicken meat and pork was 64.6% (372/576), 74.2% (299/403), and 61.5% (480/780), respectively. These were noted as reported higher than the reported prevalence of *E. coli* in the 2007 Australian AMR pilot study (beef 29.7% [121 isolates], chicken meat 69.0% [290 isolates], pork 18.1% [92 isolates]) (Barlow & Gobius 2008). The studies are not directly comparable and the different detection rates could be due to detection methods, differences in sampling and processing methods, differences in meat cuts targeted, or evolving agricultural and retail practices over time.

Representativeness of data and testing for antibiotic resistance

The broth MIC method was used to determine the antibiotic susceptibility profiles of 1,151 *E. coli* isolates (beef n = 372, chicken meat n = 299, pork n = 480) for 14 antibiotics covering 9 antibiotic classes (Table 2). There was a 4-fold increase in the number of isolates tested compared to the 2007 pilot study by Barlow and Gobius (2008) that was facilitated using a robotic antibiotic susceptibility platform (RASP) (Truswell et al. 2021).

More than the targeted 200 *E. coli* isolates were collected for each commodity. This enabled robust estimation of AMR prevalence in *E. coli* from raw retail beef, chicken meat and pork. The AMR data for *E. coli* are considered representative of populations present in raw beef, chicken meat and pork sold in retail outlets within the greater metropolitan areas of Australian capital cities, which collectively comprise over 60% of the national population. However, not all cuts available were tested, so the results may not reflect all raw meat products available in these areas.

The key results for *E. coli* are discussed below; results have been summarised by commodity (Key results summarised by commodity section below), and tables and figures presented in the *E. coli* tables and figures section below. Comprehensive MIC distributions based on ECOFF, EUCAST clinical and CLSI clinical breakpoints are provided in Supplementary tables 2, 3 and 4 for raw retail beef, chicken meat and pork, respectively.

Microbiological resistance to antibiotics

The rates of microbiological resistance and clinical resistance are presented in Figure 3 for each commodity.

High-importance antibiotics

Overall, not detected to low microbiological resistance to high-importance rated antibiotics was observed among *E. coli* isolates from raw retail beef, chicken meat and pork in this study. Microbiological resistance to amikacin, colistin and meropenem was not detected in any *E. coli* isolate in any raw retail meat in this study. Extended-spectrum β-lactamase (ESBL) and carbapenemase-producing *E. coli* are of public health interest due to their ability to resist critical antibiotics and the challenges this poses for managing human infections (Aljohni, Harun-Ur-Rashid & Selim 2025). The absence of meropenem (a carbapenem) resistance in this study indicates that *E. coli* isolates from these retail meats with acquired resistance is rare. Microbiological resistance to cephalosporins (cefotaxime and ceftazidime), which could indicate ESBL *E. coli* (WHO 2021b), was very low across all commodities; detected in only 6 isolates in total (beef 0.5% [2/372], chicken meat 0.7% [2/299], and pork 0.4% [2/480]). Microbiological resistance to ciprofloxacin was also low across all raw meats (beef 0.8% [3/372], chicken meat 2.7% [8/299], pork 2.5% [12/480]). Ciprofloxacin is useful for treatment of human *E. coli* infections that are resistant to other lower importance/first-line antibiotics (ACSQHC 2023).

These results indicate that microbiological resistance to the last-line antibiotics tested for in this study remains low overall for *E. coli* from raw retail beef, chicken meat and pork. This study was designed to detect low-levels of resistance to high-importance antibiotics and reinforces the importance of continuing to promote antibiotic stewardship across all sectors to maintain low levels of resistance. Ongoing surveillance is required to detect emergence of resistance occurring through the food chain. Nonselective culture methods were prioritised in this study, and the obtained results reflect the chosen methodology. Future studies applying selective media to specifically detect ESBL and carbapenemase-producing *E. coli* in these commodities would be of interest.

Australian human and livestock context: Resistance to amikacin, colistin, meropenem, cefotaxime, ceftazidime and ciprofloxacin were tested in *E. coli* because they are considered last-line antibiotics in Australia (ASTAG 2018) and serve as indicators for key resistance mechanisms. AMR 2021 rates among human clinical *E. coli* specimens from Australian national reporting were < 0.1% for meropenem, ~6–11% for cefotaxime/ceftriaxone, and ~11–14% for ciprofloxacin (ACSQHC 2023). As none of these high-importance antibiotics are registered for use in Australian food-producing animals (ASTAG 2018), there should be minimal selective pressure for resistance development in the animal microbiota. AMR in *E. coli* isolates from Australian livestock have recently been reported to range from not detected to < 4% (Abraham et al. 2019; ACMF 2022; Barlow et al. 2022; Kidsley et al. 2018; Laird et al. 2022; MLA 2020).

Medium-importance antibiotics

Microbiological resistance to gentamicin (beef – not detected [0/372], chicken meat 0.7% [2/299], pork 0.6% [3/480]) and amoxicillin-clavulanate (beef 1.3% [5/372], chicken meat 2.7% [8/299], pork 2.5% [12/480]) was seen at low rates overall across all raw retail meats indicating that the majority of *E. coli* have not acquired resistance in this study.

Australian human and livestock context: In Australia, the aminoglycoside gentamicin and the β-lactam amoxicillin-clavulanate are used as part of first-line treatment of *E. coli* infections in humans (ACSQHC 2023). AMR 2021 rates among *E. coli* human specimens were reported as ~6–8% (gentamicin) and ~12–17% (amoxicillin-clavulanate) (ACSQHC 2023). Gentamicin is not registered for use in food-producing animals but other aminoglycosides are, and amoxicillin-clavulanic acid only has limited registration for some treatment of cattle (ASTAG 2018). AMR rates among Australian livestock *E. coli* have recently been reported as not detected to ~1% for gentamicin and not detected to 10% for amoxicillin-clavulanate (Abraham et al. 2019; ACMF 2022; Barlow et al. 2022; Kidsley et al. 2018; Laird et al. 2022; MLA 2020).

Other antibiotics

The use of antibiotics, in both human and veterinary medicine, exerts selective pressure that contributes to the development and persistence of AMR in bacteria. Evidence from animal studies, and AMR monitoring generally, shows that antibiotics more frequently used in veterinary settings tend to exhibit higher levels of resistance in bacterial isolates from animals. However, the presence of resistant bacteria on meat does not necessarily indicate that the animal was the direct source. Other potential sources including humans, environmental contamination and animal feed may also contribute to the bacterial profile observed. Resistance levels are further influenced by factors such as bacterial species, historical use, production type and management practices.

In this study, microbiological resistance to ampicillin and trimethoprim was detected across the retail meats (pork isolates 40% and 20.6%, respectively, chicken meat 20.7% and 7%, respectively and beef 9.7% and 4.6%, respectively). Similar patterns were seen for microbiological resistance to tetracycline (pork isolates 37.7%, chicken meat isolates 18.1%, and beef isolates 12.6%). Microbiological resistance to chloramphenicol was observed in 16% of pork isolates and 2.7% of isolates for both chicken meat and beef. Microbiological resistance to florfenicol was observed in 1.3% of beef isolates, 0.7% of chicken meat isolates, and 6.5% of pork isolates.

Australian human and livestock context: The low-importance antibiotic ampicillin (approved for use in humans) is common in surveillance systems and can signal resistance to other low-importance penicillins such as amoxicillin (approved for use in food-producing animals). Antibiotic sulfamethoxazole is only approved for use in Australia in combination with trimethoprim to treat humans in a medium-importance combination antibiotic (ASTAG 2018). Trimethoprim is only registered for use in food producing animals in Australia in combination with some registered sulphonamide class combinations, which all rate as medium-importance (ASTAG 2018). Trimethoprim and sulfamethoxazole were tested separately in this study due to the different related genes. Ampicillin and trimethoprim are still considered important and common antibiotics for *E. coli* related infection treatment in humans (ACSQHC 2023). AMR rates of ~41–49% for ampicillin, ~22% for trimethoprim, and ~27 for trimethoprim–sulfamethoxazole among *E. coli* human clinical specimens were reported in 2021 (ACSQHC 2023). These antibiotics are classified VCIAA by WOAH due to their wide range of applications, the nature of the diseases these antibiotics are able to treat, and a lack of suitable alternatives for animals (WOAH 2024a). Varying AMR rates have been reported, depending on the livestock animal, for ampicillin (~4–60%) and this has been attributed to differences in historical usage, husbandry requirements, types of disease to be treated, and availability of alternatives (Abraham et al. 2019; ACMF 2022; Barlow et al. 2022; Kidsley et al. 2018; Laird et al. 2022; MLA 2020). This has also been the case for AMR to the tetracycline class (~15–68%) and phenicol class (not detected to ~47%) found in *E. coli* isolated from livestock (Abraham et al. 2019; ACMF 2022; Barlow et al. 2022; Kidsley et al. 2018; Laird et al. 2022; MLA 2020), with antibiotics in these classes registered for use in humans or animals (ASTAG 2018). Trimethoprim and sulfamethoxazole rates alone were rarely reported, while reporting of trimethoprim-sulfamethoxazole is more frequent, with ranges for all three differing by animal from ~2–34%.

Historical comparison with 2007 results

Trend analysis over time (for example, increasing resistance) was not possible in this study, as different methods and sampling approaches from those in the survey by Barlow and Gobius (2008) were used. These changes were made to align with current international guidance. However, a cautious comparison of similarities and differences is still possible and may offer useful insights. To provide some context, the MIC data from Barlow and Gobius (2008) were reanalysed for antibiotics tested at sufficient concentrations. This was done using the current ECOFFs and the same method for calculating CIs as in the current study. For most antibiotics tested, the reported prevalence of resistant isolates appeared similar for the two studies (Figure 6). The only notable exceptions[1] were lower reported levels of tetracycline and ampicillin resistance in chicken-meat-derived *E. coli* in the current study (Figure 6). These observations should be interpreted cautiously given the methodological differences.

Microbiological complete susceptibility and multidrug resistance

Microbiological complete susceptibility indicates that a bacterium should not have acquired resistance mechanisms to any of the antibiotics tested. In this study, the rates of complete susceptibility among *E. coli* were high overall across the raw retail meats (beef 80.9% [301/373] chicken meat 65.6% [196/299], and pork 44.6% [214/480]) (Figure 4).

This means that the majority of *E. coli* isolates among raw retail beef, chicken meat and pork have not acquired resistance to any of the antibiotics tested. Source attribution was beyond the scope of this study, and additional studies are required to identify the sources and likely pressures influencing the different rates of AMR detected in food associated isolates.

MDmR refers to isolates with microbiological resistance to 3 or more classes of antibiotics, indicating that infections from these isolates may be harder to treat, especially when high-importance rated antibiotics are present in the resistance pattern. In this study, MDmR (Figure 4) was observed at low rates in beef (4%; 15/372) and chicken meat (8.1%; 24/299) and higher in pork (25.4%; 122/480). Notably, MDmR involving high-importance antibiotics was low across all commodities (< 3%).

Australian context: Microbiological complete susceptibility and resistance to multiple antibiotics have been suggested as key summary indicators for AMR (ECDC, BIOHAZ & CVMP 2017). However, data from humans and livestock in Australia are not included here for context due to methodological differences and lack of harmonisation in antibiotic panels, which limit comparability. This is also true for the survey by Barlow and Gobius (2008). MDmR was defined in the current study as microbiological resistance to three or more antibiotic classes, with resistance to a single antibiotic assumed to represent resistance to the entire class. In contrast, Barlow and Gobius (2008) reported resistance phenotypes based on combinations of individual antibiotics without grouping them by class. To improve future surveillance, studies should harmonize antibiotic panels and apply consistent class-based definitions of MDmR. The values reported in Barlow and Gobius (2008) for *E. coli* among retail meats were: in beef 81% (81/100) showed no resistance to any antibiotic tested and 5% (5/100) were resistant to three or more antibiotics; in chicken, 35% (35/100) showed no resistance and 22% (22/100) were resistant to three or more antibiotics; in pork, 46% (42/92) had no resistance and 22% (20/92) were resistant to three or more antibiotics.

[1] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

Known genetic resistance determinants

This report focused its genetic analysis on isolates exhibiting resistance to high-importance antibiotics and MDmR. These isolates were subject to short-read WGS to detect known resistance determinants. More comprehensive analyses are planned for future publications.

A total of 172 *E. coli* isolates were selected for WGS to detect known resistance genes based on being either:

- microbiologically resistant to high-importance antibiotics, and/or
- MDmR (microbiologically resistant to 3 or more antibiotic classes).

Genotypes of all 172 *E. coli* isolates are presented in Supplementary tables 5, 6 and 7. A summary of the sequence types for each commodity is provided in Figure 5. Overall, 73 different multilocus sequence types (STs) were identified. For beef, 12 different STs were identified with ST58 having the highest proportion (5/17). Chicken meat had 16 different STs identified, with the most frequently detected being ST354 (6/32), ST58 (5/32), and ST16353 (3/32). For pork, 59 different STs were identified and the highest proportions were for ST10 (19/123), ST58 (13/123), ST101 (9/123).

Twenty-two out of 23 *E. coli* isolates that were resistant to the quinolone ciprofloxacin had genes associated with quinolone resistance detected through WGS. ST354 was the most prevalent ST associated with ciprofloxacin resistance. ST354 and ST744 are livestock-associated quinolone resistant STs (Lee 2021; Laird et al. 2022; Truswell et al. 2023; Abraham et al. 2015). ST354 has also been detected among various environments as well as in human and animal hosts, with some exhibiting notable resistance mechanisms (Manges et al. 2019; Guo et al. 2015).

Of the 6 isolates (2 from beef, 2 from chicken meat and 2 from pork) that were resistant to cephalosporins only one had genes known to confer the resistance detected and 5/6 were ST58. ST58 is an emerging multidrug-resistant sequence type and uropathogen found among humans, animals (for example, poultry, cattle, wildlife), and the environment (Reid et al. 2022; Li et al. 2023; Wyrsch et al. 2024). While the origin of bacteria was not determined in this study, foodborne transmission of uropathogens is possible, though direct evidence for this is lacking (George & Manges 2010; Vincent et al. 2010; Nordstrom, Liu, & Price 2013), which highlights the importance of not only adequate food preparation and cooking, but also of proper primary production, processing and handling of raw meat by primary producers through to consumers.

Of the 143 isolates that were MDmR, all but 4 isolates had genetic elements associated with the resistance that was phenotypically observed. ST58 and ST10 were the most prevalent in this group. ST10 is an international ST found in various settings, including humans, animals and the environment (Manges et al. 2019; Silva et al. 2023). It has been identified in food-producing animals such as pigs, poultry and cattle. ST10 strains often exhibit resistance to multiple antibiotics, including tetracycline and ampicillin (Silva et al. 2023; Lee et al. 2021).

There were a small number of isolates with a phenotype of resistance to third-generation cephalosporins and quinolones, without an associated genotype. Many of the isolates without the associated genes had a phenotype just above the ECOFF and the resultant phenotype might be an instance of ‘MIC drift’ rather than true microbiological resistance (Abraham et al. 2019).

Genetic elements associated with quinolone resistance

Of the 22 quinolone microbiological resistant isolates, 11 isolates (1 beef and 10 pork isolates) had the *qnrS, qnrS1 or qnrS13* gene known to confer resistance to quinolones. Nine of these isolates also had genes for efflux pumps known to reduce susceptibility to quinolones: *acrF* and *mtdM*. The other 2

qnrS1 positive isolates also encoded the *acrF* pump but not the *mtdM* pump and had a quinolone associated point mutation: *parE*_I529L.

Ten isolates had one or more point mutations in locations associated with quinolone resistance. This included all 8 chicken meat derived isolates, and 2 pork derived isolates. These isolates also harboured the *acrF* and *mtdM* efflux pump genes.

One isolate derived from beef with quinolone microbiological resistance only had the *acrF* and *mtdM* efflux pumps with no identified quinolone genes or point mutations present.

Seven ST354 isolates (6 chicken meat and 1 pork isolate) all had D87N and S83L mutations in the *gyrA* gene and E48G and S80I mutations in the *parC* gene, a combination that was not seen in any other isolates.

Genetic elements associated with cephalosporin resistance

There were 6 isolates with microbiological resistance for third-generation cephalosporins. A single isolate, which was also microbiologically resistant for β-lactams, folate pathway inhibitors and tetracyclines, had a $bla_{\text{CTX-M-1}}$ gene known to confer resistance to cephalosporins and an *acrF* gene encoding an efflux pump associated with cephalosporin resistance. This isolate was the only one in the collection that also had clinical resistance to third-generation cephalosporins, but only to cefotaxime (not ceftazidime). The remaining 5 isolates encoded efflux pumps (*acrF*) associated with cephalosporin resistance, but other cephalosporin resistance genes were not identified.

In summary, the genetic analysis of *E. coli* isolates that were MDmR or resistant to high-importance rated antibiotics showed a wide variety of bacterial types across different meat products. Two sequence types – ST58 and ST10 – were commonly found among the MDmR samples. Most bacteria resistant to ciprofloxacin carried known resistance genes, particularly those in the ST354 group. Although this study didn't investigate direct links to human infections, the presence of bacteria types known to cause urinary tract infections, and those found internationally, emphasises the importance of robust food safety practices, future source attribution and continued genomic surveillance.

Key results summarised by commodity

The key results presented above are collated here by commodity for interested readers.

Beef

The rates of microbiological resistance among 372 *E. coli* isolates from raw retail beef (Figure 3) were as follows:

- High-importance antibiotics: resistance was rare to very low (amikacin, colistin and meropenem not detected in any isolate [< 0.1%, rare]; cefotaxime and ceftazidime very low [0.5%]; and resistance very low for ciprofloxacin [0.8%]).
- Medium-importance antibiotics: resistance ranged from rare to low (gentamicin not detected [< 0.1%, rare], and amoxicillin/clavulanate low [1.3%]).
- Low-importance antibiotics: resistance ranged from low to moderate (chloramphenicol [2.7%], florfenicol [1.3%] and ampicillin [9.7%] all low; tetracycline moderate [12.6%]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (Figure 6) the only notable results[2] were resistance to ampicillin and

[2] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

tetracycline was lower in the current dataset. These observations should be interpreted cautiously given methodological differences.

No microbiological resistance was seen in an extremely high proportion of *E. coli* isolates (301/372 isolates, 80.9%) (Figure 4).

MDmR (microbiological resistance to 3 or more antibiotic classes) was low (15 isolates, 4.2%). This included a very low number of MDmR isolates resistant to high-importance antibiotics (3 isolates, 0.8%) (Figure 4).

Fourteen unique patterns of resistance for up to 5 of the 9 antibiotic classes were observed among isolates. The 4 most prevalent antibiotic-class resistance patterns in beef *E. coli* isolates were: tetracyclines only (22 isolates, 5.9%); folate pathway inhibitors only (9 isolates, 2.4%); β-lactams only (7 isolates, 1.9%); and β-lactams + tetracyclines (8 isolates, 2.2%).

Chicken meat

The rates of microbiological resistance among 299 *E. coli* isolates from retail chicken meat (Figure 3) were as follows:

- High-importance antibiotics: resistance ranged from rare to low (amikacin, colistin and meropenem not detected [< 0.1%, rare]; cefotaxime and ceftazidime very low [0.7%], and ciprofloxacin low [2.7%]).
- Medium-importance antibiotics: resistance was very low to low (gentamicin very low [0.7%], and amoxicillin/clavulanate low [2.7%]).
- Low-importance antibiotics: resistance ranged from very low to high (florfenicol very low [0.7%], chloramphenicol low [2.7%], trimethoprim low [7%], tetracycline moderate [18.1%], and ampicillin high [20.7%]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (Figure 6) there were no notably different results[3].

The rate of microbiological complete susceptibility among chicken meat *E. coli* isolates to all antibiotics tested was very high (196/299 isolates, 65.6%) (Table 2, Figure 4).

MDmR was low, present in 22 isolates (7.4%), and only 2 of these isolates (0.7%) were also resistant to high-importance antibiotics (Figure 4).

Seventeen unique patterns of resistance were observed among isolates involving up to 4 of the 9 antibiotic classes. The 4 most prevalent antibiotic-class resistance patterns among chicken meat *E. coli* isolates were: tetracyclines only (18 isolates, 6.0%); β-lactams + tetracyclines (16 isolates, 5.4%); folate pathway inhibitors only (14 isolates, 4.7%); and β-lactams + folate pathway inhibitors + tetracyclines (13 isolates, 4.3%).

Pork

The rates of microbiological resistance among 480 *E. coli* isolates from raw retail pork (Figure 3) were as follows:

- High-importance antibiotics: resistance ranged from rare to low (amikacin, colistin and meropenem not detected [< 0.1%, rare]; cefotaxime and ceftazidime very low [0.4% and 0.2% respectively], and ciprofloxacin low [2.5%]).

[3] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

- Medium-importance antibiotics: resistance ranged from very low to low (gentamicin very low [0.6%], and amoxicillin/clavulanate low [2.5%]).
- Low-importance antibiotics: resistance ranged from low to high (florfenicol low [6.5%]; chloramphenicol moderate [16%]; and trimethoprim, tetracycline and ampicillin high [20.6%, 37.7%, and 40% respectively]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (Figure 6) there were no notably different results [4].

The rate of microbiological complete susceptibility for pork *E. coli* isolates to all antibiotics tested was high (214/480 isolates, 44.6%) (Table 2, Figure 4).

MDmR was high (122 isolates, 25.4%); however, MDmR with resistance to high-importance antibiotics was low (13 isolates, 2.7%) (Figure 4).

Twenty-two unique patterns of resistance to up to 5 of the 9 antibiotic classes were observed. The 4 most prevalent antibiotic-class resistance patterns in pork *E. coli* isolates were: MDmR resistance pattern β-lactams + folate pathway inhibitors + phenicols + tetracyclines (45 isolates, 9.4%); MDmR resistance pattern β-lactams + folate pathway inhibitors + tetracyclines (41 isolates, 8.5%); β-lactams + tetracyclines (37 isolates, 7.7%); and tetracyclines only (25 isolates, 5.2%).

Key messages

The rare (not detected) to low microbiological resistance rates to high-importance rated antibiotics and high complete susceptibility levels among indicator *E. coli* isolated from raw retail beef, chicken and pork meat indicate a reduced risk of foodborne transmission of bacteria that may become involved in resistant human infections or spread resistance to other bacteria.

Rates of MDmR were generally low, but where higher rates were seen, the majority of MDmR were to low-importance antibiotics for human treatment, suggesting that effective alternatives from other classes remain available. Overall, these results show support for the effectiveness of Australian antibiotic stewardship programs and prescribing guidelines. Resistance levels to antibiotics that are important in veterinary medicine but considered low-importance for humans (although can be common first-line treatments) remain consistent with expectations based on findings from Barlow and Gobius (2008). While the results do not indicate recent increases, they reinforce the interconnectedness of animal health, human health, food safety and food security. The absence of fully comparable historical data limits our ability to confidently assess long-term trends. Establishing coordinated surveillance systems that conduct repeated surveys would enable more accurate detection of both improvements in the form of reduced resistance levels and the emergence of new resistance risks.

The genotypic identification provides a valuable database to be leveraged in future studies and confirms that many strains of the *E. coli* that were MDmR or resistant to high-importance antibiotics in this study are STs that are internationally distributed.

Because this study did not investigate the source of the *E. coli* detected among the retail meats (that is, whether it is from animal, human, or environmental origin), this is an area that would benefit from future cross-sector studies to help robustly identify sources and potential AMR pressures.

[4] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

Effective, sustained, and cooperative efforts are needed not only among the One Health sectors but also among all stakeholders in the farm-to-fork pathway to ensure food safety practices are implemented through the complete chain and appropriate antibiotic use is practiced.

E. coli tables and figures

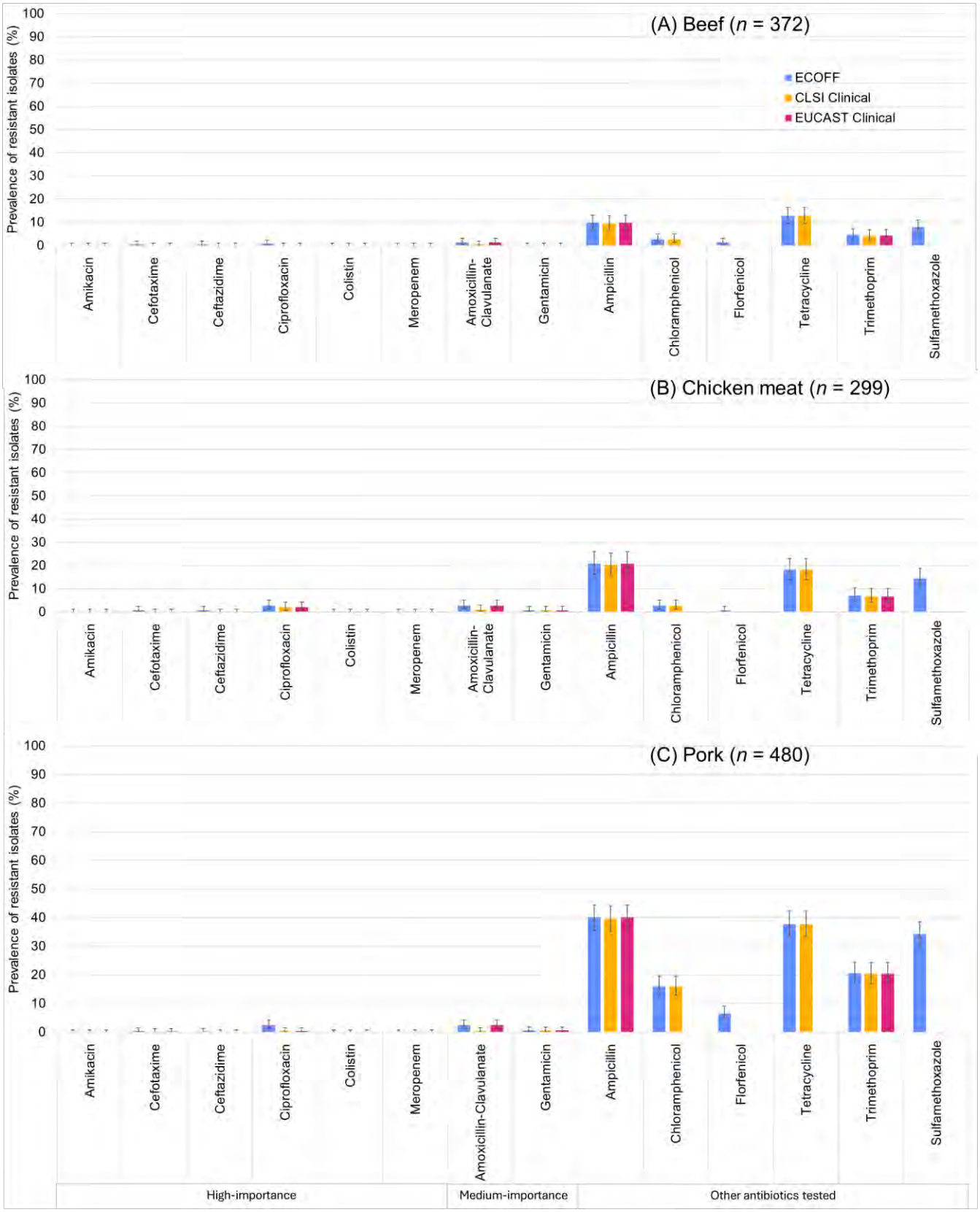


Figure 3: The rates of resistance to high-importance rated (ASTAG 2018), medium-importance rated (ASTAG 2018), and other antibiotics among *E. coli* isolates from raw retail meat. A) beef (n = 372 isolates), B) chicken meat (n = 299 isolates), and C) pork (n = 480 isolates). Prevalence of microbiological resistance based on ECOFF (blue), and clinical resistance based on CLSI (yellow) and EUCAST (pink) clinical breakpoints. 95% CIs shown as error bars.

Table 2: Prevalence of microbiological resistance patterns for different antibiotic classes among *E. coli* isolated from raw retail beef, chicken meat and pork.

Beef (n = 372)			Chicken meat (n = 299)			Pork (n = 480)		
Pattern (Phenotype)	*n*	Prevalence (%)	Pattern (Phenotype)	*n*	Prevalence (%)	Pattern (Phenotype)	*n*	Prevalence (%)
0: nil	301	80.7	0: nil	196	65.6	0: nil	214	44.6
1: bla	7	1.9	1: bla	11	3.7	1: bla	23	4.8
1: fpi	9	2.4	1: fpi	14	4.7	1: fpi	22	4.6
1: tet	22	5.9	1: qui	3	1	1: tet	25	5.2
2: bla_c3g	1	0.3	1: tet	18	6	2: bla_c3g	1	0.2
2: bla_fpi	6	1.6	2: bla_c3g	1	0.3	2: bla_fpi	22	4.6
2: bla_tet	8	2.2	2: bla_fpi	10	3.3	2: bla_tet	37	7.7
2: fpi_qui	1	0.3	2: bla_qui	3	1	2: fpi_phe	6	1.3
2: fpi_tet	2	0.5	2: bla_tet	16	5.4	2: fpi_tet	7	1.5
3: bla_c3g_tet	1	0.3	2: fpi_qui	1	0.3	2: phe_tet	1	0.2
3: bla_fpi_tet	3	0.8	2: fpi_tet	2	0.7	3: bla_fpi_phe	10	2.1
3: bla_qui_tet	1	0.3	3: bla_c3g_tet	1	0.3	3: bla_fpi_tet	41	8.5
3: fpi_phe_tet	1	0.3	3: bla_fpi_phe	6	2	3: bla_phe_tet	1	0.2
4: bla_fpi_phe_tet	8	2.2	3: bla_fpi_tet	13	4.3	3: bla_qui_tet	7	1.5
5: bla_fpi_phe_qui_tet	1	0.3	3: fpi_phe_tet	1	0.3	3: fpi_phe_tet	10	2.1
			4: ami_bla_fpi_tet	1	0.3	4: ami_bla_fpi_phe	1	0.2
			4: ami_fpi_qui_tet	1	0.3	4: ami_fpi_qui_tet	1	0.2
			4: bla_fpi_phe_tet	1	0.3	4: bla_c3g_fpi_tet	1	0.2
						4: bla_fpi_phe_tet	45	9.4
						4: bla_fpi_qui_tet	2	0.4
						4: fpi_phe_qui_tet	1	0.2
						5: ami_bla_fpi_phe_tet	1	0.2
						5: bla_fpi_phe_qui_tet	1	0.2

Phenotype indicates the number of antibiotic classes with resistance present and each class. Abbreviations: n – number of isolates with associated phenotype, bla – B-lactams, c3g – third-generation cephalosporins, fpi – folate pathway inhibitors, phe – phenicols, tet – tetracyclines, qui – quinolones, ami – aminoglycosides. Phenotypes are based on ECOFFs.

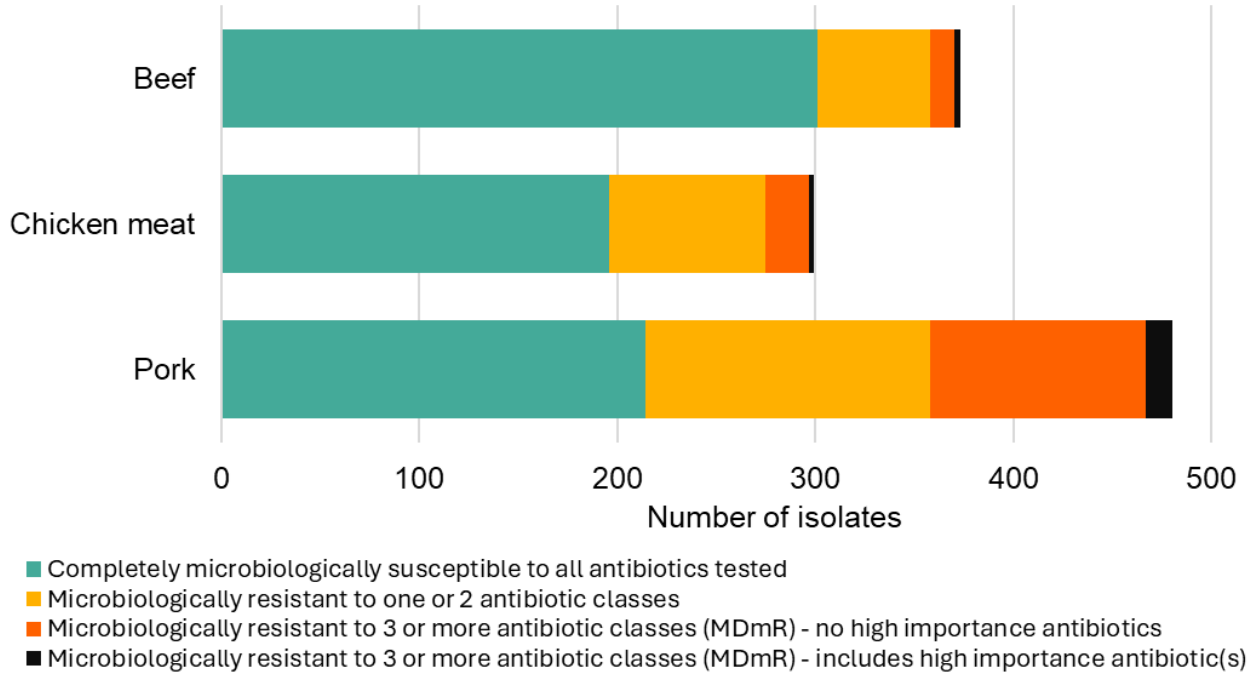


Figure 4: The number of isolates with complete microbiological susceptibility and MDmR for *E. coli* isolated from raw retail beef (n = 372 isolates), chicken meat (n = 299 isolates) and pork (n = 480 isolates). Completely microbiologically susceptible (green), resistant to one or 2 classes of antibiotics tested (yellow), resistant to 3 or more antibiotic classes tested, not including high-importance antibiotics (red), and resistant to 3 or more antibiotic classes tested including high-importance antibiotics (black).

Figure 5: The diversity of different STs determined by WGS among *E. coli* isolated from raw retail beef that were either microbiologically resistant to at least one high-importance rated antibiotic and/or were MDmR. The STs with the highest proportions have been labelled.

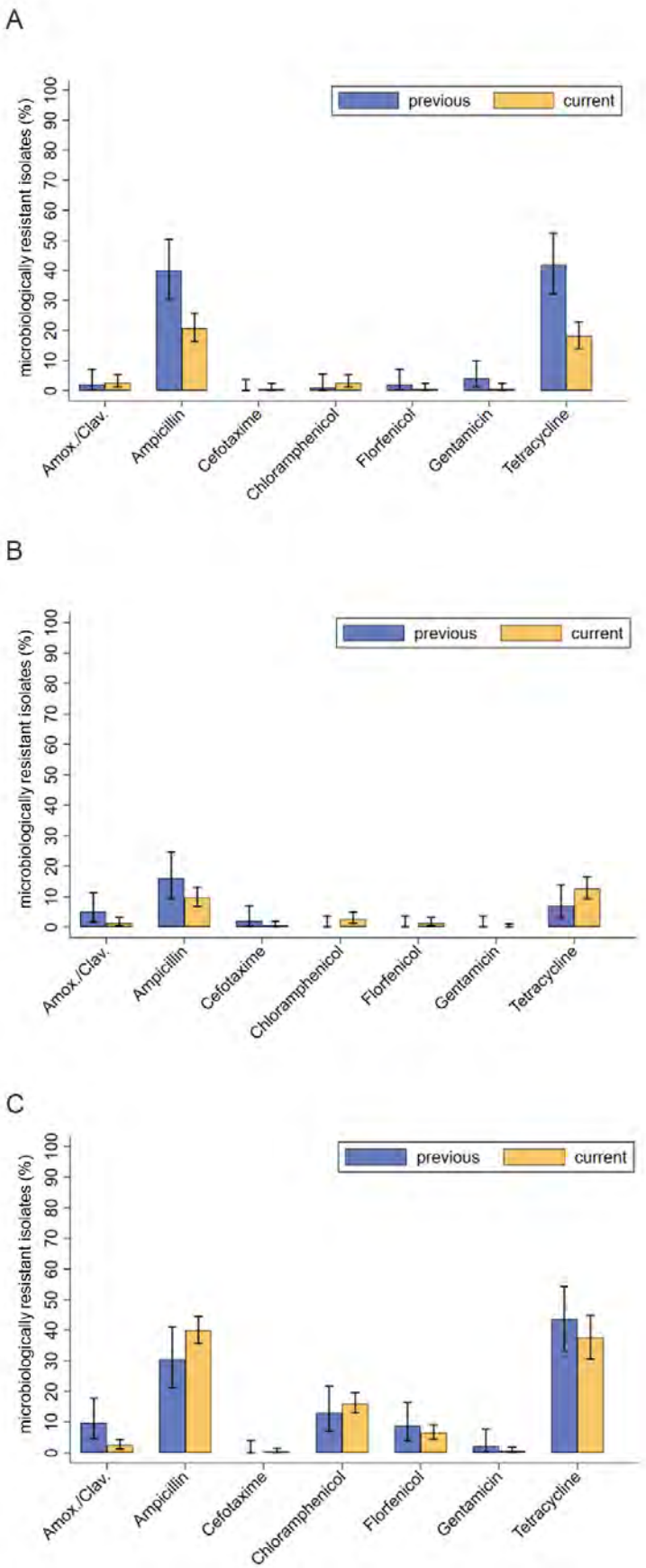


Figure 6: Reported rates of microbiological resistance among *E. coli* from the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (blue, previous) and this study (yellow, current) from A) beef isolates (previous n = 100, current n = 372), B) chicken meat isolates (previous n = 100, current n = 299), and C) pork isolates (previous n = 92, current n = 480). 2007 results were reanalysed against the same ECOFFs used in the current study and 95% CIs for each data set shown as error bars (presented for information only and no statistical comparison was undertaken).

Enterococcus spp.

Enterococcus spp. were included as target microorganisms in AMR surveillance of raw retail beef, chicken meat and pork because:

- *Enterococcus* was expected to be sufficiently prevalent in raw meat to provide robust estimates of AMR within the population. Raw meat provides a direct snapshot of the bacterial populations, including resistant strains, that have entered the meat production chain (either from food animals, humans, or the environment) before being destroyed by effective cooking.
- *Enterococcus* species, especially *E. faecium* and *E. faecalis*, are widespread in nature, natural inhabitants of the human and animal gut, opportunistic pathogens (primarily involved in urinary tract infections as well as infections of other body parts, but rarely a cause of foodborne illness), and considered reservoirs of resistance genes. In Australia, *Enterococcus* spp. are considered opportunistic pathogens for which the impact of AMR is substantial for human health in both hospital and community settings (ACSQHC 2023).
- *Enterococcus* spp. are key indicators of AMR trends and can disseminate resistance to other bacteria. Monitoring these bacteria provides insights into AMR dynamics, supporting global One Health strategies to combat the spread of resistance and protect public health (WHO 2017).

Raw retail meat sampling and detection of target bacteria

Collection and testing of 288 beef, 291 chicken, and 291 pork raw retail meat samples for *Enterococcus* occurred over 40 weeks between 19 September 2022 and 30 July 2023. Sampling and testing were designed to ensure geographical representativeness and even distribution through time.

The species identity of 695 *Enterococcus* isolates from the commodities were confirmed by MALDI-TOF MS. In the method applied, up to 10 presumptive colonies of *Enterococcus* were identified until an *E. faecium* or *E. faecalis* isolate was confirmed. If by the 10th isolate no *E. faecium* or *E. faecalis* were identified, the species of the 10th isolate was confirmed and recorded.

Table 3 summarises the number of species detected among each commodity. Because *E. faecalis* and *E. faecium* are of the most public health significance, these isolates were taken forward for AST and the results thereof are presented and discussed below.

Table 3: Number and proportion of *Enterococcus* spp. detected among each commodity.

	Beef (N = 288)	Chicken meat (N = 291)	Pork (N = 291)
Enterococcus faecalis	154 (53.5%)	189 (64.9)	198 (68.0%)
Enterococcus faecium	53 (18.4%)	54 (18.6)	27 (9.3%)
Enterococcus gallinarum	0	2 (0.7%)	0
Enterococcus hirae	15 (5.2%)	0	3 (1.0%)
Total	**222 (77.1%)**	**245 (84.2%)**	**228 (78.4%)**

The reported rate of detection for *E. faecalis* was lower in the current study than that of Barlow and Gobius (2008) in the 2007 Australian AMR pilot study (beef: 53.5% current, 87.9% previous; chicken meat: 64.9% current, 92.0% previous; pork: 68.0% current, 83.1% previous). Barlow and Gobius (2008) did not detect *E. faecium* in beef, pork or chicken meat. In contrast, *E. faecium* was detected first in 9.3–18.6% of samples in this study, depending on the commodity.

Representativeness of data and testing for antibiotic resistance

The broth MIC method was used to determine the antibiotic susceptibility profiles of:

- 541 *E. faecalis* isolates (beef n = 154, chicken meat n = 189, pork samples n = 198) for 12 antibiotics representing 10 antibiotic classes (Table 12)
- 134 *E. faecium* isolates (beef n = 53, chicken meat n = 54, pork n = 27) for 13 antibiotics representing 11 antibiotic classes (Table 12).

The method used was designed to detect a total of 200 *Enterococcus* isolates per commodity, rather than to ensure the detection of at least 200 isolates of each species (i.e., *E. faecalis* and *E. faecium*) per commodity. Nevertheless, AMR results are reported separately for *E. faecalis* and *E. faecium* due to their distinct relevance to human AMR infections. As fewer than 55 *E. faecium* isolates were recovered from each commodity, these results should be interpreted with caution. The limited sample size results in wider confidence intervals and less precise estimates (see Sample sizes section). Due to these limitations, only the number of resistant z isolates were reported.

More than 150 *E. faecalis* isolates were collected for each commodity. This enabled sufficiently robust estimation of AMR prevalence in *E. faecalis* from raw retail beef, chicken meat and pork. The AMR data for *E. faecalis* are considered representative of populations present in raw beef, chicken meat and pork sold in retail outlets within the greater metropolitan areas of Australian capital cities, which collectively comprise over 60% of the national population. However, not all cuts available were tested so the results may not reflect all raw meat products available in these areas.

The key results for *Enterococcus* spp. are discussed below. Results have been summarised by commodity (Key results summarised by commodity section below), and tables and figures presented in the E. faecalis tables and figures and E. faecium tables and figures sections below. A comprehensive distribution of MICs based on ECOFF, EUCAST, and CLSI clinical breakpoints is provided in Supplementary Tables 8, 9 and 10 for *E. faecalis* and Supplementary Tables 12, 13 and 14 for *E. faecium* isolates for beef, chicken meat and pork, respectively. The rates of microbiological resistance and clinical resistance are presented in Figure 7 for *E. faecalis* and the number of resistant isolates in Figure 11 for *E. faecium* for each commodity. Table 4 and Table 5 present the resistance patterns based on antibiotic class for each commodity for *E. faecalis* and *E. faecium*, respectively.

Microbiological resistance to antibiotics

High-importance antibiotics

Overall, microbiological resistance to high-importance antibiotics among *E. faecalis* and *E. faecium* isolates from all raw retail meat commodities was either not detected or detected at low levels in this study:

- Microbiological resistance was not detected in any isolate for teicoplanin and vancomycin across all commodities.

- For *E. faecalis*, resistance was not detected to low levels across all raw retail meats tested for ciprofloxacin (not detected in any *E. faecalis* isolate), nitrofurantoin (beef 0.6%, chicken meat 0.5%, pork not detected), linezolid (beef not detected, chicken meat 1.1%, pork 3.0%) and daptomycin (beef 0.6%, chicken meat 0.5%, pork 1.0%).
- For *E. faecium*, microbiological resistance for linezolid was not detected in any *E. faecium* isolate, ciprofloxacin (beef not detected, chicken meat and pork 1 isolate each), daptomycin (beef 1 isolate, chicken meat not detected, pork not detected), and nitrofurantoin (beef 4 isolates, chicken meat 3 isolates, pork 1 isolate) were observed. AMR detection among *E. faecium* was the only instance in this study where detections of clinical resistance (CLSI for ciprofloxacin, daptomycin, and nitrofurantoin) were notably higher than the microbiological resistance based on the ECOFF. This likely reflects that CLSI and EUCAST are separate agencies and can apply different breakpoint criteria and interpretive standards. WGS provides useful information in these scenarios by identifying if known underlying resistance mechanisms are present. All *E. faecium* isolates with MICs above the CLSI clinical breakpoint for these antibiotics were analysed using AMRFinder+ (data not shown). No ciprofloxacin or nitrofurantoin associated resistance genes or mutations were detected in any isolates. For daptomycin, 3 isolates (MIC 8 µg/mL) carried a LiaR_E57K point mutation, while the remaining 3 had no daptomycin-associated genes or mutations identified.
- Virginiamycin (only tested for in *E. faecium* because *E. faecalis* is intrinsically resistant) is registered for use in cattle, chickens and pigs but is not registered for use in humans (ASTAG 2018) and not detected to low levels of microbiological resistance were detected in this study (beef 2 isolates, chicken meat 2 isolates, pork not detected).

These results suggest that the vast majority of *Enterococcus* isolates in this study have not acquired resistance to high-importance rated antibiotics that could make treatment of infections harder or that could spread to other bacteria.

Australian human and livestock context: Ciprofloxacin, daptomycin, linezolid, nitrofurantoin, teicoplanin and vancomycin were tested against *Enterococcus* spp. because they are last-line antibiotics in Australia (ASTAG 2018). In Australia serious human *Enterococcus* spp. infections, and patients allergic to penicillins, are treated with vancomycin (ACSQHC 2023). Vancomycin-resistant *Enterococcus* spp. are of public health significance as they are harder to treat and can require treatment with last-line antibiotic agents, including teicoplanin or daptomycin (ACSQHC 2023). AMR for key antibiotics in the treatment of human enterococcal infections has been reported in Australia and rates are generally higher in *E. faecium*. AMR among *E. faecalis* and *E. faecium* human specimens in 2021 respectively were ~7% and 95% for ciprofloxacin; 0.3–0.5% for linezolid; ~0.1% and 14% teicoplanin; and ~0.1% and 35% for vancomycin (ACSQHC 2023). None of these antibiotics are registered for use in food-producing animals in Australia (ASTAG 2018), indicating that selective pressure for resistance in any animal-derived meat isolates should be low. Overall, AMR among *Enterococcus* spp. from Australian livestock has recently been reported to be not detected to low for these antibiotics (ACMF 2022; Barlow et al. 2017; Lee et al. 2021; O'Dea et al. 2019; MLA 2020).

Medium-importance antibiotics

In this study, gentamicin microbiological resistance was not detected to very low among *E. faecalis* isolates (beef not detected, chicken meat not detected, pork 0.5%). No gentamicin resistance was detected in any *E. faecium* isolate from any retail meat commodity. Gentamicin is used in the treatment of endocarditis from *Enterococcus* spp. for humans (ACSQHC 2023) and it is not registered for use in food-producing animals in Australia but other antibiotics in the same class are (ASTAG 2018).

Other antibiotics

Evidence from animal studies and AMR monitoring consistently shows that antibiotics more frequently used in veterinary settings tend to exhibit higher levels of resistance in bacterial isolates from animals. While resistant bacteria can be found on meat, this doesn't mean the animal was the direct source. This survey did not aim to determine the origin of bacteria found on meat, and other potential sources – such as human handling, environmental contamination and animal feed – may also contribute to the bacterial profile observed. Resistance levels are further influenced by factors such as bacterial species, historical use, production type and management practices, and should be interpreted within a broader epidemiological context.

In this study, no ampicillin microbiological resistance was detected in *E. faecalis* isolates from any retail meat commodity, and only observed in some *E. faecium* (beef one isolate, chicken meat one isolate, pork 2 isolates). Similar results, with different rates of microbiological resistance, were observed for erythromycin, streptomycin and tetracycline by commodity for both *E. faecalis* or *E. faecium* isolates.

- In *E. faecalis,* tetracycline resistance was highest followed by erythromycin and streptomycin:
  - beef: tetracycline 21.4%, erythromycin 4.5%, streptomycin 1.3%
  - pork: tetracycline 32.8%, erythromycin 11.6%, streptomycin 5.1%
  - chicken meat: tetracycline 47.6%, erythromycin 22.8%, streptomycin 1.1%.
- In *E. faecium*, resistance was detected in the following number of isolates:
  - pork: erythromycin 11 isolates, tetracycline 9 isolates, streptomycin 7 isolates.
  - chicken meat: tetracycline 20 isolates, erythromycin 18 isolates, streptomycin 7 isolates.
  - beef: erythromycin 7 isolates, tetracycline 6 isolates, streptomycin 5 isolates.

Australian context: In Australia, the penicillins ampicillin (registered for use in humans) and amoxicillin (registered for use in food-producing animals); macrolides erythromycin (registered for use in food producing animals) and azithromycin (registered for use in humans); tetracyclines tetracycline (registered for use in humans) and chlortetracycline/oxytetracycline (registered for use in food-producing animals); and aminoglycoside streptomycin (registered for use in food-producing animals) are low-importance antibiotics (ASTAG 2018). Ampicillin is still used against enterococcal infections in humans and is commonly used in first-line treatment (ACSQHC 2023). AMR rates in human clinical specimens have been reported as ~0.1 to 0.2% among *E. faecalis* and ~86 to 90% among *E. faecium* for ampicillin in 2021 (ACSQHC 2023). Penicillins, macrolides, streptomycin and tetracyclines are also important for veterinary medicine and are classified as VCIAA by WOAH (WOAH 2024a). Similarly, AMR rates in Australian livestock have been reported to vary from <0.1 to 90% depending on the antibiotic, enterococcal species and animal species (ACMF 2022; Barlow et al. 2017; Lee et al. 2021; O'Dea et al. 2019; MLA 2020). As with *E. coli,* these differences are attributed to different historical usage, husbandry requirements, types of disease that need to be treated, and availability of alternatives (Abraham et al. 2019; ACMF 2022; Barlow et al. 2022; Kidsley et al. 2018; Laird et al. 2022; MLA 2020).

Historical comparison with 2007 results

Trend analysis over time (for example, increasing resistance) was not possible in this study, as different methods and sampling approaches from those in the survey by Barlow and Gobius (2008) were used. These changes were made to align with current international guidance. However, a cautious comparison of similarities and differences is still possible and may offer useful insights. To provide some context, the MIC data from Barlow and Gobius (2008) were reanalysed for antibiotics tested at sufficient concentrations. This was done using the current ECOFFs and the same method

for calculating CIs as in the current study. For most antibiotics tested, the reported prevalence of resistant isolates appeared similar for the two studies. There only notable exceptions[5] were lower reported rates of resistance to erythromycin and tetracycline observed in the current study for *E. faecalis* among raw retail chicken meat.

Microbiological complete susceptibility and multidrug resistance

Complete susceptibility of *Enterococcus* spp. isolates to the panel of antibiotics tested indicates that each antibiotic tested was able to effectively inhibit or kill the organism (at the ECOFF) and the bacteria have not acquired resistance.

In this study, the rates of microbiological complete susceptibility for *E. faecalis* isolates were high overall among raw retail meat commodities (beef 77.9% [120/154], pork 64.6% [128/198], and chicken meat 47.1% [89/189]). Microbiological complete susceptibility was detected in *E. faecium* isolates from all commodities (beef 33/53, pork 14/27, and chicken meat 24/54 isolates).

MDmR refers to isolates with microbiological resistance to 3 or more classes of antibiotics, indicating that infections caused by these isolates may be more difficult to treat, particularly when resistance includes last-line, high-importance antibiotics. In this study, MDmR was generally low across all commodities for *E. faecalis* isolates. MDmR involving high-importance rated antibiotics was not detected in any beef isolate but was detected in chicken meat and pork derived isolates.

For *E. faecalis*, MDmR was observed in all commodities. MDmR rates in chicken meat and pork *E. faecalis* isolates were 1.1% (2/189) and 5.0% (10/198), respectively. This indicates a low risk for the spread of MDmR *E. faecalis* isolates with acquired resistance to multiple classes of antibiotics that may be harder to treat.

For *E. faecium*, MDmR was observed in isolates from all commodities (pork 6/27 isolates, chicken meat 4/54 isolates and beef 2/53 isolates). Due to the low number of isolates, these results need to be interpreted with caution.

Australian context: Microbiological complete susceptibility and resistance to multiple antibiotics have been suggested as key summary indicators for AMR (ECDC, BIOHAZ & CVMP 2017). However, data from humans and livestock in Australia are not included here due to methodological differences and lack of harmonisation in antibiotic panels, which limit comparability. This is also true for the survey by Barlow and Gobius (2008). MDmR was defined in the current study as microbiological resistance to 3 or more antibiotic classes, with resistance to a single antibiotic assumed to represent resistance to the entire class. In contrast, Barlow and Gobius (2008) reported resistance phenotypes based on combinations of individual antibiotics without grouping them by class. To improve future surveillance, studies should harmonize antibiotic panels, apply consistent class-based definitions of MDmR and transparently document assumptions about cross-resistance and classification criteria. The values reported in Barlow and Gobius (2008) for *E. faecalis* among retail meat (*E. faecium* was not detected) were; in beef, 73% (73/100) had no resistance to any antibiotic tested and 1% (1/100) were resistant to three or more antibiotics; in chicken, 19% (19/100) showed no resistance and 11% (11/100) were resistant to three or more antibiotics; and in pork, 78% (78/100) had no resistance and 3% (3/100) were resistant to three or more antibiotics.

[5] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

Known genetic determinants of resistance

This report focused the genetic analysis on isolates exhibiting resistance to high-importance antibiotics and MDmR. These isolates were subject to short-read WGS to detect known resistance determinants. However, more comprehensive analyses are planned for future publications from this survey.

A total of 18 *E. faecalis* isolates were selected for short-read WGS and genotypes of all 18 *E. faecalis* isolates are presented in Supplementary Table 11. A summary of the STs for each commodity is provided in Figure 9. ST506 was the most prevalent, with 7 isolates detected in total from pork and chicken samples. These isolates were also MDmR to linezolid + phenicols + tetracyclines + macrolides. ST506 has been previously identified in international pork derived *E. faecalis* (Huang et al. 2022). There were 6 *E. faecalis* isolates with resistance to high-importance rated antibiotics (daptomycin and nitrofurantoin) tested in this study. None of the 4 daptomycin (lipopeptide) resistant isolates had corresponding known resistance genes detected. Neither did the 2 nitrofurantoin (nitrofuran) resistant isolates. In contrast, all of the 12 MDmR *E. faecalis* isolates selected for sequencing had genes detected known to be associated with the MDmR that was phenotypically observed. This included 9 MDmR isolates that included resistance to high-importance linezolid (oxazolidinone) (n = 9), in all of which was detected an *optrA* gene known to confer resistance to phenicols and oxazolidinones. All but one of the linezolid-resistant isolates belonged to ST506 (n = 8) and were also MDmR for phenicols + tetracyclines + macrolides + oxazolidinones. The majority of the ST506 isolates were of pork origin (n = 6) and the remaining isolates were derived from chicken meat. The phenotype of the linezolid microbiologically resistant isolates was confirmed by genetic identification of an *optr*A gene, which is known to confer resistance to both linezolid as well as to chloramphenicol.

A total of 24 *E. faecium* isolates were selected for short-read WGS and genotypes of all 24 *E. faecium* isolates are presented in Supplementary Table 15. A summary of the STs for each commodity is provided in Figure 13. For *E. faecium*, the STs identified were diverse among all commodities. None of the major STs reported in the Australian enterococcal 2023 blood stream (ST78, ST1424, ST17, ST80, ST796, ST1421, and ST555) (Coombs et al. 2024) and 2020 sepsis (ST17, ST1424, ST80, ST796, ST78, ST1421, ST555, and ST117) (Coombs et al. 2022) infection reports were identified in this study. There were 14 *E. faecium* isolates with resistance for high-importance rated antibiotics: virginiamycin (n = 4), ciprofloxacin (n = 2), nitrofurantoin (n = 7), and daptomycin (n = 1). One isolate that was resistant for streptogramin and virginiamycin harboured a known resistance gene. This was an ST2044 isolated from chicken meat that carried *vat*(E). None of the 2 ciprofloxacin-resistant isolates had a known quinolone-resistance gene identified. No known genes conferring nitrofurantoin resistance were identified in the 7 isolates sequenced. Six of 10 MDmR isolates that were sequenced had genes associated with all resistance that was phenotypically observed. Five out of 6 MDmR aminoglycoside + macrolides + tetracycline isolates had genes associated with the resistance that was phenotypically observed. No known genes conferring nitrofurantoin resistance were identified in 3 MDmR isolates.

In summary, genetic analysis of *E. faecalis* and *E. faecium* isolates that were microbiologically resistant to high-importance antibiotics or MDmR revealed diverse STs. *E. faecium* STs did not match major STs previously reported in Australian bloodstream or sepsis infection cases. High-importance antibiotic resistance in both *E. faecalis* and *E. faecium* often lacked known corresponding resistance genetic elements. In contrast, most MDmR isolates had identifiable genes matching observed resistance, particularly in *E. faecalis* where all linezolid-resistant isolates carried the optrA gene. There are several possible reasons for discrepancies between phenotypic AMR and the presence of known resistance genes, including MIC drift, novel resistance mechanisms (mutations in target genes), efflux pump activity, and plasmid loss.

Key results summarised by commodity

The key results presented above are collated here by commodity for interested readers.

Beef

E. FAECALIS

The rates of microbiological resistance for 12 antibiotics among 154 *E. faecalis* isolates from beef (Figure 7) were as follows:

- High-importance antibiotics: resistance was rare to very low (ciprofloxacin, teicoplanin, vancomycin, and linezolid not detected [< 0.1%, rare], and daptomycin and nitrofurantoin very low [both 0.6%]).
- Medium-importance antibiotics: resistance was rare (gentamicin not detected [< 0.1%, rare]).
- Low-importance antibiotics: resistance was rare to high (ampicillin and chloramphenicol not detected [< 0.1%, rare], streptomycin low [1.3%], erythromycin low [4.5%], and tetracycline high [21.4%]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study, (Figure 10) there were no notably different results[6].

The rate of microbiological complete susceptibility among beef *E. faecalis* isolates to all antibiotics and antibiotic classes tested was extremely high (120/154 isolates, 77.9%) (Table 4, Figure 8). MDmR (microbiological resistance to 3 or more antibiotic classes) was not detected.

Five unique patterns of resistance for 1–2 of the 10 antibiotic classes were observed. The 4 most prevalent antibiotic-class resistance combinations in beef *E. faecalis* isolates included classes with high-importance antibiotics (tetracyclines [23 isolates, 14.9%], macrolides + tetracyclines [7 isolates, 4.5%], aminoglycosides + tetracyclines [2 isolates, 1.3%], and nitrofurans or lipopeptides [each one isolate, 0.6%]).

E. FAECIUM

The rates of microbiological resistance for 13 antibiotics among 53 *E. faecium* isolates from beef (Figure 11) were as follows:

- High-importance antibiotics: resistance for teicoplanin, vancomycin, linezolid, and ciprofloxacin not detected in any isolate, daptomycin 1/53 isolates, virginiamycin 2/53 isolates, and nitrofurantoin 4/53 isolates.
- Medium-importance antibiotics: resistance was not detected for gentamicin in any isolate.
- Low-importance antibiotics: resistance was detected in ampicillin 1/53 isolates, chloramphenicol 1/53 isolates, streptomycin 5/53 isolates, tetracycline 6/53 isolates, and erythromycin 7/53 isolates.

Microbiological complete susceptibility to all tested antibiotics and antibiotic classes among beef *E. faecium* isolates was detected in 33/53 isolates (Table 5, Figure 12).

MDmR (resistance to 3 or more antibiotic classes) was detected in 2/53 isolates, and MDmR with resistance involving high-importance antibiotics was not detected.

Among the resistant isolates, 12 unique patterns of resistance to 1–3 antibiotic classes were observed. Resistance to one or 2 antibiotic classes was detected in 18/53 isolates. The 4 most prevalent antibiotic-class resistance combinations in beef *E. faecium* isolates were macrolides (5

[6] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

isolates), followed by nitrofurans (3 isolates), aminoglycosides and streptogramins (2 isolates each), and aminoglycosides + tetracyclines (2 isolates).

Chicken meat

E. FAECALIS

The rates of microbiological resistance for 12 antibiotics among 189 *E. faecalis* isolates from chicken meat (Figure 7) were as follows:

- High-importance antibiotics: resistance ranged from rare to low (ciprofloxacin, teicoplanin, and vancomycin not detected (< 0.1%, rare), daptomycin and nitrofurantoin very low (both 0.5%), and linezolid low (1.1%).
- Medium-importance antibiotics: resistance was rare (gentamicin not detected [< 0.1%, rare]).
- Low-importance antibiotics: resistance ranged from rare to high (ampicillin not detected [< 0.1%, rare], chloramphenicol low [1.6%], streptomycin low [1.1%], and erythromycin and tetracycline high [22.8% and 47.6%, respectively]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study, (Figure 10) the only notable results[7] were the lower reported resistance for erythromycin and tetracycline observed in the current study.

The rate of microbiological complete susceptibility among chicken meat *E. faecalis* isolates to all 12 antibiotics tested for was high (89/189 isolates, 47.1%) (Table 4, Figure 8).

MDmR (resistance to 3 or more antibiotic classes) was low (2/189 isolates, 1.1%). Both isolates detected showed MDmR with resistance to high-importance antibiotics.

Nine unique patterns of resistance for 1–4 of the 10 antibiotic classes were observed. Resistance to one or 2 antibiotic classes was detected in 51.9% (98/189) of isolates. The four most prevalent antibiotic-class resistance patterns among chicken meat *E. faecalis* isolates included classes with high-importance rated antibiotics. These combinations were MDmR combinations: macrolides + oxazolidinones + phenicols + tetracyclines (2 isolates, 1.1%); and non-MDmR combinations: tetracyclines (52 isolates, 27.5%), macrolides + tetracyclines (34 isolates, 18%), and macrolides (7 isolates, 3.7%).

E. FAECIUM

The rates of microbiological resistance for 13 antibiotics tested among 54 *E. faecium* isolates from chicken meat (Figure 11) were as follows:

- High-importance antibiotics: resistance was not detected in any isolate for teicoplanin, vancomycin, linezolid, and daptomycin. Resistance for ciprofloxacin, virginiamycin, and nitrofurantoin was detected among 1/54, 2/54, and 3/54 isolates respectively.
- Medium-importance antibiotics: resistance for gentamicin was not detected among any isolate.
- Low-importance antibiotics: resistance was for chloramphenicol not detected, ampicillin 1/54 isolates, streptomycin moderate 7/54 isolates, tetracycline 20/54 isolates and erythromycin 18/54 isolates.

Microbiological complete susceptibility among chicken meat *E. faecium* isolates to all antibiotics and antibiotic classes tested was detected in 24/54 isolates (Table 5, Figure 12).

[7] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

MDmR (resistance to 3 or more antibiotic classes) was detected in 4/54 isolates, with MDmR including resistance to high-importance antibiotics accounting for half of these isolates (2/54 isolates).

Among the resistant isolates, 13 unique patterns of resistance to 1–4 different antibiotic classes were observed. Resistance to 1–2 antibiotic classes was detected in 26/54 isolates. The 4 most prevalent antibiotic-class resistance patterns in chicken meat *E. faecium* isolates included classes with high-importance rated antibiotics (macrolides + tetracyclines [9 isolate], tetracyclines [6 isolates], aminoglycosides [3 isolates], and macrolides [2 isolates]).

Pork

E. FAECALIS

The rates of microbiological resistance among 198 *E. faecalis* isolates from pork (Figure 7) were as follows:

- High-importance antibiotics: resistance ranged from rare to low (ciprofloxacin, teicoplanin, vancomycin, and nitrofurantoin not detected [< 0.1%, rare], daptomycin very low [1%], and linezolid low [3%]).
- Medium-importance antibiotics: resistance was very low (gentamicin 0.5%).
- Low-importance antibiotics: resistance ranged from rare to high (ampicillin not detected [< 0.1%, rare], chloramphenicol low [4.5%], streptomycin low [5.1%], erythromycin moderate [11.6%], and tetracycline high [32.8%]).
- Of the antibiotics that were tested in this study and the 2007 Australian AMR pilot study, (Figure 10) there was no notable resistance levels reported[8].

The rate of microbiological complete susceptibility among pork *E. faecalis* isolates to all antibiotics tested was very high (128/198 isolates, 64.6%) (Table 4, Figure 8).

MDmR (resistance to 3 or more antibiotic classes) was low (10/198 isolates, 5.0%). Seven of the detected isolates detected showed MDmR with resistance to high-importance antibiotics (7/198 isolates, 3.5%).

Nine unique patterns of resistance were observed among isolates for 1–4 of the 10 antibiotic class combinations. The 4 most prevalent antibiotic-class resistance combinations in pork *E. faecalis* isolates included classes with high-importance rated antibiotics. These combinations were MDmR combination: macrolides + oxazolidinones + phenicols + tetracyclines (6 isolates, 3%); and non-MDmR combinations: tetracyclines (37 isolates, 18.7%), macrolides + tetracyclines (10 isolates, 5%), and aminoglycosides + tetracyclines (8 isolates, 4%).

E. FAECIUM

The rates of microbiological resistance among 27 *E. faecium* isolates from pork (Figure 11) were as follows:

- High-importance antibiotics: teicoplanin, vancomycin, virginiamycin, linezolid and daptomycin resistance was not detected (0/27). Nitrofurantoin and ciprofloxacin resistance was detected in one isolate each.
- Medium-importance antibiotics: resistance was rare (gentamicin resistance not detected in any isolate.

[8] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

- Low-importance antibiotics: resistance for chloramphenicol 0/27, ampicillin 2/27, streptomycin 7/27, tetracycline 9/27, and erythromycin 11/27.

Microbiological complete susceptibility among pork *E. faecium* isolates to all 13 antibiotics and 11 antibiotic classes tested was detected in 14/27 isolates (Table 5, Figure 12).

MDmR (resistance to 3 or more antibiotic classes) was detected in 6/27 isolates, with one isolate thereof showing MDmR involving resistance to high-importance antibiotics.

Eight unique patterns of resistance to 1–4 different antibiotic classes were observed among the resistant isolates. The 4 most prevalent antibiotic-class resistance combinations in pork *E. faecium* isolates included classes with high-importance rated antibiotics; the highest being MDmR aminoglycosides + macrolides + tetracyclines (4 isolates, 14.8%); followed by macrolides + tetracyclines (2 isolates), macrolides (2 isolates), and aminoglycosides (2 isolates).

Key messages

The rare (not detected) to low resistance rates to high-importance antibiotics and high rates of complete susceptibility among *E. faecalis* isolates from raw retail beef, chicken and pork meat indicate a reduced risk of foodborne transmission of bacteria that may become involved in resistant infections or spread resistance.

These results show support for the effectiveness of Australian antibiotic stewardship programs and prescribing guidelines. Additionally, resistance levels to antibiotics that are important in veterinary medicine but considered low-importance for humans (although can be common first-line treatments) remain consistent with expectations based on findings from Barlow and Gobius (2008). While the results do not indicate recent increases, they reinforce the interconnectedness of animal health, human health, food safety and food security. The absence of fully comparable historical data limits our ability to confidently assess long-term trends. Establishing coordinated surveillance systems that conduct repeated surveys would enable more accurate detection of both improvements in the form of reduced resistance levels and the emergence of new resistance risks.

Effective, sustained and cooperative efforts are needed not only among the One Health sectors but also among all stakeholders in the farm-to-fork pathway to ensure food safety practices are implemented through the whole chain and appropriate antibiotic use is practiced.

This will be critical for maintaining or reducing the rates observed in this study in the future. Because this study did not investigate the source of the *E. faecalis* detected among the retail meats (that is, whether it is from animal, human, or environmental origin), this is an area that would benefit from future cross-sector studies to help robustly identify sources and the potential AMR pressures.

For *E. faecium*, more isolates would need to be tested to provide more precise estimates of the true proportion of resistance to these antibiotics among isolates from raw retail beef, chicken meat and pork. However, the costs and benefits of generating that data via different methodologies would need to be considered for future studies.

E. faecalis tables and figures

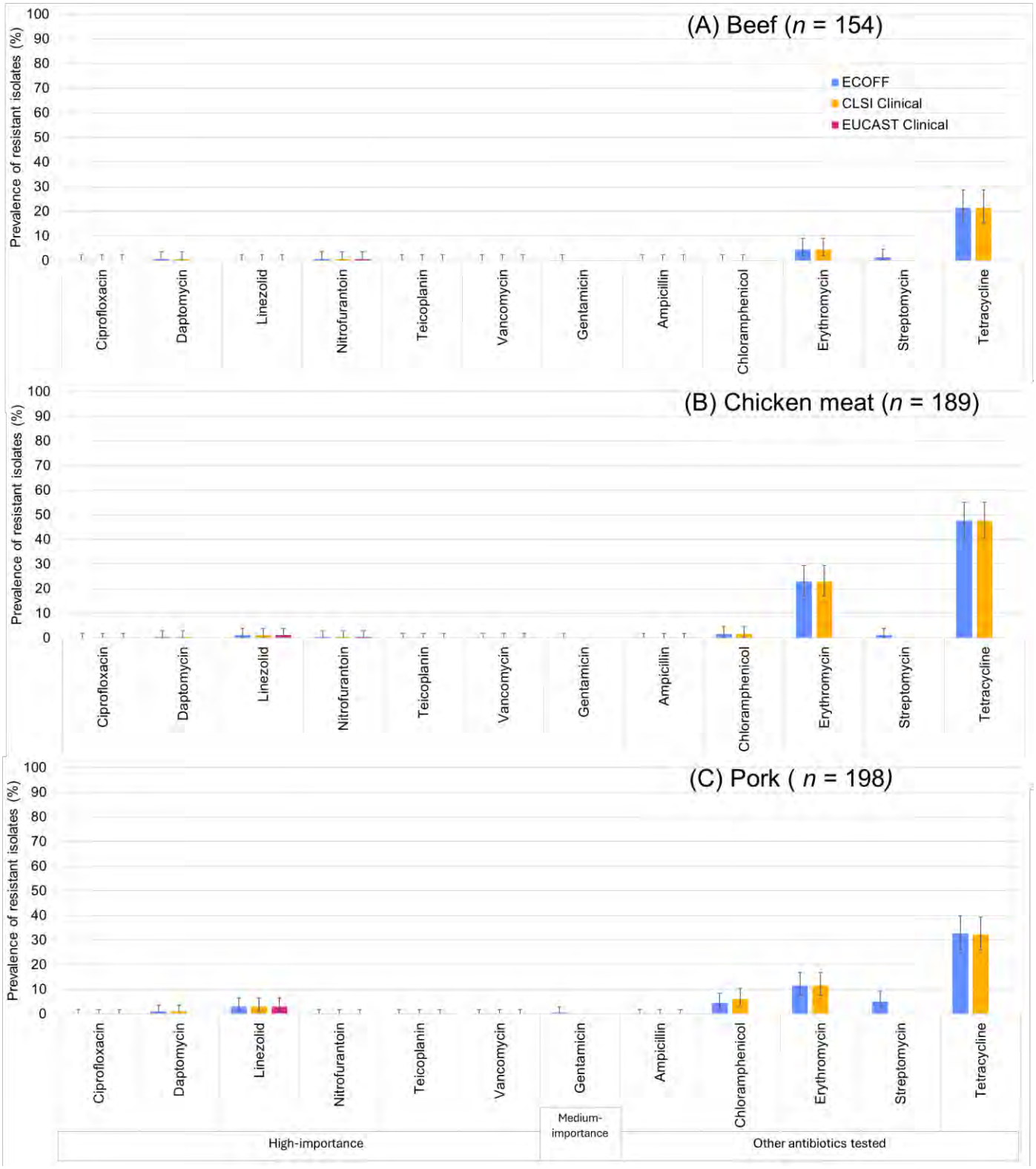


Figure 7: The rates of resistance to high-importance rated (ASTAG 2018), medium-importance rated (ASTAG 2018) and other antibiotics among *E. faecalis* isolates from retail A) beef (n = 154 isolates), B) chicken meat (n = 189 isolates), and C) pork (n = 198 isolates). Prevalence of microbiological resistance based on ECOFF (blue), and clinical resistance based on CLSI (yellow) and EUCAST (pink) clinical breakpoints. 95% CIs shown as error bars.

Table 4: Prevalence of microbiological resistance patterns for different antibiotic classes among *E. faecalis* isolated from raw retail beef, chicken meat and pork.

Beef (n = 154)			Chicken meat (n = 189)			Pork (n = 198)		
Pattern (Phenotype)	*n*	Prevalence (%)	Pattern (Phenotype)	*n*	Prevalence (%)	Pattern (Phenotype)	*n*	Prevalence (%)
0: nil	120	77.9	0: nil	89	47.1	0: nil	128	64.6
1: nit	1	0.6	1: ami	1	0.5	1: lip	2	1
1: tet	23	14.9	1: lip	1	0.5	1: mac	3	1.5
2: ami_tet	2	1.3	1: mac	7	3.7	1: tet	37	18.7
2: lip_tet	1	0.6	1: nit	1	0.5	2: ami_tet	8	4
2: mac_tet	7	4.5	1: tet	52	27.5	2: mac_tet	10	5
			2: ami_tet	1	0.5	3: mac_oxa_tet	1	0.5
			2: mac_tet	34	18	3: mac_phe_tet	1	0.5
			2: phe_tet	1	0.5	4: ami_mac_phe_tet	2	1
			4: mac_oxa_phe_tet	2	1.1	4: mac_oxa_phe_tet	6	3

Phenotype indicates the number of antibiotic classes with resistance present and each class. Abbreviations: n – number of isolates with associated phenotype, ami – aminoglycosides, lip – lipopeptides, mac – macrolides, nit – nitrofurans, phe – phenicols, tet – tetracyclines, oxa – oxazolidinones.

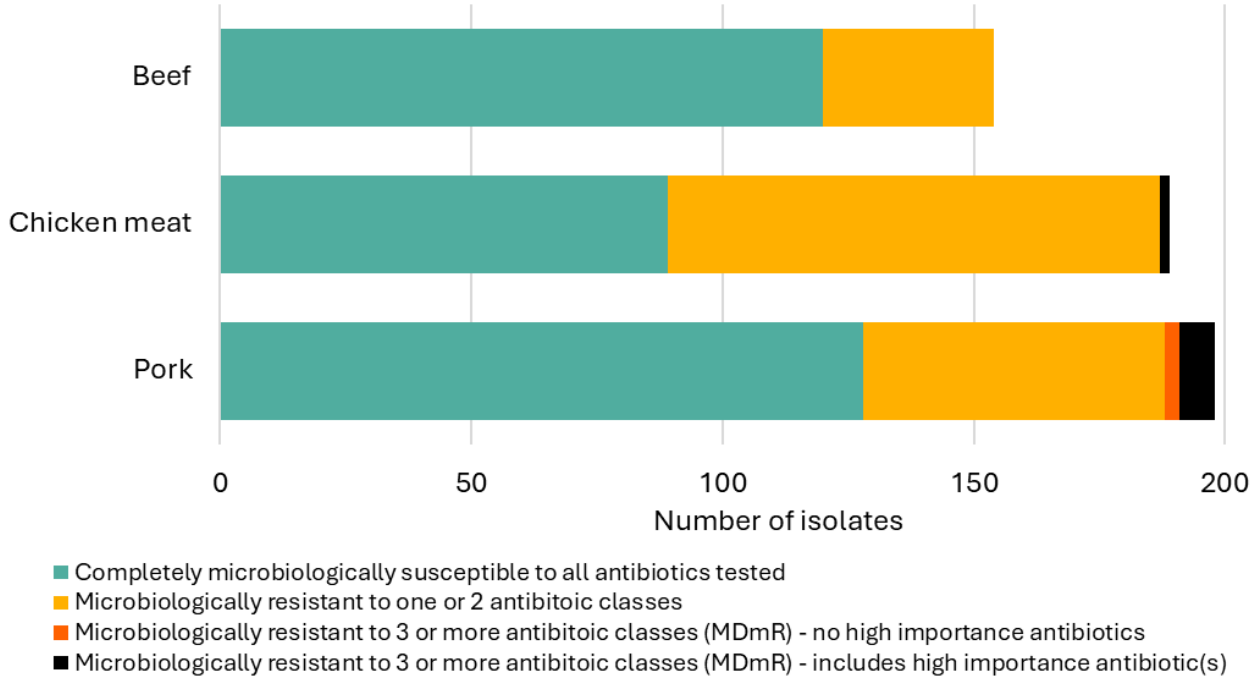


Figure 8: The number of isolates with complete microbiological susceptibility and MDmR for *E. faecalis* isolated from raw retail beef (n = 154 isolates), chicken meat (n = 189 isolates) and pork (n = 198 isolates). Completely microbiologically susceptible (green), resistant to one or 2 classes of antibiotics tested (yellow), resistant to 3 or more antibiotic classes tested, not including high-importance antibiotics (red), and resistant to 3 or more antibiotic classes tested including high-importance antibiotics (black).

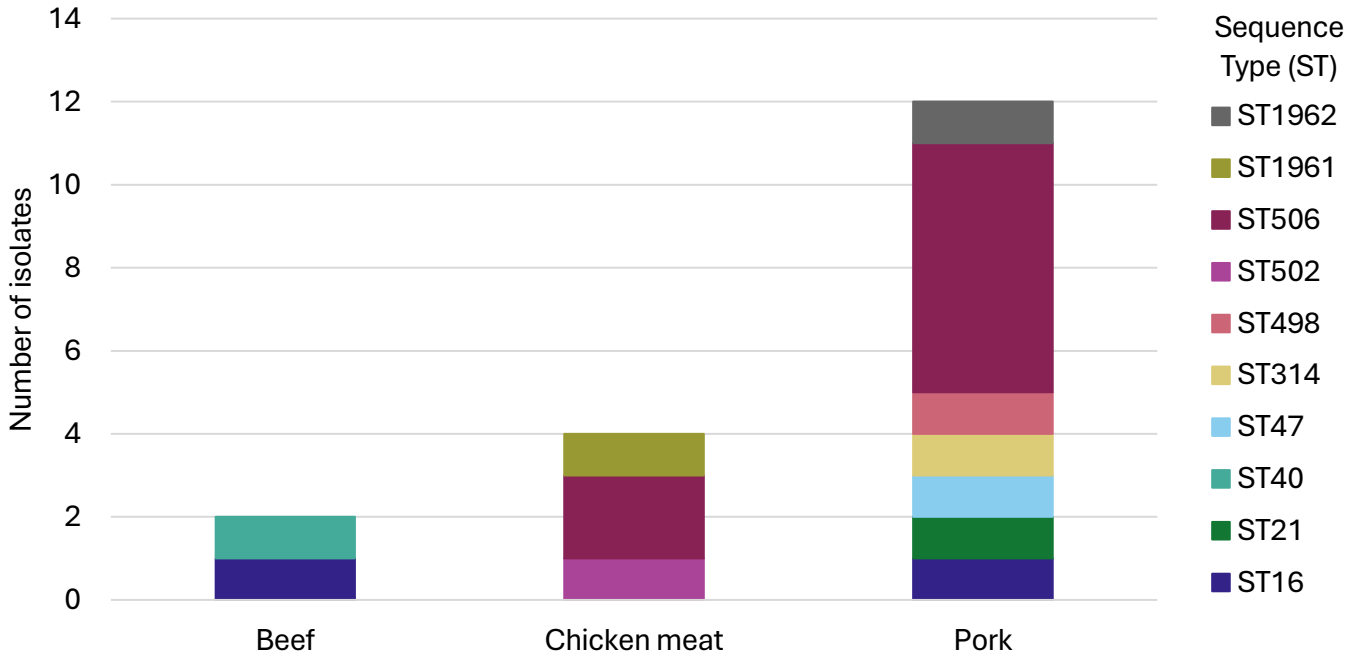


Figure 9: The number of different STs determined by WGS among *E. faecalis* isolated from raw retail beef, chicken meat and pork that were either microbiologically resistant to at least one high-importance rated antibiotic and/or were MDmR.

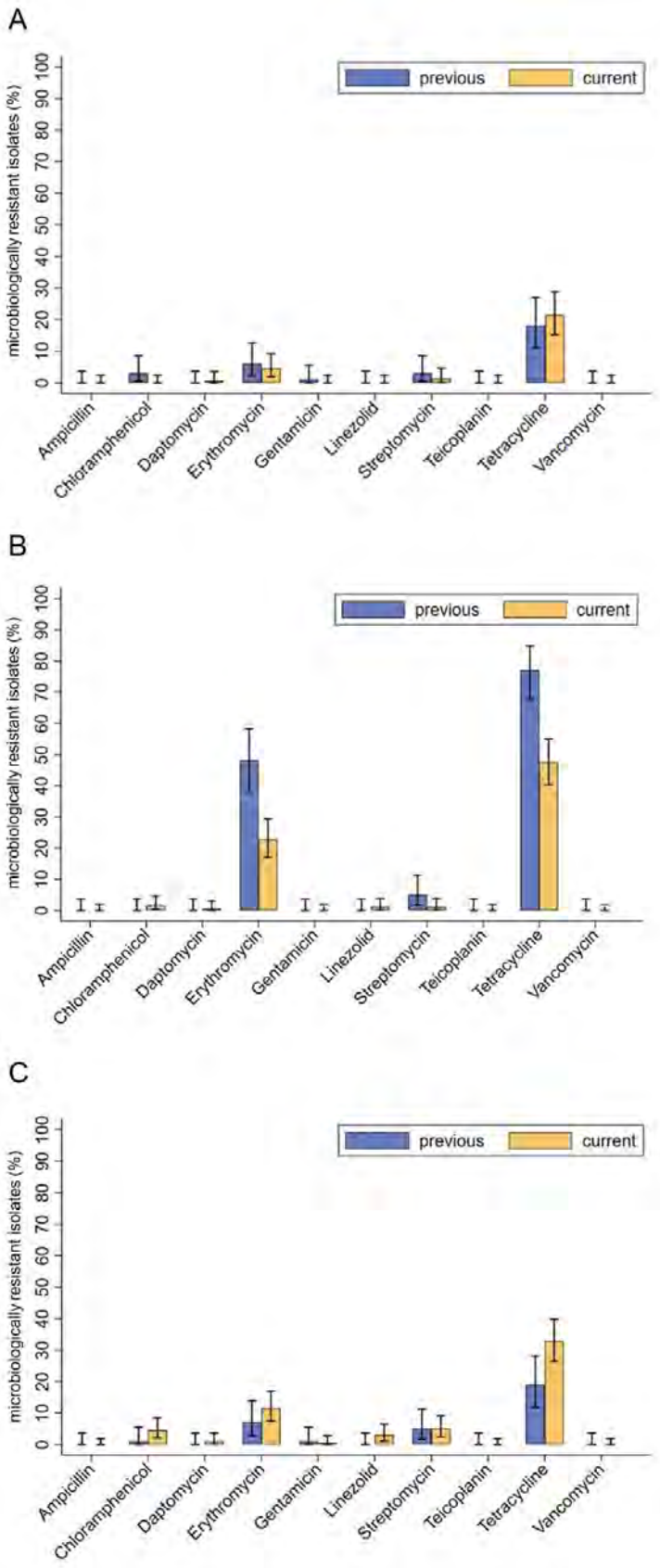


Figure 10: Reported rates of microbiological resistance among *E. faecalis* from the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (blue, previous) and this study (yellow, current). *E. faecalis* isolated from A) beef isolates (previous n = 100, current n = 154), B) chicken meat isolates (previous n = 100, current n = 189), and C) pork isolates (previous n = 100, current n = 198. 2007 results were reanalysed against the same ECOFFs used in the current study and 95% CIs for each data set shown as error bars (presented for information only and no statistical comparison was undertaken).

E. faecium tables and figures

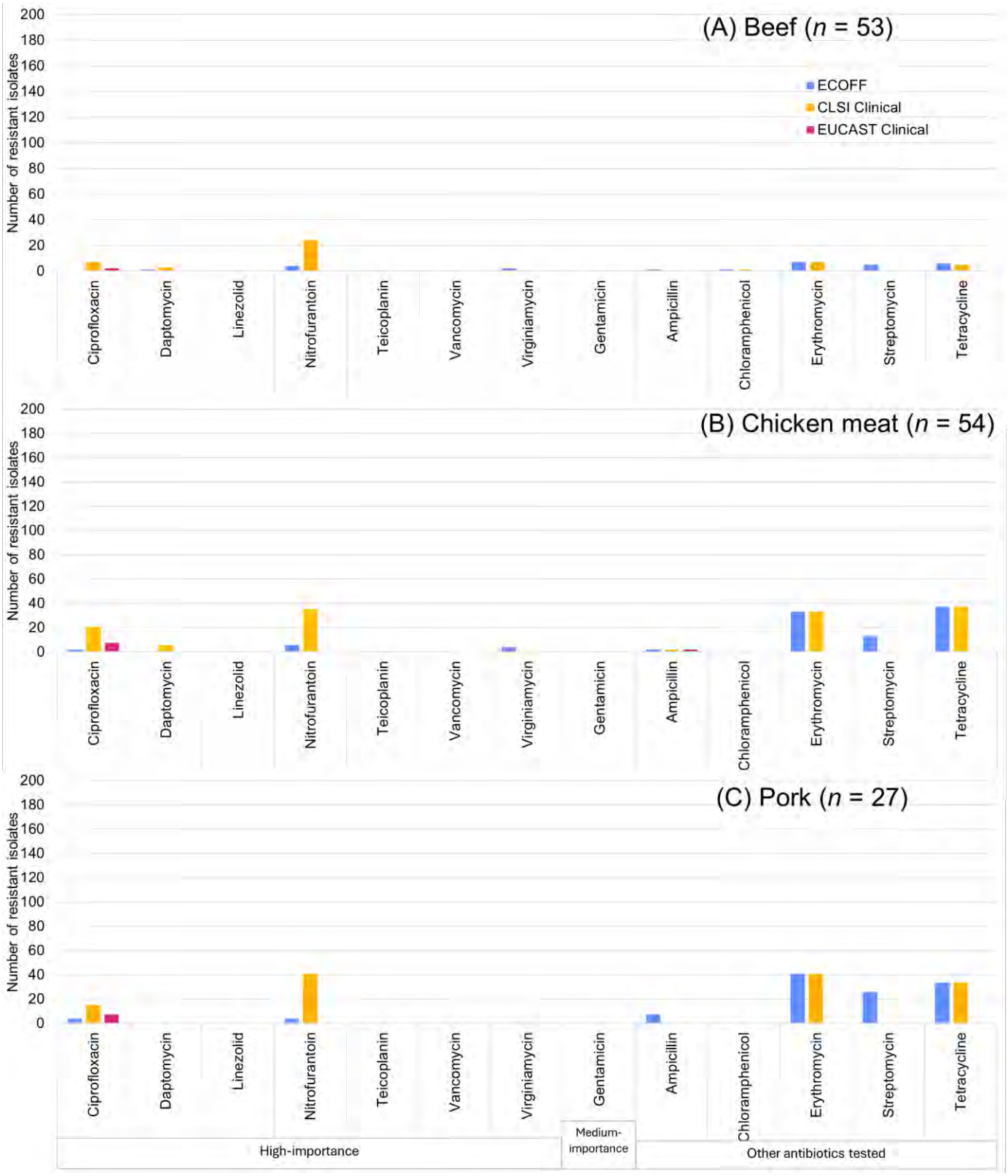


Figure 11: The number of resistance to high-importance rated (ASTAG 2018), medium-importance rated (ASTAG 2018) and other antibiotics among *E. faecium* isolates from retail A) beef (n = 53 isolates), B) chicken meat (n = 54 isolates), and C) pork (n = 27 isolates). Microbiological resistance based on ECOFF (blue), and clinical resistance based on CLSI (yellow) and EUCAST (pink) clinical breakpoints.

Table 5: The number of microbiological resistance patterns for different antibiotic classes among *E. faecium* isolated from raw retail beef, chicken meat and pork.

Beef (n = 53 isolates)		Chicken meat (n = 54 isolates)		Pork (n = 27 isolates)	
Pattern (Phenotype)	*n*	Pattern (Phenotype)	*n*	Pattern (Phenotype)	*n*
0: nil	33	0: nil	24	0: nil	14
1: ami	2	1: ami	3	1: mac	2
1: lip	1	1: mac	2	1: qui	1
1: mac	5	1: qui	1	2: ami_mac	1
1: nit	3	1: str	1	2: ami_tet	1
1: phe	1	1: tet	6	2: mac_tet	2
1: str	2	2: ami_mac	1	3: ami_mac_tet	4
1: tet	1	2: ami_tet	1	4: ami_bla_mac_tet	1
2: ami_tet	2	2: mac_nit	1	4: bla_mac_nit_tet	1
2: nit_tet	1	2: mac_str	1		
3: ami_mac_tet	1	2: mac_tet	9		
3: bla_mac_tet	1	3: ami_mac_tet	1		
		3: mac_nit_tet	2		
		4: ami_bla_mac_tet	1		

Phenotype indicates the number of antibiotic classes with resistance present and each class. Abbreviations: n – number of isolates with associated phenotype, ami – aminoglycosides, lip – lipopeptides, mac – macrolides, nit – nitrofurans, phe – phenicols, str – streptogramins, tet – tetracyclines, qui – quinolones, bla – beta lactams.

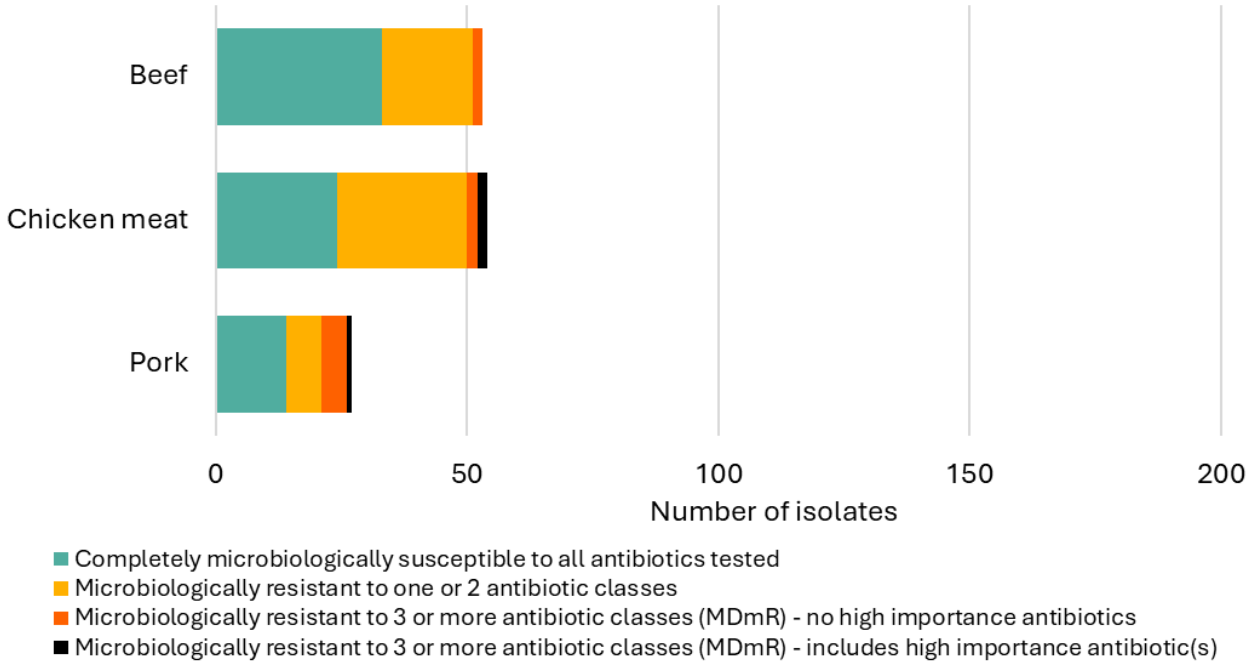


Figure 12: The number of isolates with complete microbiological susceptibility and MDmR for *E. faecium* isolated from raw retail beef (n = 53), chicken meat (n = 54) and pork (n = 27). Completely microbiologically susceptible (green), resistant to one or 2 classes of antibiotics tested (yellow), resistant to 3 or more antibiotic classes tested, not including high-importance antibiotics (red), and resistant to 3 or more antibiotic classes tested including high-importance antibiotics (black).

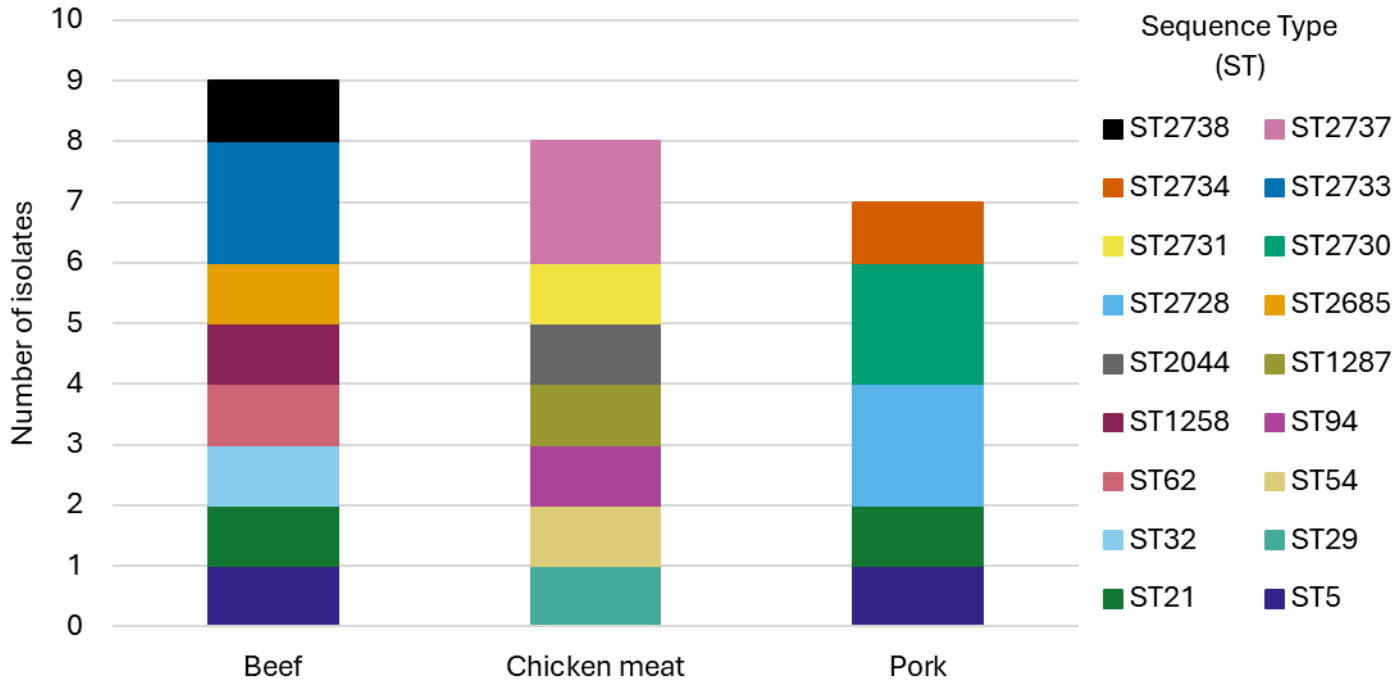


Figure 13: The number of different STs determined by WGS among *E. faecium* isolates from raw retail beef, chicken meat and pork that were either microbiologically resistant to at least one high-importance rated antibiotic and/or were MDmR.

Salmonella

Salmonella was included in the AMR surveillance of raw retail chicken meat and pork because:

- *Salmonella* was expected to be sufficiently prevalent in raw chicken meat (however, not in pork or beef) to provide robust estimates of AMR within the population. A smaller number of pork samples were included to demonstrate the anticipated low prevalence. Raw meat provides a direct snapshot of the bacterial populations, including resistant strains, that have entered the meat production chain (either from food animals, humans or the environment) before being destroyed by effective cooking.
- Non-typhoidal *Salmonella* include serovars that are significant pathogens responsible for foodborne illnesses in Australia (Ford et al. 2019; Glass et al. 2023) and can act as reservoirs of resistance genes. Although most foodborne illness caused by *Salmonella* does not need antibiotic treatment, it may be necessary for people with severe symptoms or more vulnerable groups like the young, old, and people with weakened immune systems (ACSQHC 2023). In Australia, *Salmonella* are considered of high public health importance and pathogens for which the impact of resistance is substantial in hospital and community settings (ACSQHC 2023).
- Monitoring *Salmonella* helps track AMR trends, inform control measures and support efforts to mitigate the public health risks associated with the spread of resistant foodborne pathogens (WHO 2017).

Salmonella is a genus of bacteria divided into two species, six subspecies and over 2,600 serovars. In this report, the term '*Salmonella*' refers to *Salmonella enterica* subsp. *enterica* and specifically the 'non-typhoidal' *Salmonella* serovars. Typhoidal *Salmonella enterica* subsp. *enterica* serovars include *Salmonella* Typhi and *Salmonella* Paratyphi that cause typhoid fever, a serious systemic infection with high fever, while non-typhoidal *Salmonella* refers to other *Salmonella enterica* subsp. *enterica* serovars that typically cause milder gastrointestinal illness like diarrhoea and stomach cramps, which are more commonly seen in foodborne illness. In Australia, non-typhoidal *Salmonella* serovars are common, but typhoidal *Salmonella* serovars are rare, and infection usually occurs in people returning from travel in regions where typhoid is more prevalent.

Raw retail meat sampling and detection of target bacteria

Raw retail chicken meat samples were collected across all states and territories during 40 sampling weeks between 19 September 2022 and 30 July 2023. Sampling was conducted evenly over time to ensure temporal balance and reduce potential biases related to seasonal or periodic variations in the data.

A total of 174 *Salmonella* isolates were collected from 2,005 chicken meat samples, which was slightly less than the target of 200 isolates. The prevalence of *Salmonella* isolates from raw retail chicken meat was lower in the current study than that reported in the 2007 pilot Australian AMR survey by Barlow and Gobius (8.7% (174/2,005) current, 21.9% (174/794) previous) (Barlow & Gobius 2008). The studies are not directly comparable and the different detection rates could be due to differences in sampling methods, differences in meat cuts targeted, or evolving agricultural and retail practices over time.

Collection and testing of retail pork meat samples for *Salmonella* was only included in the study to provide evidence of an expected low prevalence of the bacteria. This was confirmed and only a small number of 20 *Salmonella* isolates were collected from the 809 pork samples tested (2.5% prevalence of *Salmonella*).

Representativeness of data and testing for antibiotic resistance

The broth MIC method was used to determine the antibiotic susceptibility profiles of 194 *Salmonella* isolates collected for 14 antibiotics representing 10 antibiotic classes (Table 11).

From raw retail chicken meat, 174 *Salmonella* isolates were collected. This enabled sufficiently robust estimation of AMR prevalence in *Salmonella* from raw retail chicken meat. The AMR data for *Salmonella* are considered representative of populations present in chicken meat sold in retail outlets within the greater metropolitan areas of Australian capital cities, which collectively comprise over 60% of the national population. However, not all cuts available were tested so the results may not reflect all raw meat products available in these areas.

As expected only a small number of 20 *Salmonella* isolates were collected from pork, and this means there was not enough data to reliably estimate how common antibiotic resistance is among them. The results have wide confidence intervals, are less precise, and may not represent the true situation (see Sample sizes). Unlike chicken meat, this means there is considerable uncertainty regarding how well the data reflect the true proportion of AMR among *Salmonella* from raw retail pork. Many more pork meat samples would need to be tested to generate enough isolates to provide more reliable estimates of the true proportion of AMR to these antibiotics. But results are included, even when numbers are small, to ensure the report is open and complete. Although testing for *Salmonella* in pork wasn't part of the original study plan, it was added to provide extra insight when planning future studies. Sharing these findings helps readers understand both what was found and the study's limits. Because of the limitations only the number of AMR bacteria detected are reported (Table 11), not the proportions.

The key *Salmonella* results from raw retail chicken meat are discussed below, results have been summarised by commodity (see Key results summarised by commodity), and tables and figures are presented in the *Salmonella* tables and figures section.

A comprehensive distribution of MICs based on ECOFF, EUCAST and CLSI clinical breakpoints is provided in Supplementary Tables 16 and 17 for chicken meat and pork, respectively. The rates of microbiological resistance and clinical resistance are presented in Figure 14 for each commodity. The key results for each commodity are presented and then discussed below.

Microbiological resistance to antibiotics

High-importance antibiotics

No microbiological resistance to amikacin, colistin, meropenem, cefotaxime, ceftazidime or ciprofloxacin was detected among *Salmonella* isolates from raw retail chicken meat and pork.

Australian human and livestock context: Amikacin, colistin, meropenem, cefotaxime, ceftazidime, and ciprofloxacin are considered last-line antibiotics in Australia (ASTAG 2018). Ciprofloxacin and ceftriaxone are both considered important treatments for severe *Salmonella* infections, and some resistance to these antibiotics (0.3–2.2%) was reported among different human clinical non-typhoidal *Salmonella* specimens in 2021 (ACSQHC 2023). These antibiotics are not registered for use in chickens or pigs in Australia (ASTAG 2018), and AMR among live broiler chickens and pig *Salmonella* isolates is generally not detected to extremely low (Abraham et al. 2019; ACMF 2022; Kidsley et al. 2018)

Medium-importance antibiotics

Gentamicin is not permitted for use in food-producing animals (though other aminoglycosides are), and amoxicillin-clavulanate has limited approval for treatment of cattle but not for other food

producing animals including chickens and pigs (ASTAG 2018). In this study, microbiological resistance to gentamicin and amoxicillin-clavulanate was not detected in *Salmonella* isolates from raw chicken meat, indicating a low contribution to the spread of resistance.

Other antibiotics

The number of *Salmonella* isolates from pork that were microbiologically resistant were: azithromycin 0/20, florfenicol and trimethoprim 2/20 isolates, chloramphenicol 3/20 isolates, tetracycline 11/20, and ampicillin 15/20 isolates.

No microbiological resistance to azithromycin, and low resistance to ampicillin (4%) was detected among *Salmonella* isolates from raw retail chicken meat in this study indicating that acquired resistance to these first-line treatments options is low. No microbiological resistance was detected to trimethoprim, chloramphenicol or florfenicol. Low microbiological resistance was detected to tetracycline (4.6%) in chicken meat *Salmonella* isolates, indicating a low likelihood of contribution to the spread of resistance for these antibiotics.

Australian human and livestock context: In Australia, the penicillins ampicillin (registered for use in humans) and amoxicillin (registered for use in food-producing animals); macrolides erythromycin (registered for use in food producing animals) and azithromycin (registered for use in humans), tetracyclines tetracycline (registered for use in humans) and chlortetracycline/oxytetracycline (registered for use in food-producing animals); and phenicols chloramphenicol (registered for humans) and florfenicol (registered for use in cattle and pigs) are low-importance antibiotics (ASTAG 2018). Generally one representative was inlcuded in the study. Azithromycin is considered an important antibiotic for *Salmonella*-related infection treatment in humans (ACSQHC 2023). Azithromycin AMR in non-typhoidal *Salmonella* human specimens has been reported at 9.1%, ampicillin at ~1–6%, and trimethoprim ~2% in 2021(ACSQHC 2023). Amoxicillin, erythromycin, tetracyclines, and florfenicol are all considered an important treatment option for livestock (WOAH 2024a). AMR resistance rates in Australian chickens and pig isolates have been reported to vary by animal and range from absent to ~20% (Abraham et al. 2019; ACMF 2022; Kidsley et al. 2018).

Historical comparison with 2007 results

Trend analysis over time (for example, increasing resistance) was not possible in this study, as different methods and sampling approaches from those in the survey by Barlow and Gobius (2008) were used. These changes were made to align with current international guidance. However, a cautious comparison of similarities and differences is still possible and may offer useful insights. To provide some context, the MIC data from Barlow and Gobius (2008) were reanalysed for antibiotics tested at sufficient concentrations. This was done using the current ECOFFs and the same method for calculating CIs as in the current study. The reported rates of microbiological resistance were generally similar between the two studies[9] (Figure 16) except for lower reported resistance to tetracycline in the current dataset. These observations should be interpreted cautiously given methodological differences.

Microbiological complete susceptibility and multidrug resistance

Microbiological complete susceptibility indicates that a bacterium has not acquired resistance to any of the antibiotics tested. In this study, an extremely high number (160/174 isolates, 92%) of *Salmonella* isolates detected from chicken meat samples were microbiologically completely susceptible.

[9] Notable differences were based on the simplistic measure of confidence intervals that did not overlap, and do not represent statistical significance.

No MDmR was observed in *Salmonella* isolates from chicken meat samples. For pork, 3 MDmR isolates were detected but none with microbiological resistance for high-importance antibiotics.

Australian context: Microbiological complete susceptibility and resistance to multiple antibiotics have been suggested as key summary indicators for AMR (ECDC, BIOHAZ & CVMP 2017). However, data from humans and livestock in Australia are not included here due to methodological differences and lack of harmonisation in antibiotic panels, which limit comparability. This is also true for the survey by Barlow and Gobius (2008). MDmR was defined in the current study as microbiological resistance to three or more antibiotic classes, with resistance to a single antibiotic assumed to represent resistance to the entire class. In contrast, Barlow and Gobius (2008) reported resistance patterns based on combinations of individual antibiotics without grouping them by class. To improve future surveillance, studies should harmonize antibiotic panels, apply consistent class-based definitions of MDmR and transparently document assumptions. The values reported in Barlow and Gobius (2008) for *Salmonella* among retail chicken meat were 77% (77/100) with no resistance to any antibiotic tested and 6% (6/100) resistant to three or more antibiotics. *Salmonella* was not tested for in pork.

Known genetic resistance determinants

The scope of genetic analysis was limited to analysis of isolates with microbiological resistance to high-importance antibiotics and MDmR in this report. Future publication of more in-depth analyses is planned. These findings help pinpoint the resistant strains present in the food supply, clarify the genetic mechanisms behind resistance and support future surveillance and risk assessment efforts.

Only 3 MDmR *Salmonella* isolates derived from pork were sequenced. Known genes corresponding to resistance were found for all classes for all isolates. The sequence types were ST515, ST19 and ST34. Genotypes of the isolates are presented in Supplementary Table 18. ST515 was MDmR for β-lactams + phenicols + tetracyclines. ST515 has previously been detected among pigs in Australia (Kidsley et al. 2018).

ST19 was MDmR for β-lactams + phenicols + tetracyclines. ST34 was MDmR for aminoglycosides + β-lactams + folate pathway inhibitors + phenicols + tetracyclines. ST19 is known to be a globally dispersed sequence type of *Salmonella enterica* Typhimurium (Gómez-Baltazar et al. 2023).

Key results summarised by commodity

Chicken meat

The rates of microbiological resistance for 14 antibiotics among 174 *Salmonella* isolates from chicken meat (Figure 14) were as follows:

- High-importance antibiotics: resistance was not detected (amikacin, cefotaxime, ceftazidime, ciprofloxacin, colistin and meropenem all not detected [< 0.1%, rare]).
- Medium-importance antibiotics: resistance was not detected (amoxicillin/clavulanate and gentamicin not detected [< 0.1%, rare]).
- Low-importance antibiotics: resistance was rare to low (azithromycin, chloramphenicol, florfenicol, and trimethoprim not detected [< 0.1%, rare], ampicillin low [4%], and tetracycline low [4.6%]).
- Of the antibiotics tested in both this study and the 2007 Australian AMR pilot study, (Figure 16) the lower reported resistance to tetracycline in the current dataset was the only notable

result[10]. These observations should be interpreted cautiously given methodological differences.

The rate of microbiological complete susceptibility for chicken meat *Salmonella* isolates to all antibiotics tested was extremely high (160/174 isolates, 92%) (Table 6, Figure 15).

MDmR (resistance to 3 or more antibiotic classes) was not detected. The most prevalent antibiotic-class resistance combinations in chicken meat *Salmonella* isolates did not include classes with high-importance rated antibiotics.

Three unique patterns of resistance to 1–2 different classes of antibiotics were observed. Resistance to one or 2 antibiotic classes was detected in 8% (14/174) of isolates. These combinations were: tetracyclines (7 isolates, 4%), β-lactams (6 isolates, 3.4%), and β-lactams + tetracyclines (1 isolate, 0.6%).

Pork

As expected, the number of isolates collected from pork (n = 20) was not sufficient to provide robust predictions of the true proportion of *Salmonella* isolates from retail pork meat that are susceptible or resistant. As such, the data provided below for *Salmonella* isolates from pork samples is for information only and must be interpreted with caution. However, the number of isolates with AMR have been summarised below.

The rates of microbiological resistance detected for 14 antibiotics among 20 *Salmonella* isolates from pork (Figure 14) are summarised below:

- High-importance antibiotics: resistance was not detected (amikacin, cefotaxime, ceftazidime, ciprofloxacin, colistin and meropenem).
- Medium-importance antibiotics: resistance was not detected for amoxicillin/clavulanate and was detected in 1/20 isolates for gentamicin.
- Low-importance antibiotics: azithromycin not detected, florfenicol and trimethoprim 2/20 isolates, chloramphenicol 3/20 isolates, tetracycline 11/55, and ampicillin 15/20 isolates.

Four out of 20 *Salmonella* pork isolates were completely microbiologically susceptible (Table 6, Figure 15).

MDmR (resistance to 3 or more antibiotic classes) was detected among 3/20 isolates and MDmR with resistance to high-importance antibiotics was not detected.

Six unique patterns of resistance for 1–5 different antibiotic classes were observed. Three *Salmonella* isolates were selected for sequencing, all derived from pork. Genotypes of the isolates are presented in Supplementary Table 18. ST515 was MDmR for β-lactams + phenicols + tetracyclines. ST19 was MDmR for β-lactams + phenicols + tetracyclines. ST34 was MDmR for aminoglycosides + β-lactams + folate pathway inhibitors + phenicols + tetracyclines. Known genes corresponding to resistance were found for all classes for all isolates.

Key messages

The rare (not detected) microbiological resistance to high-importance antibiotics, the extremely high microbiological complete susceptibility rates, and overall low microbiological resistance to low-importance rated antibiotics among *Salmonella* detected from raw retail chicken meat indicate a

[10] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

reduced risk of foodborne transmission of resistant bacteria that may become involved in infections or spread resistance. Additionally, resistance levels to high-importance antibiotics for humans and those that are important in veterinary medicine but considered low-importance for humans (although can be common first-line treatments) remain consistent with expectations based on findings from Barlow and Gobius (2008). However, the absence of fully comparable historical data limits our ability to confidently assess long-term trends. Establishing coordinated surveillance systems that conduct repeated surveys would enable accurate detection of improvements, both in the form of reduced resistance levels and the emergence of new resistance risks.

These results show support for the effectiveness of Australian antibiotic stewardship programs, and prescribing guidelines. The genotypic identification provides a valuable database to be leveraged in future studies. But effective, sustained, and cooperative efforts are needed not only among the One Health sectors but also among all stakeholders in the farm-to-fork pathway to ensure food safety practices are implemented through the whole chain and appropriate antibiotic use is practiced. This will be critical for maintaining or reducing the rates observed in this study in the future. Because this study did not investigate the source of resistant *Salmonella* detected among the retail meats (that is, whether it is from animal, human, or environmental origin) this is an area that would benefit from future cross-sector studies to help robustly identify sources and the potential AMR pressures.

For pork, far more isolates would need to be tested to provide more precise estimates of the true proportion of resistance to these antibiotics among *Salmonella* isolates. While the costs and benefits of generating that data would need to be considered for future studies, nationally representative prevalence and concentration data of any foodborne pathogen in a retail food commodity is of high value and benefit to Australia. This data is critical for accurate assessment and mitigation of foodborne risks (WHO 2021a). However, contemporary nationally representative data are lacking for Australian retail commodities. Generating this data for target foodborne pathogens and commodities over several years will significantly enhance the precision and reliability of risk assessments, leading to more effective and targeted interventions. This will improve public health protection, enhance regulatory frameworks and support the food industry.

Salmonella tables and figures

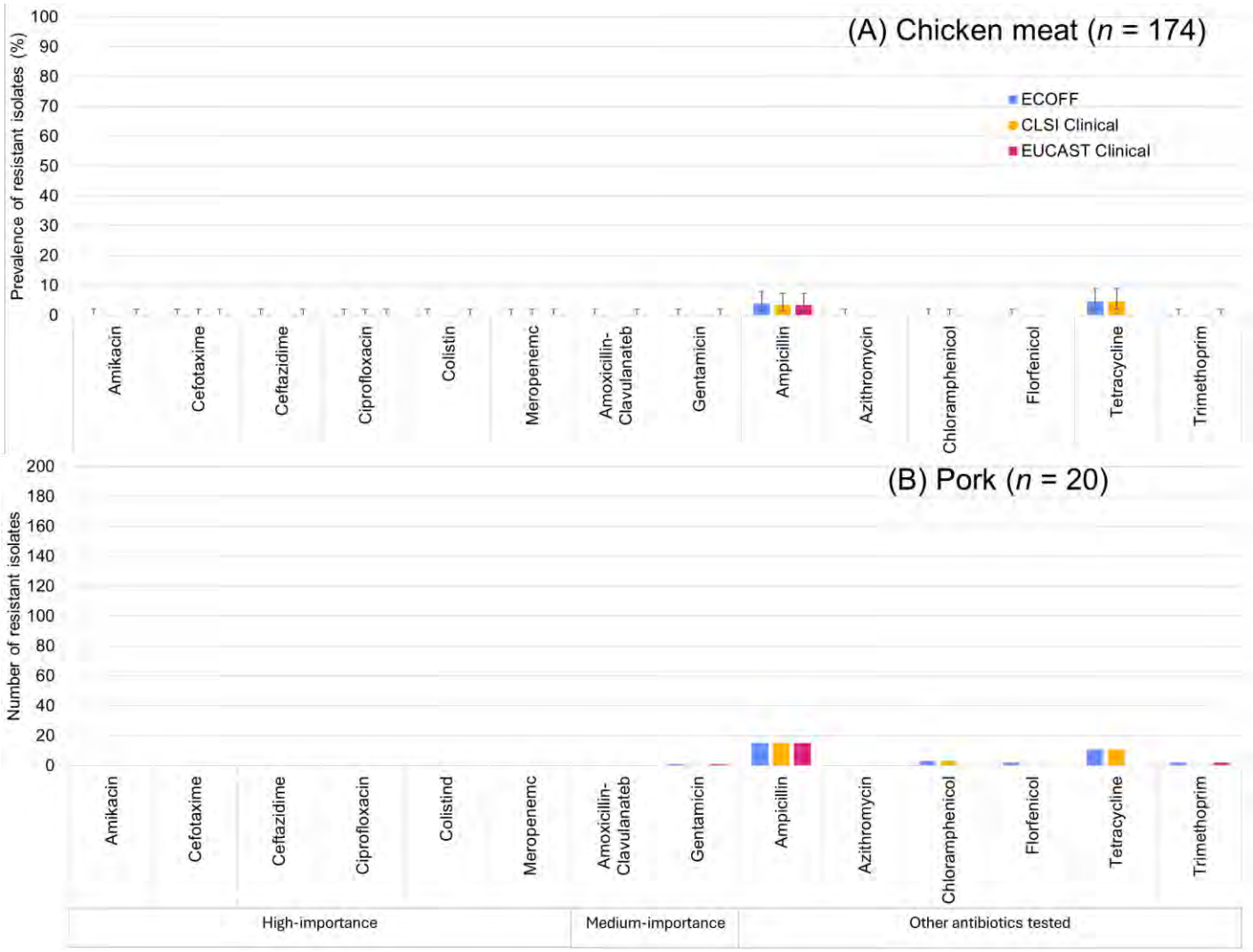


Figure 14: The rates of resistance to high-importance rated (ASTAG 2018), medium-importance rated (ASTAG 2018) and other antibiotics among *Salmonella* isolates from retail A) chicken meat (n = 174 isolates) with 95% CIs shown as error bars, B) The number of resistant isolates among 20 *Salmonella* isolates detected from raw retail pork. Prevalence of microbiological resistance based on ECOFF (blue), and clinical resistance based on CLSI (yellow) and EUCAST (pink) clinical breakpoints.

Table 6: Prevalence of microbiological resistance patterns for different antibiotic classes among *Salmonella* isolated from raw chicken meat and number of isolates for pork.

Chicken meat (*n* = 174 isolates)			Pork (*n* = 20 isolates)	
Phenotype (Pattern)	*n*	Prevalence (%)	Phenotype (Pattern)	*n*
0: nil	160	92.0	0: nil	4
1: bla	6	3.4	1: bla	4
1: tet	7	4.0	1: tet	1
2: bla_tet	1	0.6	2: bla_tet	8
			3: bla_fpi_phe	1
			3: bla_phe_tet	1
			5: ami_bla_fpi_phe_tet	1

Phenotype indicates the number of antibiotic classes with resistance present and each class. Abbreviations: n – number of isolates with associated phenotype, bla – B-lactams, tet – tetracyclines, fpi – folate pathway inhibitors, phe – phenicols, ami – aminoglycosides.

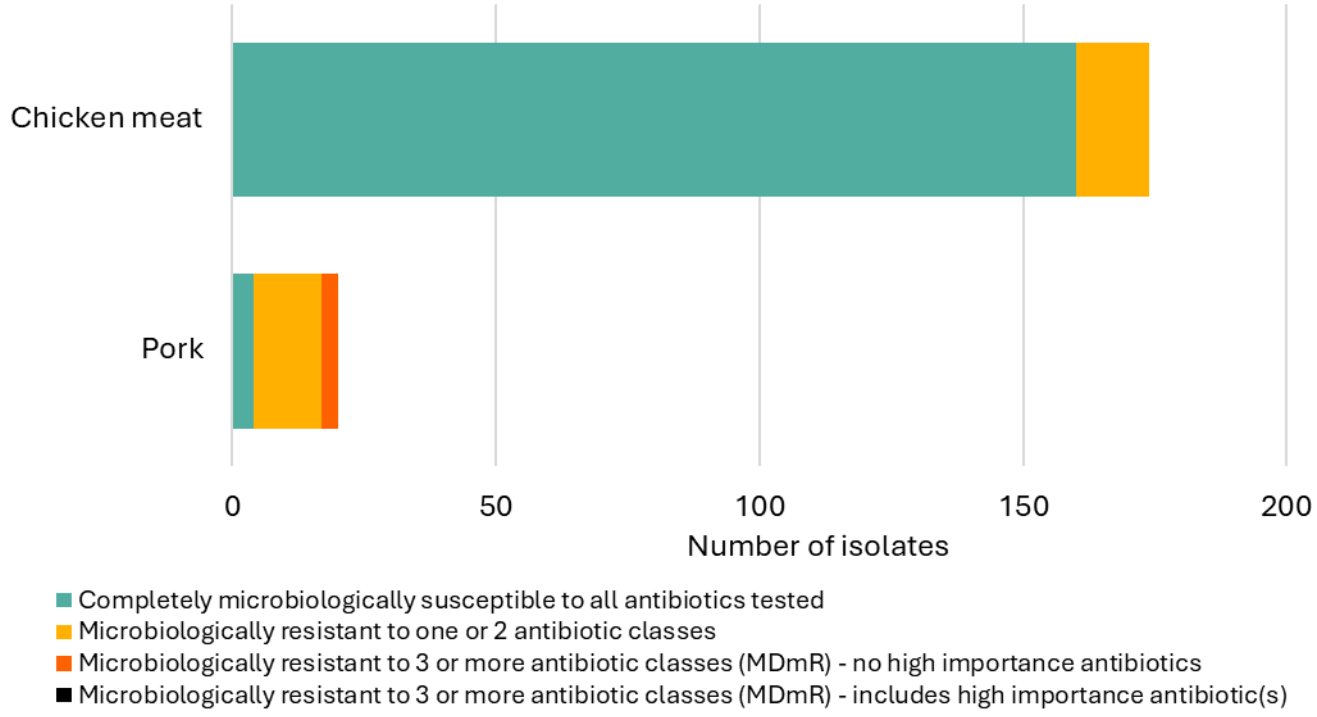


Figure 15: The number of isolates with complete microbiological susceptibility and MDmR for *Salmonella* isolates from raw retail chicken meat (n = 174) and pork (n = 20). Completely microbiologically susceptible (green), resistant to one or 2 classes of antibiotics tested (yellow), resistant to 3 or more antibiotic classes tested, not including high-importance antibiotics (red), and resistant to 3 or more antibiotic classes tested including high-importance antibiotics (black). MDmR with resistance to high-importance antibiotics was not detected in any *Salmonella* isolates.

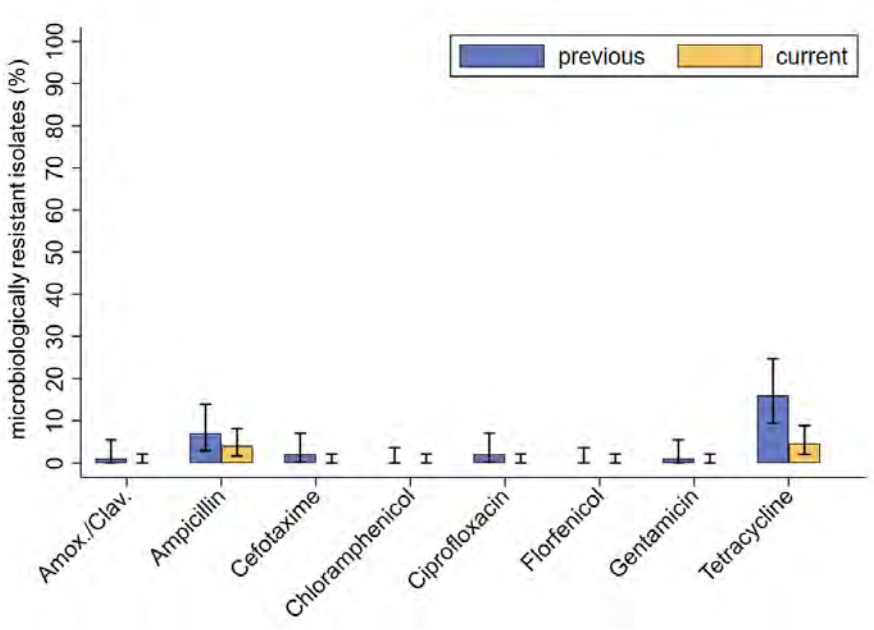


Figure 16: Reported rates of microbiological resistance among *Salmonella* spp. isolated from chicken meat between the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (n = 100) (blue, previous) and this study (n = 174) (yellow, current). 2007 results were reanalysed against the same ECOFFs used in the current study and 95% CIs for each data set shown as error bars (presented for information only and no statistical comparison was undertaken).

Campylobacter spp.

Campylobacter was included in AMR surveillance among raw retail chicken meat because:

- *Campylobacter* was expected to be sufficiently prevalent in raw chicken meat to provide robust estimates of AMR within the population; however, less so in pork or beef. No additional samples to investigate prevalence in beef and pork were included for this survey. Raw meat provides a direct snapshot of the bacterial populations, including resistant strains, that have entered the meat production chain (either from food animals, humans, or the environment) before being destroyed by effective cooking.
- *Campylobacter* species, particularly *Campylobacter coli* and *Campylobacter jejuni*, are leading causes of bacterial foodborne illness in Australia (Glass et al. 2023) and can act as reservoirs of resistance genes. These bacteria commonly inhabit the gastrointestinal tracts of food animals, especially poultry, and can contaminate meat during processing. While campylobacteriosis is typically self-limiting and does not require antibiotic treatment, antibiotics may be prescribed for immunocompromised individuals, young children, or the elderly who are at higher risk for severe illness (Wallace, Bulach et al. 2021).
- Monitoring *Campylobacter* in retail meat helps track AMR trends, inform control measures, and support efforts to mitigate the public health risks associated with the spread of resistant foodborne pathogens (WHO 2017).

Raw retail chicken meat sampling and detection of target bacteria

Raw retail chicken meat samples collected during the period between 27 March 2023 and 30 July 2023 were tested for *Campylobacter*. Nationally, *Campylobacter* was detected among 535 of 860 (62.2%) raw retail chicken meat samples. This was higher than the prevalence of 40.0% reported by Barlow and Gobius (2008) in the 2007 Australian AMR pilot study. A previous survey by FSANZ reported that 84.3% of carcass rinse samples collected postprocessing were positive for *Campylobacter* (FSANZ 2010). A study conducted between October 2016 and October 2018 across New South Wales, Queensland and Victoria detected *Campylobacter* spp. in 90% of 552 retail chicken meat samples (Walker et al. 2019). It was also reported that the Australian chicken meat industry typically finds an approximate division of 70% *C. jejuni* and 30% *C. coli* in chicken samples (Walker et al. 2019). However, as noted by Walker et al. (2019), different standard methods can be used to detect *Campylobacter* in food and variations in implementation that may affect recovery rates can occur between laboratories. Different enrichment broths and agars may affect recovery rates and these methodological differences make direct comparisons with other prevalence studies *of Campylobacter* among retail meat challenging (Walker et al. 2019).

Of the 535 *Campylobacter* isolates detected, 231 were confirmed as *C. coli* and 304 as *C. jejuni* by MALDI-TOF MS and these isolates were taken forward to AST.

Representativeness of data and testing for antibiotic resistance

Antimicrobial susceptibility profiles were successfully collected for 432 of the 535 *Campylobacter* isolates from chicken meat. The remaining isolates did not grow under the assay conditions in this study.

The broth MIC method was successful in determining the antibiotic susceptibility profiles of 207 *C. coli* isolates for 10 antibiotics representing 6 antibiotic classes (Table 13) and 225 *C. jejuni* isolates for 11 antibiotics representing 7 antibiotic classes (Table 13).

The testing period for *Campylobacter* from chicken meat (March 2023 to July 2023) was shorter than for the other target bacteria in this survey. This was because of recovery rate issues identified by jurisdictions and FSANZ at the beginning of 2023. In response, corrective actions were implemented between January and March 2023 to improve the applied methodology. Recovery rates subsequently improved to acceptable levels across all laboratories, supported by collaboration with contracted analytical labs. Modifications to the Australian Standard method, along with enhanced staff awareness and training, likely contributed to these improvements.

As noted previously, other Australian studies have noted the challenges related to *Campylobacter* isolation from different methods and implementation. Similarly, EFSA (2019) recently raised the challenges with the isolation of *Campylobacter* due to the use of varying methods between laboratories. This included differences in sample size, enrichment steps, temperature and culture media which can all affect how many bacteria are recovered, which species are detected and the diversity and antibiotic resistance profiles found. EFSA noted the lack of standardisation makes it hard to compare results across studies or countries and can lead to inconsistent estimates of *Campylobacter* prevalence and resistance. EFSA proposed that EU monitoring of *Campylobacter* should use harmonised methods for both isolation and antibiotic susceptibility testing, specifically recommending a protocol based on the European standard EN ISO 10272-1, to improve data comparability between their member states.

Greater than 200 isolates were collected for both *C. coli* and *C. jejuni*. This enabled robust estimation of AMR prevalence in *E. faecalis* from raw retail beef, chicken meat and pork. The AMR data for *Campylobacter* are considered representative of populations present in raw chicken meat sold in retail outlets within the greater metropolitan areas of Australian capital cities, which collectively comprise over 60% of the national population. However, not all cuts available were tested so the results may not reflect all raw meat products available in these areas.

The key results for *Campylobacter* spp. are discussed below; results have been summarised in the Key results summarised by species section below, and tables and figures presented in the *Campylobacter* tables and figures section below.

Comprehensive MIC distributions based on ECOFF, EUCAST and CLSI clinical breakpoints are provided in Supplementary Tables 19 and 20 for *C. coli* and *C. jejuni*, respectively. The rates of microbiological resistance and clinical resistance are presented in Figure 17 for each species. Figure 18 presents the rates of microbiological complete susceptibility and MDmR for each species.

Microbiological resistance to antibiotics

High-importance antibiotics

Ciprofloxacin is a quinolone which is an important antibiotic class for the treatment of not only campylobacteriosis in humans but also infections caused by other bacteria (ACSQHC 2023). Although nalidixic acid is not registered for any use in Australia (ASTAG 2018), it is also a quinolone, and its results are considered together with ciprofloxacin in this report.

In this study, low rates of microbiological resistance for ciprofloxacin and nalidixic acid (both 1.4%) were detected among raw retail chicken meat *C. coli* isolates. In contrast, moderate rates for ciprofloxacin (16.0%) and nalidixic acid (13.3%) were observed in *C. jejuni* isolates. The persistence and spread of quinolone resistant *Campylobacter* has been observed internationally, and although there are various hypotheses about why this occurs, a low fitness cost of resistance is likely a key contributor (Luo et al. 2005; Zeitouni & Kempf 2011; Goulart, Zhang & Sahin 2023; Zhang, Lin & Pereira 2003; Whelan et al. 2019; Piddock et al. 2003; Price et al. 2007; Han et al. 2012). When bacteria develop resistance to antibiotics, it can come with a trade-off – meaning the mutation or mechanism

that provides resistance also reduces the bacteria's overall fitness (for example, slower growth, reduced ability to compete with non-resistant strains, or increased energy costs) (Bengtsson-Palme, Kristiansson & Larsson 2018; Koutsoumanis et al. 2021; Vanacker, Lenuzza & Rasigade 2023). *C. jejuni* acquires quinolone resistance through point mutations in QRDR regions in *gyr*A (for example, Thr86Ile) (Piddock et al. 2003), and horizontal gene transfer is unlikely to play a significant role in spreading this type of resistance between bacterial species (Jeon et al. 2008). This resistance has not been linked to a fitness cost for quinolone resistant *C. jejuni,* and studies have demonstrated that resistant *C. jejuni* strains may have a unique advantage to persist and spread even without antibiotic pressure (Luo et al. 2005; Zeitouni & Kempf 2011; Goulart, Zhang & Sahin 2023; Zhang, Lin & Pereira 2003; Whelan et al. 2019; Han et al. 2012).

These results support the need for ongoing surveillance of *Campylobacter* across the food chain. The regular reporting of AMR associated with human infections from *Campylobacter* would be welcomed by FSANZ to assist in determining any evolving foodborne risk of AMR from this microorganism.

Australian human and live broiler chicken context: AMR rates among *Campylobacter* from human clinical specimens in Australia were not reported in the national 2021 report (ACSQHC 2023). A recent Australian study investigated AMR in 137 *C. jejuni* and 27 *C. coli* isolates collected from gastroenteritis patients across 8 Australian states and territories between October 2018 and February 2019. Similar rates of AMR were reported for the 2 species for ciprofloxacin (*C. coli* ~22%, *C. jejuni* ~20%) (Wallace, Bulach et al. 2021). Quinolones have never been registered for use in food-producing animals in Australia (ASTAG 2018). Despite this, ~14–24% and ~3–5% AMR for ciprofloxacin and similar levels for nalidixic acid have been reported among *C. jejuni* and *C. coli* isolates, respectively, from Australian live broiler chickens (Abraham et al. 2020; ACMF 2022). Australia's ban on using quinolones in food-producing animals, strict border controls and the country's uniquely isolated geographic location have been suggested to have contributed to in comparatively lower prevalence of quinolone resistant *C. jejuni* in both human and poultry isolates than in other countries (Owiredu et al. 2025; Wallace, Bulach et al. 2021).

Medium-importance antibiotics

Microbiological resistance to gentamicin was not detected among any *Campylobacter* species from retail chicken meat in this study. Very low rates of clindamycin microbiological resistance were detected for both *Campylobacter* species (0.9–1.0%). This suggests a low risk of *Campylobacter* among raw retail chicken meat contributing to the spread of AMR related to these antibiotics.

Australian human and livestock context: Gentamycin is not registered for use in food-producing animals in Australia and not detected to low AMR rates have been reported in Australian human and livestock isolates (Abraham et al. 2020; ACMF 2022; Wallace, Bulach et al. 2021). Clindamycin is registered for use in both humans and chickens in Australia (ASTAG 2018).

Other antibiotics

The macrolides azithromycin and erythromycin are common registered treatments for certain bacterial infections in humans and food-producing animals respectively (ASTAG 2018). Macrolides are also effective antibiotics for treating human campylobacteriosis (Moffatt et al. 2021). In this study, microbiological resistance to azithromycin or erythromycin was low overall for both *Campylobacter* species (0.4–1.4%), indicating that these antibiotics should be effective against the majority of *Campylobacter* isolates detected from chicken meat. Additionally, rates of microbiological resistance to chloramphenicol, florfenicol, streptomycin, and telithromycin (tested in *C. jejuni* only) were also low overall for both species (not detected–0.5%).

Tetracycline microbiological resistance was observed at moderate levels (16.4%) for *C. jejuni* and low levels (1.4%) for *C. coli* isolates for raw retail chicken meat in this study.

Australian context: Azithromycin, chloramphenicol, erythromycin, florfenicol, streptomycin, tetracycline, and telithromycin (tested in *C. jejuni* only) are currently low-importance antibiotics in Australia (ASTAG 2018). Among these, erythromycin, florfenicol, streptomycin, and certain tetracyclines are approved for use in animals in Australia and also classified as VCIA by WOAH (WOAH 2024a; ASTAG 2018). Not detected to low AMR rates have been reported for azithromycin, erythromycin, chloramphenicol, florfenicol, streptomycin and telithromycin among both Australian human clinical and livestock *Campylobacter* isolates for these antibiotics (Abraham et al. 2020; ACMF 2022; Wallace, Bulach et al. 2021). AMR rates among human *C. jejuni* and *C. coli* isolates for tetracyclines have been reported at ~15% and ~11% respectively, and ~22% and ~3% respectively for live broiler chickens (Abraham et al. 2020; ACMF 2022; Wallace, Bulach et al. 2021).

Historical comparison with 2007 results

Trend analysis over time (e.g. increasing resistance) was not possible in this study, as different methods and sampling approaches from those in the survey by Barlow and Gobius (2008) were used. These changes were made to align with current international guidance. However, a cautious comparison of similarities and differences is still possible and may offer useful insights. To provide some context, the MIC data from Barlow and Gobius (2008) were reanalysed for antibiotics tested at sufficient concentrations. This was done using the current ECOFFs and the same method for calculating CIs as in the current study. Based on this reanalysis, ciprofloxacin and nalidixic acid resistance rates for *C. jejuni* were noted[11] as reported higher in the current dataset than those reported in 2007 (Figure 20). These observations should be interpreted with caution given methodological differences.

Microbiological complete susceptibility and multidrug resistance

Microbiological complete susceptibility rates (all antibiotics tested were effective at inhibiting growth at the ECOFF) among *C. coli* and *C. jejuni* from raw retail chicken meat samples were very high (97.0%; 201/207, and 79.6%; 179/225, respectively). This means the majority of *Campylobacter* isolates detected in this study should not have acquired resistance to any of the antibiotics tested.

MDmR was only observed in two *Campylobacter* isolates from chicken meat samples (one each for *C. coli* [0.5%; 1/207] and *C. jejuni* [0.4%; 1/225]) in this study. These isolates were resistant to macrolides, quinolones and tetracyclines meaning the usual treatment options may be limited for these isolates should they cause infection, but the prevalence is very low. The *C. coli* isolate did not have any known resistance genes that matched the phenotype detected. The *C. jejuni* isolate harboured resistance determinants matching the phenotype.

Australian context: Microbiological complete susceptibility and resistance to multiple antibiotics have been suggested as key summary indicators for AMR (ECDC, BIOHAZ & CVMP 2017). However, data from humans and livestock in Australia are not included here due to methodological differences and lack of harmonisation in antibiotic panels, which limit comparability. This is also true for the survey by Barlow and Gobius (2008). MDmR was defined in the current study as microbiological resistance to three or more antibiotic classes, with resistance to a single antibiotic assumed to represent resistance to the entire class. In contrast, Barlow and Gobius (2008) reported resistance phenotypes based on combinations of individual antibiotics without grouping them by class. To improve future surveillance, studies should harmonize antibiotic panels, apply consistent class-

[11] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

based definitions of MDmR, and transparently document assumptions about cross-resistance and classification criteria. The values reported in Barlow and Gobius (2008) for *C. jejuni* among retail chicken meat were 95% (57/60) with no resistance to any antibiotic tested and 3% (2/60) resistant to three or more antibiotics. The values reported for *C. coli* among retail chicken meat were 95% (38/40) with no resistance to any antibiotic tested and 2.5% (1/40) resistant to three or more antibiotics.

Known genetic resistance determinants

This report focused the genetic analysis on isolates exhibiting resistance to high-importance antibiotics and MDmR. These isolates were subject to short-read WGS to detect known resistance determinants. Additional comprehensive analyses are planned for future publications.

Three *C. coli* isolates were selected for WGS sequencing, all microbiologically resistant for quinolones. The *C. coli* isolates were ST6775, ST6184 and ST1181. ST1811 was also MDmR for aminoglycosides + macrolides + phenicols + quinolones + tetracyclines. Only ST6184 had a known associated resistance mutation for quinolones: *gyr*_T86I. ST1811 only had a *bla*OXA-193 mutation detected. Limited background information was found on these STs.

A total of 38 *C. jejuni* isolates were selected for sequencing, all resistant for quinolones. There were 11/38 isolates that failed sequencing quality control (QC). These isolates had estimated genome sizes that were twice the size expected and were either unable to be assigned a ST, or multiple STs were identified in the single sample. This indicates that these samples likely included multiple strains of *C. jejuni* that could not be separated in the laboratory due to the swarming nature of *Campylobacter* growth.

A summary of the *C. jejuni* STs for each commodity is provided in Figure 19. Of the 27 isolates that passed QC, there was a diverse range of STs with the STs with the highest proportion being ST1078, ST7323 and ST2895. These STs have previously been isolated from Australian chicken livestock and have been associated with quinolone resistance (ACMF 2022). While quinolone resistant ST7323 isolates have been identified from Australian chicken caeca in the past, ST2895 and ST1078 were not identified in that study (Abraham et al. 2020). *C. jejuni* ST50 accounted for 1 of the isolates tested. This ST is associated with disease in humans, but previous studies have identified Australian isolates to be unique as they did not carry the resistance genes or mutations seen on international isolates (tetracycline, β-lactam, and quinolone) (Wallace, Cribb et al. 2021; Cribb et al. 2024). While the isolate had quinolone and tetracycline microbiological resistance, no mutations in associated quinolone-resistance genes were identified; however, a tetracycline-resistance associated gene, *tet*(O), was present. Of the 27 *C. jejuni* isolates, 25 had the *gyr*A_T86I mutation identified, known to confer quinolone resistance. The single MDmR isolate was ST2895, and was MDmR for macrolides + quinolones + tetracyclines and had known genetic determinants associated with resistance: *gyrA*_T86I, *rplV*_A103V, and *tet*(O).

In summary, genetic analysis of *Campylobacter* isolates from raw chicken meat that were microbiologically resistant to high-importance antibiotics or MDmR revealed diverse strains. Most *C. jejuni* isolates carried a known mutation linked to quinolone resistance. Some STs are internationally distributed, while others are less commonly reported. Genomic surveillance enables tracking of these strains across regions and commodities, helping to monitor emerging resistance.

Key results summarised by species

The key results from above are summarised here by *Campylobacter* species for interested readers.

Campylobacter coli

The rates of resistance for 10 antibiotics representing 6 antibiotic classes among 207 *C. coli* isolates from chicken meat (Figure 17) were:

- High-importance antibiotics: resistance was low (ciprofloxacin low [1.4%], nalidixic acid low [1.4%]).
- Medium-importance antibiotics: resistance was very low to low (gentamicin very low [0.5%], and clindamycin low [1%]).
- Low-importance antibiotics: resistance was rare to low (chloramphenicol not detected [< 0.1%, rare], florfenicol and streptomycin very low [both 0.5%], erythromycin low [1%], and azithromycin and tetracycline low [both 1.4%]).
- Of the antibiotics tested in both this study and the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (Figure 20), there were no notable results[12].

The rate of microbiological complete susceptibility among chicken meat *C. coli* isolates to all antibiotics tested was extremely high (201/207 isolates, 97.1%) (Table 7, Figure 18).

MDmR (resistance to 3 or more antibiotic classes) was very low (one of 207 isolates, 0.5%), with this single isolate MDmR resistant to aminoglycosides + macrolides + phenicols + quinolones + tetracyclines.

Five unique patterns of resistance for 1–5 of the 6 antibiotic classes were observed among isolates. The 4 most prevalent antibiotic-class resistance combinations in chicken meat *C. coli* isolates included classes with high-importance rated antibiotics (macrolides [2 isolates, 1%], quinolones, tetracyclines, and quinolones + tetracyclines [each 1 isolate, 0.5%]).

Campylobacter jejuni

The rates of microbiological resistance for 11 antibiotics representing 7 antibiotic classes among 225 *C. jejuni* isolates from chicken meat (Figure 17) were:

- High-importance antibiotics: resistance was moderate (ciprofloxacin moderate [16%] and nalidixic acid moderate [13.3%]).
- Medium-importance antibiotics: resistance was rare to very low (gentamicin not detected [< 0.1%, rare], and clindamycin very low [0.9%]).
- Low-importance antibiotics: resistance was rare to moderate (chloramphenicol, florfenicol, streptomycin, and telithromycin not detected [< 0.1%, rare], erythromycin and azithromycin very low [both 0.4%], and tetracycline moderate [16.4%]).
- Of the antibiotics tested in both this study and the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (Figure 20), ciprofloxacin and nalidixic acid resistance were noted[13] as reported higher in the current dataset when reanalysed using current ECOFFs. As mentioned earlier, these observations are indicative only and should not be interpreted as a formal trend analysis.

[12] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

[13] Notable results were based on the simplistic measure of confidence intervals of the two studies that did not overlap, and do not represent statistical significance.

The rate of microbiological complete susceptibility for chicken meat *C. jejuni* isolates to all antibiotics tested was extremely high (179/225 isolates, 79.6%) (Table 7, Figure 18).

MDmR (resistance to 3 or more antibiotic classes) was very low (one of 224 isolates, 0.4%) with this single isolate resistant to macrolides + quinolones + tetracyclines.

Five unique patterns of resistance to 1–3 antibiotic classes were observed. The 4 most prevalent antibiotic-class resistance combinations in chicken meat *C. jejuni* isolates included classes with high-importance rated antibiotics. These combinations were quinolones + tetracyclines (28 isolates, 12.4%); quinolones only (8 isolates, 3.6%); tetracyclines only (8 isolates, 3.6%); and macrolides + quinolones + tetracyclines (one isolate, 0.4%).

Key messages

The rare (not detected) microbiological resistance to the majority of high-importance rated antibiotics, very high rates of microbiological complete susceptibility, and overall very low microbiological resistance to low-importance rated antibiotics among *Campylobacter* spp. detected from raw retail chicken meat indicate a reduced risk of foodborne transmission of resistant bacteria that may become involved in infections or spread resistance.

The moderate levels of ciprofloxacin microbiological resistance detected in *C. jejuni* isolates from raw retail chicken meat align with similar findings in Australian human and live broiler chickens (meat chickens raised for consumption) samples. The fact that Australia has never registered quinolones (i.e., ciprofloxacin) for use in food-producing animals, along with Australia's strict border controls and isolated geographic location have been suggested to have contributed to comparatively lower prevalence of quinolone resistant *C. jejuni* in both human and poultry isolates than in other countries. However, the apparent increase in resistance indicates the need for continued surveillance, strong antibiotic stewardship and effective implementation of food safety measures relevant to this foodborne pathogen.

For consumers, these bacteria are common causes of campylobacteriosis because they live in the intestines of animals, especially poultry, and can contaminate food, water and surfaces. However, they are also easily killed in raw chicken by appropriate cooking methods, and cross contamination can be limited by safe food handling. For most people, campylobacteriosis infections are mild and do not require antibiotics. Antibiotics are only recommended for cases of severe illness or for people with risk factors for severe illness. Moderate resistance to ciprofloxacin in *C. jejuni* means that some bacteria found in chicken meat may not respond to this antibiotic, However, the macrolide – azithromycin – is an effective common treatment of human campylobacteriosis and resistance to macrolides was not detected or very low for *Campylobacter* in this study.

Both the prevalence data on *Campylobacter* in food and the AMR data generated by this study are critical for accurate assessment and mitigation of foodborne risks (WHO 2021a). Generating this data over several years will significantly enhance the precision and reliability of Australian risk assessments, leading to more effective and targeted interventions. This will contribute to improving public health protection, enhance regulatory frameworks and support the food industry.

Campylobacter tables and figures

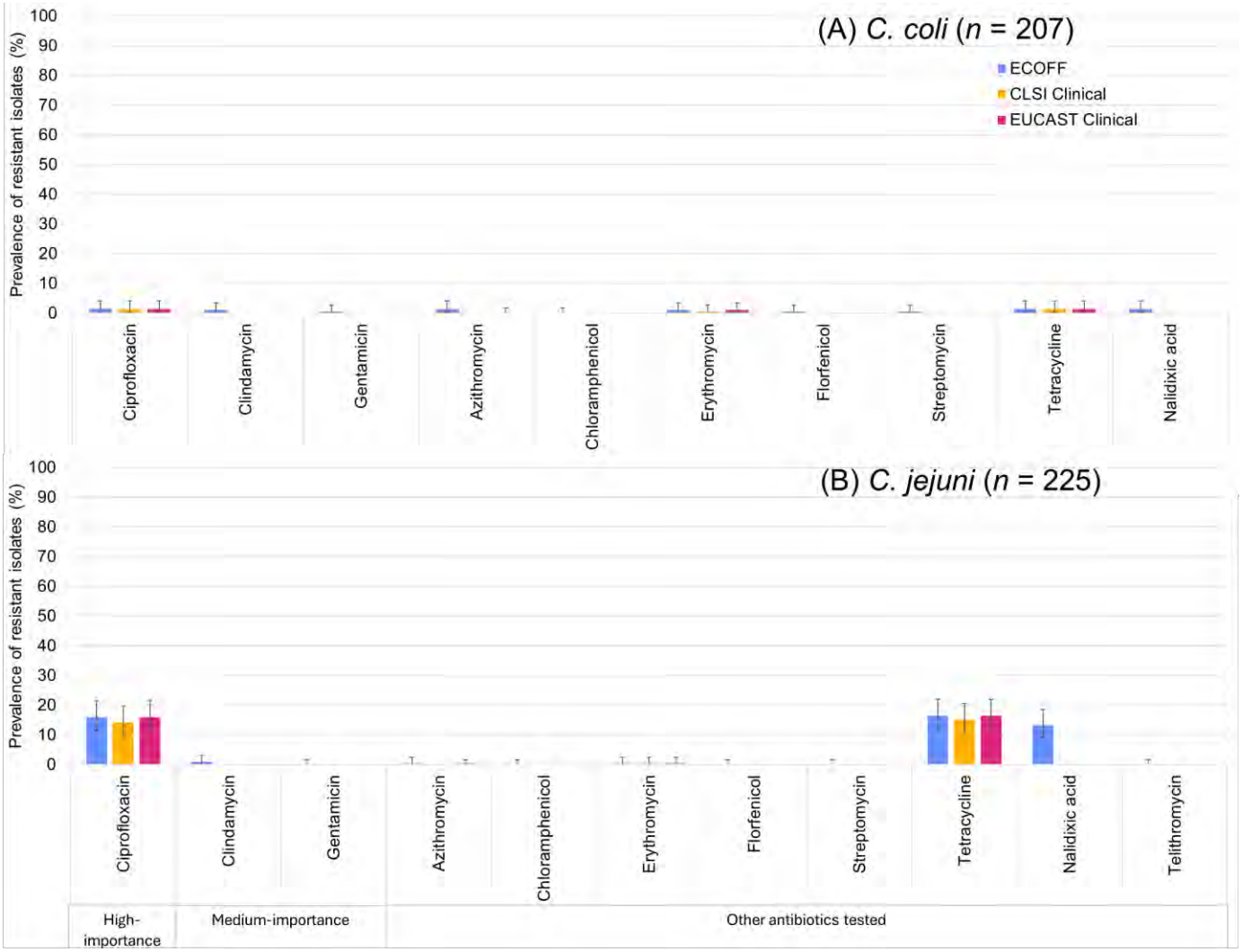


Figure 17: The rates of resistance to high-importance rated (ASTAG 2018), medium-importance rated (ASTAG 2018) and other antibiotics among chicken meat *Campylobacter* isolates for A) C. coli (n = 207 isolates), B) C. jejuni (n = 225 isolates). Prevalence of microbiological resistance based on ECOFF (blue), and clinical resistance based on CLSI (yellow) and EUCAST (pink) clinical breakpoints. 95% CIs shown as error bars.

Table 7: Prevalence of microbiological resistance patterns for different antibiotic classes among *Campylobacter* isolated from raw retail chicken meat.

C. coli (n = 206)			*C. jejuni* (n = 225)		
Phenotype (Pattern)	*n*	Prevalence (%)	Phenotype (Pattern)	*n*	Prevalence (%)
0: nil	201	97.1	0: nil	179	79.6
1: mac	2	1	1: qui	8	3.6
1: qui	1	0.5	1: tet	8	3.6
1: tet	1	0.5	2: mac_qui	1	0.4
2: qui_tet	1	0.5	2: qui_tet	28	12.4
5: ami_mac_phe_qui_tet	1	0.5	3: mac_qui_tet	1	0.4

Phenotype indicates the number of antibiotic classes with non-wild type/resistance present and each class.
Abbreviations: n – number of isolates with associated phenotype, mac – macrolides, qui – quinolones, tet – tetracyclines, ami – aminoglycosides, phe – phenicols.

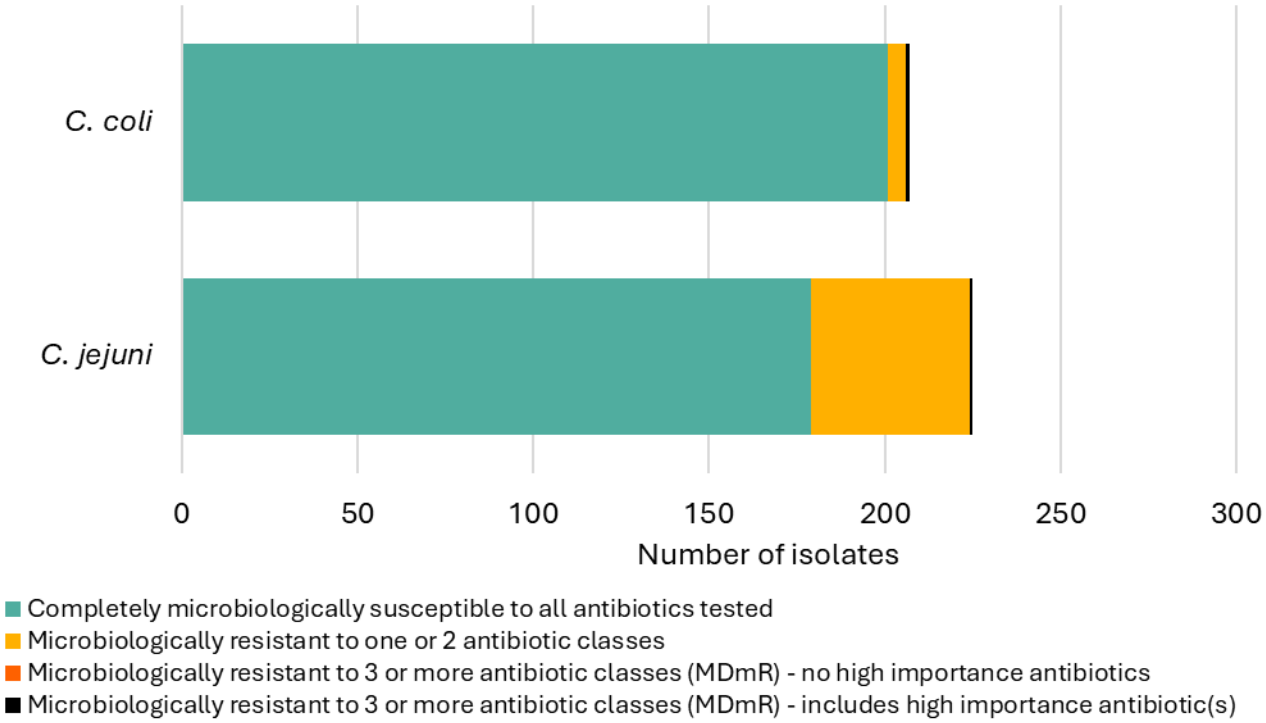


Figure 18: The number of isolates with complete microbiological susceptibility and MDmR for *Campylobacter* isolated from raw retail chicken meat (*C. coli* [n = 207 isolates], *C. jejuni* [n = 225 isolates]).Completely microbiologically susceptible (green), resistant to one or 2 classes of antibiotics tested (yellow), resistant to 3 or more antibiotic classes tested, not including high-importance antibiotics (red), and resistant to 3 or more antibiotic classes tested including high-importance antibiotics (black). MDmR without resistance to high-importance antibiotics was not detected in any *Campylobacter* samples.

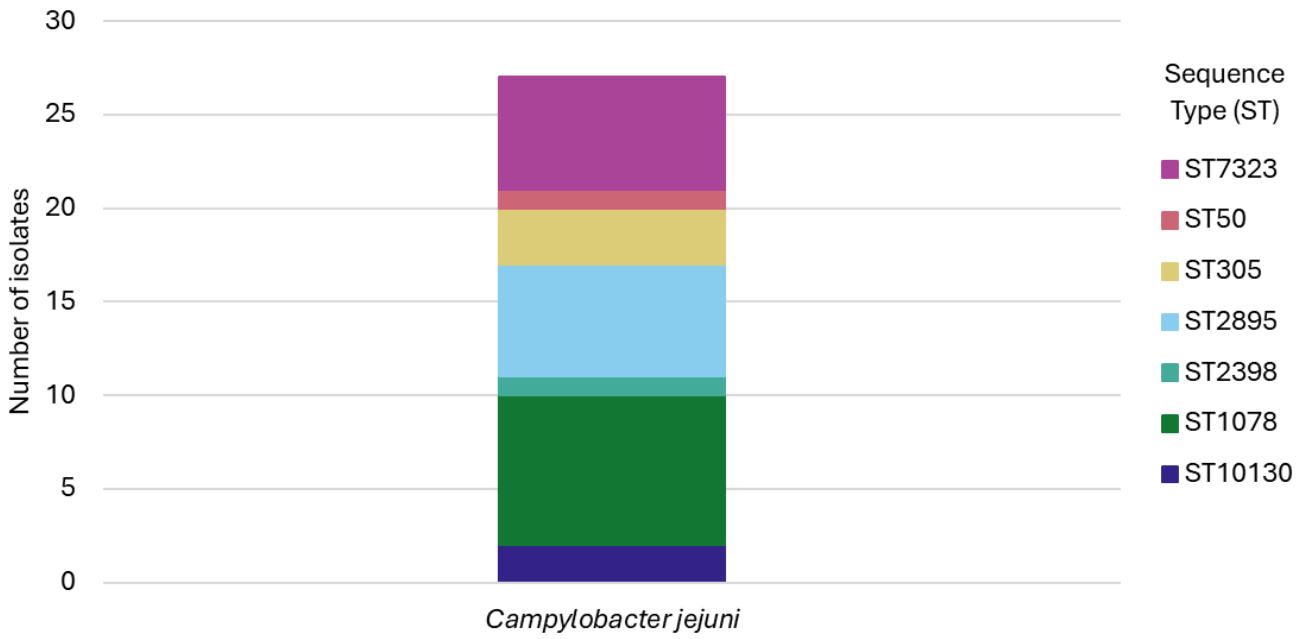


Figure 19: The number of different STs determined by WGS among *Campylobacter jejuni* isolated from raw retail chicken meat that were either microbiologically resistant to at least one high-importance rated antibiotic and/or were MDmR.

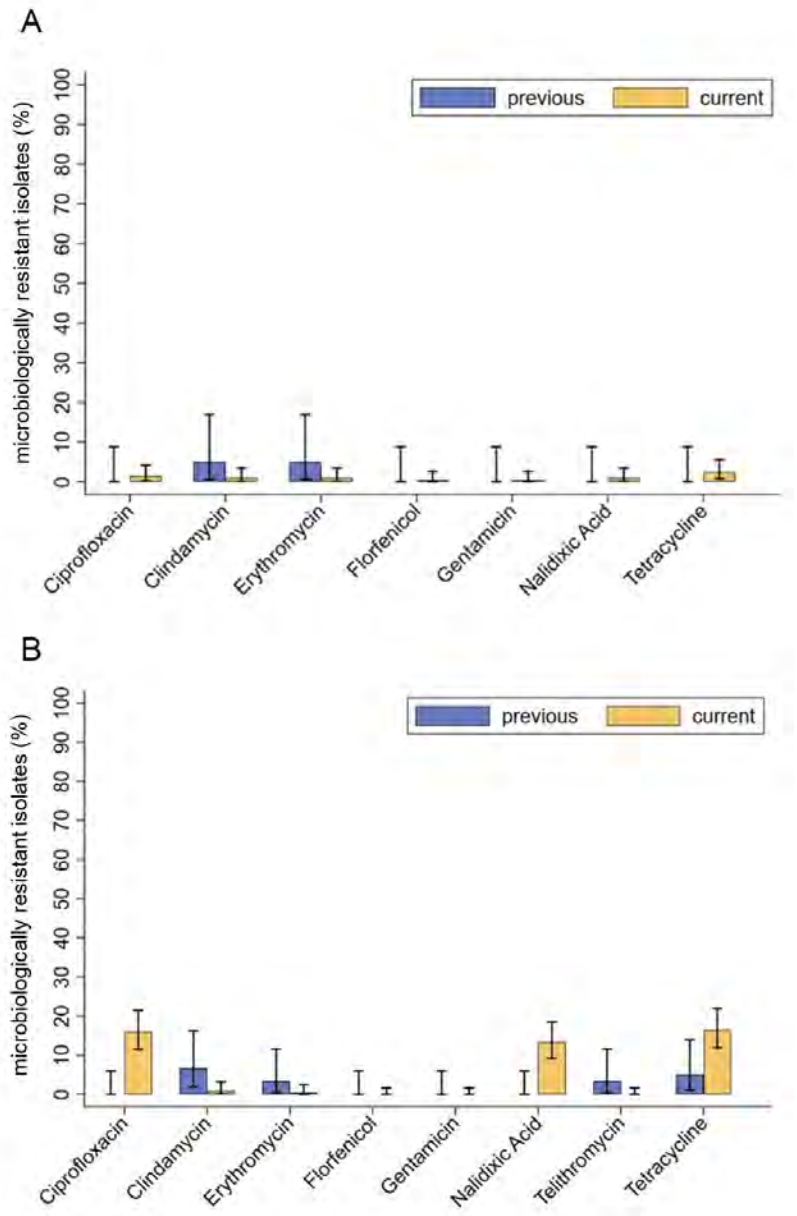


Figure 20: Reported rates of microbiological resistance among *Campylobacter* spp. isolated from chicken meat between the 2007 Australian AMR pilot study (Barlow & Gobius 2008) (blue, previous) and this study (yellow, current). (A) Reported rates of microbiological resistance among *C. coli* isolates from the 2007 study (n = 40) and the current study (n = 207). (B) Reported rates of microbiological resistance among *C. jejuni* isolates from the 2007 study (n = 60) and the current study (n = 225). Only antibiotics tested in both studies are included. 2007 results were reanalysed against the same ECOFFs used in the current study and 95% CIs shown as error bars (presented for information only and no statistical comparison was undertaken).

Conclusion

A nationwide survey of AMR among bacteria isolated from Australian raw retail beef, chicken and pork meat samples was completed between September 2022 and July 2023. The study provides a comprehensive national snapshot of AMR in foodborne and commensal bacteria among raw retail beef, chicken and pork meat. *Escherichia coli* and *Enterococcus* spp. were isolated as indicator organisms for AMR because they are common commensal bacteria of the human and animal gut. These bacteria can be indicators of emerging and persistent resistance and can contribute to the tracking of AMR across sectors. The study also targeted two key foodborne pathogens, *Salmonella* (chicken meat and pork) and *Campylobacter* spp. (chicken meat only).

Overall, there is a low risk of bacteria from these raw retail meats being involved in resistant infections or spreading resistance when safe primary production, processing, cooking and food handling is practiced. Key findings included:

- Rare to low microbiological resistance was detected for high-importance antibiotics critical for treating human infections. The only exception was moderate ciprofloxacin resistance observed among *C. jejuni*, but resistance to other macrolide antibiotics commonly used to treat human campylobacteriosis was rare to low.
- High rates of complete microbiological susceptibility to all antibiotics tested were common across all bacteria and commodities.
- Low levels of multidrug microbiological resistance (MDmR) were mostly observed. Most MDmR in *E. coli* was linked to low-importance antibiotics, indicating that alternative treatment options remain available.
- Resistance to antibiotics considered to be low-importance antibiotics for human medicine but that are often critical in veterinary contexts was consistent with expectations based on the 2007 pilot study by Barlow and Gobius.

These findings are broadly consistent with the survey by Barlow and Gobius (2008) and recent surveillance of Australian livestock animals, showing support for the effectiveness of current antibiotic stewardship practices in food-producing animals.

Note:

High-importance antibiotics means these are essential antibiotics for the treatment or prevention of infections in humans where there are few or no treatment alternatives for infections.

Low-importance antibiotics means there are a reasonable number of alternative antibiotics in different classes available to treat or prevent most human infections even if AMR develops.

When describing resistance rates ‘rare’ means not detected, ‘low’ means < 10%, and ‘moderate’ means 10–20%.

Moderate levels of quinolone (ciprofloxacin) microbiological resistance were detected in *Campylobacter jejuni* isolates from raw retail chicken meat. This aligns with global trends and findings from Australian human clinical and live broiler chicken samples. Importantly, quinolones have never been registered for use in Australian livestock, highlighting the unique global challenge of quinolone-resistant *Campylobacter*.

Terminology note:

Microbiological resistance means an antibiotic did not work against bacteria, based on a defined cut-off, suggesting acquired resistance.

Complete microbiological susceptibility means all antibiotics tested worked against the bacteria.

MDmR means bacteria were microbiologically resistant to three or more antibiotic classes.

While the earlier study by Barlow and Gobius (2008) reported similar AMR levels, the two studies differ in design and methodology, and the datasets are not directly comparable. Therefore, no definitive trends can be concluded. For antibiotics tested in both studies, resistance levels were generally similar, except for quinolone resistance in *C. jejuni*, which were noted as reported higher in the current dataset. These observations should be interpreted cautiously given methodological differences. This underscores the need for more frequent and harmonised surveillance to accurately detect both improvements in the form of reduced resistance levels and the emergence of new resistance risks.

The study also highlights the interconnectedness of human, animal and environmental health. Low-importance human antibiotics are still common first-line treatments but are often critical in veterinary medicine. Differences in resistance profiles across meat types and bacteria for these antibiotics emphasise the importance of coordinated One Health efforts. Sustained antibiotic stewardship and food safety practices from farm to fork are essential to preserve antibiotic effectiveness and protect public health.

Although this study was not designed to determine the origin of bacteria on meat products, the genomic database developed provides a valuable resource for future research. Cross-sector collaboration is encouraged to explore transmission pathways and inform holistic AMR management strategies. The database developed in this study provides a valuable resource for Australian research, and organisations are encouraged to contact FSANZ to discuss potential research projects, particularly cross-sector research, which could be of benefit nationally and internationally.

Finally, while AMR bacteria were detected in raw meat, it is important to note the same proper food safety practices used to prevent foodborne illness can effectively mitigate risks associated with AMR bacteria in food. The bacteria found in this study are easily made harmless through effective cooking, and cross-contamination is reduced through safe food handling. Public awareness initiatives on safe food production, food handling, proper cooking temperatures and cross-contamination prevention could further reduce the likelihood of both foodborne illness and foodborne AMR transmission.

This study strengthens Australia's One Health AMR surveillance framework and reinforces the need for ongoing monitoring, collaborative action and sustained stewardship to protect human and animal health, food safety and food security into the future.

Materials and methods

Sampling

The sampling design elements described by Codex Alimentarius Commission (Codex) and the World Health Organization (WHO) (WHO 2017; Codex Alimentarius Commission 2021) were considered in the development of the surveillance plan, including the sampling strategy, target commodity/organism/antibiotics, epidemiological units, frequency of sampling, statistical power, required sample size, selection of strata, metadata and procedures for storage and transport.

Prioritising food commodities

In prioritising food commodities for this study, FSANZ considered:

- epidemiological and public health factors associated with food
- consumption and production patterns
- the likely prevalence of target organisms
- the origin of food and likely prevalence of AMR
- food where data may be limited
- food where comparison to other data may be possible.

A prioritisation matrix using a scoring approach was implemented to rank eggs, dairy, seafood, horticulture, beef, chicken meat and pork. This resulted in the selection of beef, chicken meat and pork as the priority commodities that resources allowed to be assessed in this study. Other commodities are intended to be tested if funding is made available in the future.

Sample sizes

There were 3 scenarios considered when determining the required number of raw meat samples:

a) Detecting the proportion of resistant isolates in a population.
b) Detecting the emergence of resistance (the probability of detecting at least one isolate as resistant in a population).
c) Determining increasing or decreasing trends if the study is repeated in the future.

The target number of isolates required was estimated based on the assumptions of binomial probabilities, an infinite population, a prevalence of resistance (50%), a desired alpha value of 0.05 (critical value of 1.96) and a desired level of absolute precision (this refers to the margin of error around the estimate of prevalence and describes how close the estimate is likely to be to the true value in the population, based on the chosen 95% confidence level) (Cannon and Roe 1982) using the following equation:

$$sample\ size = \left(\frac{critical\ value}{absolute\ precision}\right)^2 * prevalence\ * (1 - prevalence\ )$$

A target of 200 bacterial isolates for AST per commodity/bacteria species combination was chosen to balance affordability, accuracy, precision and power for statistical analysis for this study. Increasing the target number of isolates from the 100 targeted in Barlow and Gobius (2008) to 200 can result in noticeable improvements in precision. When assuming a prevalence of 50% (P = 0.5), increasing the sample size from 100 to 200 isolates reduces the margin of error (absolute precision) from ±9.8% to ±6.93% at a 95% confidence level. This represents an improvement in precision of approximately 29%, following the inverse square root relationship between sample size and margin of error.

If the sample size is too small, this results in large margins of error and wide CIs. For example, with smaller sample sizes of 50 isolates, the wide CIs (for example, 36.1% to 63.9% for an estimate of 50% prevalence) indicate low precision, meaning the estimate of prevalence could vary widely from the true population value, which limits confidence in the result and the use of it to inform decision-making.

Additionally, increasing the targeted number of isolates from 100 to 200 raises the chance of detecting at least one positive from ~63% to ~87%, assuming a true prevalence of 1% based on the equation below:

$$Probability\ of\ detecting\ 1\ isolate = 1 - (1 - True\ prevalence)^{\ sample\ size}$$

Targeting 200 isolates for AST also represents an internationally accepted value to ensure statistical robustness and defensibility of results (WHO 2021b; EFSA et al. 2019). This is based on statistical assumptions of binomial probabilities and an infinite population (Cannon & Roe 1982).

The number of retail meat samples needed for each 'commodity + bacteria' species combination to detect 200 isolates was based on the estimated prevalence from previous studies of the bacteria in the commodity of interest and advice from the ESAG.

Sample types

Food samples were purchased at different retailers to reflect Australian consumer buying patterns. Samples were collected at proportions of 60% from large supermarkets, 20% from small supermarkets and 20% from butchers, based on industry data and advice from the ESAG (Table 8).

The types of food samples purchased were selected to reflect consumption patterns and to maximise the chance of bacterial isolation. Chicken Maryland with skin on, beef mince and pork mince were collected in the first instance, and if not available, reserve types were collected (Table 8) to reduce the need to go to a different retailer.

All raw chicken and pork collected in this survey was Australian, as imports of these products are not permitted for sale in Australia due to biosecurity restrictions. While raw boneless pork may be imported, it must be cured or processed before being released for sale. All packaged raw beef in the survey was Australian. Although raw beef imports are permitted from approved countries, volumes are small and it is unlikely the survey included imported unpackaged beef.

Sample allocation and randomisation

Raw meat samples, including beef, chicken meat and pork, were purchased from retailers across all Australian jurisdictions for analysis. The number of samples collected in each jurisdiction was weighted by population, using September 2020 population data (ABS 2020), to ensure the dataset was as nationally representative as practically achievable. Due to practical and financial constraints, statewide or territory-wide sampling was not feasible. Instead, sampling was conducted in the metropolitan (or 'greater') area of the capital city within each jurisdiction. Local government areas with population densities of less than 100 people per square kilometre were excluded from the sampling process to maintain achievable driving distances for sample collection in greater metropolitan areas.

For each jurisdiction, the different 'commodity + retailer + organism' combinations to be sampled were allocated as evenly as possible across a 40-week sampling plan. This was also considered the most practical option for planning for laboratories, jurisdictions and FSANZ.

To ensure the independence and representativeness of samples, random allocation of areas where samples were collected from different retailers was set up according to local government areas[14] or public health regions across the greater region of the capital city in each jurisdiction.

Table 8: Samples collected, outlining cuts and supermarket types.

Commodity	Large supermarkets (Coles or Woolworths) 60%	Small supermarkets (All other specialty grocery) 20%	Independent butchers not located within supermarkets 20%
Chicken meat	500 g prepackaged raw unfrozen thigh (Maryland) with skin on. If unavailable, 500 g raw unfrozen drumstick with skin on.	500 g prepackaged or unpacked raw unfrozen thigh (Maryland) with skin on. If unavailable, 500 g raw unfrozen drumstick with skin on.	500 g unpackaged raw unfrozen thigh (Maryland) with skin on. If unavailable, 500 g raw unfrozen drumstick with skin on.
Beef	500 g prepackaged raw unfrozen mince.	500 g prepackaged or unpackaged raw unfrozen mince.	500 g unpackaged raw unfrozen mince.
Pork	500 g prepackaged raw unfrozen mince. If unavailable, at least 500 g pork shoulder or loin chop.	500 g prepackaged or unpackaged raw unfrozen mince. If unavailable, at least 500 g pork shoulder or loin chop.	500 g unpackaged raw unfrozen mince. If unavailable, at least 500 g pork shoulder or loin chop.

Sample collection and transport

Food samples collected by sampling officers across all Australian jurisdictions were transported to laboratories in Brisbane, Melbourne, Perth and Sydney for preparation and bacterial isolation. To ensure the timely arrival of perishable food samples, multiple laboratory locations were utilised to mitigate potential delays in transportation because the sampling took place towards the end of the COVID-19 pandemic.

Raw meat samples with the longest time until use-by date were collected, and all samples were kept chilled to maintain a temperature < 10°C on arrival at the analysing laboratory. Microbiological analyses were commenced within 24 hrs of sample receipt. Any samples that had a temperature of > 10°C and/or had broken packaging upon arrival were notified to FSANZ to determine whether a replacement was required. If a sample was discarded, a replacement sample was collected in a different sampling run.

[14] Public health regions were used for Queensland

Sample preparation and bacterial isolation

The bacteria targets for the different retail meats are shown in Table 9.

Preparation of test samples and initial suspension for bacterial isolation was performed according to AS5013.20:2017 for chicken meat samples. Briefly, chicken meat samples (a minimum of 4 thighs, 2 Maryland, 4 drumsticks, or one whole chicken) were placed in a stomacher bag and stomached by shaking and massaging with 500 mL buffered peptone water for 2 minutes before proceeding to isolation protocols. The resulting fluid is referred to as 'chicken meat rinse fluid'.

Preparation of test samples and initial suspension for bacterial isolation was performed according to AS5013.11.1:2018 and AS5013.11.2:2017 for beef and pork cuts. Briefly, 25 g beef or pork mince samples were placed in a stomacher bag and stomached with bacteria specific enrichment media (see isolation methods) for 2 minutes before proceeding to the isolation protocol.

Table 9: Isolation of bacteria from retail meat samples. Bacterial species targeted from each collected commodity.

	***Salmonella* spp.**	***Campylobacter* spp.**	***Escherichia coli***	***Enterococcus* spp.**
Beef	N	N	Y	Y
Chicken meat	Y	Y	Y	Y
Pork	Y	N	Y	Y

Y – targeted, N – not targeted

Pork and beef alternate cut samples (skin tissue area of top layer up to 25 cm^2 to make a minimum of 25 g of sample) were placed in individual stomacher bags and stomached with bacteria specific enrichment media (see isolation methods) for 2 minutes before proceeding to isolation protocols.

Escherichia coli isolation

Isolation of *E. coli* followed AS5013.15. Briefly, 25 g of beef or pork samples were combined with 225 mL lauryl tryptose broth, or 50 mL of chicken meat rinse fluid was mixed with 50 mL lauryl tryptose broth, then samples were incubated for 48 hrs at 37°C ± 1°C. A loopful (10 µL) was transferred to 10 mL *Escherichia coli* broth and further incubated for 48 hrs at 44 ± 1°C. Samples were inoculated onto eosin methylene blue agar and incubated at 37°C for 22–24 hrs before purification on nutrient agar and confirmation of species using matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) and preserved using Microbank™ beads.

Enterococcus spp. isolation

Enterococcus isolation was completed according to the following protocol. Briefly, 25 g of beef or pork samples were combined 225 mL of Enterococcosel broth or 50 mL of chicken meat rinse fluid was mixed with 50 mL Enterococcosel broth, followed by incubation at 41°C ± 1°C for 22–26 hrs. After incubation, samples were inoculated onto Slanetz and Bartley agar and incubated at 41°C ± 1°C for 46–50 hrs. Isolates were purified on nonselective blood agar and up to 10 isolates identified until either a *E. faecium* or *E. faecalis* was identified via MALDI-TOF MS. This isolate was stored for susceptibility testing using Microbank™ beads.

Salmonella isolation

Bacterial isolation of *Salmonella* was completed according to AS5013.10. Briefly, 450–500 mL chicken meat rinse fluid was incubated at 36°C ± 2°C for 16–20 hrs. Secondary selective enrichment was performed in Rappaport-Vassiliadis *Salmonella* broth (0.1 mL fluid, 10 mL buffered peptone water) at 41.5°C for 21–27 hrs and Muller-Kauffmann Tetrathionate-Novobiocin Broth (1 mL rinse fluid; 10 mL Muller-Kauffmann Tetrathionate-Novobiocin Broth) at 37°C for 21–27 hrs. After enrichment, samples were inoculated onto xylose lysine deoxycholate or Brilliant Green Agar and incubated for 21–27 hrs at 37°C ± 1°C. Typical colonies were purified on nutrient agar, identified using MALDI-TOF MS and preserved using Microbank™ beads before dispatch to MU.

Campylobacter spp. isolation

Campylobacter analysis of chicken meat samples was undertaken during the period between 27/03/2023 and 30/07/2023.

For *Campylobacter* isolation from chicken meat samples, AS 5013.20:2017 and AS 5013.6:2017 methods were used with the following modifications: the entire package of whole chicken meat (one only) or portions, that is, Maryland (at least 2), wings (at least 6), drumsticks (at least 4) or thighs (at least 4) and any accompanying fluid from the original packaging was added to a sterile plastic bag of suitable size. Buffered peptone water (500 mL) was added, and samples were manually massaged and shaken vigorously for 2 min. ensuring the abdominal cavity for whole carcasses, and the entire surface of the bird and all chicken meat portions, were thoroughly rinsed.

Chicken meat rinse fluid was mixed with double strength Preston broth (50 mL:50 mL) and incubated aerobically at 37 ± 1°C for 2 hrs. Following incubation, 0.4 mL of Preston antibiotic supplement (prepared as per AS5013.6.2017) was mixed into each culture followed by microaerophilic incubation at 42 ± 1°C for 40–48 hrs. After incubation, samples were used to inoculate both modified charcoal cefoperazone deoxycholate agar and Skirrow agar with microaerophilic incubation at 42 ± 1°C for 40–48 hrs. The first isolate identified as *Campylobacter* using MALDI-TOF was preserved for susceptibility testing using Microbank™ beads.

Dispatch to MU Antimicrobial Resistance and Infectious Diseases Laboratory

Symbio Laboratories prepared and stored the pure culture bacterial isolates with confirmed identity testing at –80°C using the Microbank™ bead storage system. These stored bacterial isolates were transported to MU using a refrigerated courier service on Amies charcoal swabs for AST.

Antimicrobial susceptibility testing

MU Antimicrobial Resistance and Infectious Diseases Laboratory sample receival and storage

Upon receival, isolates were grown aerobically on Columbia sheep blood agar overnight at 37°C for *E. coli*, *Salmonella* and *Enterococcus*, or micro-aerobically for 48 hrs for *Campylobacter*. Species identification was confirmed using MALDI-TOF MS as per the manufacturer's instructions. Isolates were preserved in Luria Bertani broth (*E. coli*, *Enterococcus*, and *Salmonella*) or heart infusion broth (*Campylobacter*) containing 20% glycerol stored at –80°C.

Preparation of drug panels

Custom drug panels were prepared at MU using a customised Freedom EVO platform contained in a laminar flow hood. The susceptibility of *E. coli* and *Salmonella* isolates to 14 antibiotics was tested. The panel for both species included colistin, ciprofloxacin, cefotaxime, ceftazidime, trimethoprim, amikacin, gentamicin, ampicillin, tetracycline, amoxicillin-clavulanate, chloramphenicol, meropenem and florfenicol. The *E. coli* panel (Table 10) also included sulfamethoxazole, while the *Salmonella* panel included azithromycin (Table 11).

The panel for *Enterococcus* included the following 12 antibiotics: vancomycin, ampicillin, linezolid, erythromycin, ciprofloxacin, tetracycline, daptomycin, chloramphenicol, teicoplanin, gentamicin and nitrofurantoin (Table 12). Virginiamycin was also included for *E. faecium*.

The *Campylobacter* panel included 11 antibiotics: azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, streptomycin and tetracycline (Table 13). Telithromycin was also included for *C. jejuni*.

Species-specific American Tissue Culture Collection (ATCC) control strains recommended in the CLSI guidelines were used to validate each panel upon creation and with each batch of tests performed (CLSI 2015).

Antimicrobial susceptibility testing of *E. coli*, *Salmonella* and *Enterococcus*

AST for *E. coli*, *Enterococcus* and *Salmonella* was performed using the RASP (Truswell et al. 2021). ATCC 25922 (*E. coli*) or ATCC 51299 (*E. faecalis*), as well as an in-house control, were included in each run. A single colony from overnight culture on Columbia sheep blood agar (CBA) at 37°C (*E. coli* and *Salmonella*) or 48 hrs culture at 42°C on Slanetz and Bartley agar (*Enterococcus*) was selected using the SciRobotics PickoloTM system and inoculated into broth in a 96-well plate format for automated susceptibility profiling. After overnight culture, AST was performed by broth microdilution according to the CLSI ISO 20776 standards (CLSI 2015). After incubation, assay plates were imaged using a Vizion™ platform (ThermoFisher Scientific). The generated image was used to determine the MIC.

Antimicrobial susceptibility testing of *Campylobacter*

AST for *Campylobacter* was performed according the CLSI guidelines using the Sensititre AIMTM Automated Inoculation Delivery System (CLSI 2016). *Campylobacter* isolates were subcultured twice from single colonies on CSBA at 37°C overnight under microaerophilic conditions (Oxoid™ CampyGen™, ThermoFisher Scientific) before AST. *Campylobacter jejuni* ATCC 33560 was included in each batch of isolates tested. ASTs were incubated for a minimum of 48 hrs at 37°C under microaerophilic conditions (Oxoid™ CampyGen™, ThermoFisher Scientific). Afterwards, the incubation assay plates were imaged using a Vizion™ platform (ThermoFisher Scientific). The subsequent image was used to determine the MIC.

Interpretation of antibiotic susceptibility results

This study determined MIC distributions for each antibiotic according to CLSI guidelines (CLSI 2015, 2024), based on the following breakpoints:

- epidemiological cut-off values (ECOFF) (EUCAST 2020)
- EUCAST clinical breakpoints (EUCAST 2024)
- CLSI clinical breakpoints (CLSI 2016, 2024).

All breakpoints used in the interpretation of the results are presented in Table 10, Table 11, Table 12, and Table 13 for *E. coli*, *Salmonella*, *Enterococcus* and *Campylobacter*, respectively. Where ECOFFs were unavailable on the EUCAST website, cut-off values were taken from the European Food Safety Authority (EFSA) (EFSA 2024). Additionally, these tables provide the Australian 'Importance Rating' for each antibiotic, which is based on information from the Australian Government. This rating informs regulators and users about the significance of an antibacterial in treating infections in both animals and humans, as well as the potential severity of consequences if resistance develops or increases (ASTAG 2018).

Statistical analysis

CIs of proportions were calculated using exact binomial CIs using the Clopper-Pearson method performed in Stata version 16.1 (StataCorp LLC, College Station, Texas USW).

Drug, importance rating and breakpoint tables

Table 10: Breakpoints used for interpretation of *E. coli* antibiotic susceptibility data.

			Range (mg/L)		ECOFF[a] (mg/L)	Clinical Breakpoints (mg/L)	
Class	Drug	Importance	Low	High		EUCAST[a]	CLSI[a]
Aminoglycosides	Amikacin	High	1	64	8	8	8
Aminoglycosides	Gentamicin	Medium	0.25	16	2	2	4
β-lactams	Amoxicillin-Clavulanate	Medium	4	2	8	8	16
β-lactams	Ampicillin	Low	1	32	8	8	16
Carbapenem	Meropenem	High	0.008	4	0.06	8	2
Folate pathway inhibitors	Sulfamethoxazole[b]	NR	8	512	64	.	.
Folate pathway inhibitors	Trimethoprim	Low	0.25	16	2	4	8
Phenicols	Chloramphenicol	Low	2	32	16	.	16
Phenicols	Florfenicol	Low	4	32	16	.	.
Polymyxins	Colistin	High	0.25	8	2	2	2
Quinolones	Ciprofloxacin	High	0.008	2	0.06	0.5	0.5
Tetracycline	Tetracycline	Low	1	32	8	.	8
Third-generation cephalosporins	Cefotaxime	High	0.015	4	0.25	2	2
Third-generation cephalosporins	Ceftazidime	High	0.125	16	1	4	8

[a] non-wild type/resistant is greater than the value stated
[b] ECOFF not available, greater than 64 mg/L was used (EFSA 2024; EUCAST 2024)
'. ' – no breakpoint available
'NR' – no importance rating

Table 11: Breakpoints used for interpretation of *Salmonella* spp. antibiotic susceptibility data. Resistance was called where the MIC was greater than the tabled breakpoint.

			Range (mg/L)		**ECOFF[a]**	Clinical Breakpoints (mg/L)	
Class	Drug	Importance	Low	High	(mg/L)	EUCAST[a]	CLSI[a]
Aminoglycosides	Amikacin	High	0.5	256	4	8	.
Aminoglycosides	Gentamicin	Medium	0.25	16	2	2	.
β-lactams	Amoxicillin-Clavulanate[b]	Medium	4	32	8	8	.
β-lactams	Ampicillin	Low	1	32	4	8	16
Carbapenem	Meropenem[c]	High	0.008	4	0.125	8	2
Folate pathway inhibitors	Trimethoprim	Low	0.25	16	2	4	.
Macrolides	Azithromycin	Low	1	64	16	.	.
Phenicols	Chloramphenicol	Low	2	32	16	.	16
Phenicols	Florfenicol	Low	4	32	16	.	.
Polymyxins	Colistin[d]	High	0.25	8	2	2	.
Quinolones	Ciprofloxacin	High	0.03	2	0.125	0.06	0.5
Tetracycline	Tetracycline	Low	0.5	32	8	.	8
Third-generation cephalosporins	Cefotaxime	High	0.06	4	0.5	2	2
Third-generation cephalosporins	Ceftazidime	High	0.125	16	2	4	.

[a] non-wild type/resistant is greater than the value stated
[b] ECOFF not available – EUCAST clinical greater than 8 mg/L was used
[c] ECOFF not available – greater than 0.125 mg/L was used (EFSA 2024; EUCAST 2024)
[d] ECOFF not available – greater than 2 mg/L was used (EFSA 2024)
'.' – no breakpoint available

Table 12: Breakpoints used for interpretation of *E. faecium* and *E. faecalis* antibiotic susceptibility data.

					ECOFF[a]		Clinical breakpoints (mg/L)			
			Range (mg/L)		(mg/L)		EUCAST[a]		CLSI[a]	
Class	Drug	Importance	Low	High	*faecium*	*faecalis*	*faecium*	*faecalis*	*faecium*	*faecalis*
Aminoglycosides	Gentamicin	Medium	8	512	32	64	.	.	.	.
Aminoglycosides	Streptomycin	Low	128	1024	128	512	.	.	.	.
β-lactams	Ampicillin	Low	1	16	4	4	8	8	8	8
Glycopeptides	Teicoplanin	High	0.25	128	2	2	2	2	16	16
Glycopeptides	Vancomycin	High	0.06	128	4	4	4	4	16	16
Lipopeptides	Daptomycin	High	0.25	16	8	4	.	.	4	4
Macrolides	Erythromycin	Low	0.25	16	4	4	.	.	4	4
Nitrofuran	Nitrofurantoin	High	16	256	256	32	.	64	64	64
Oxazolidinones	Linezolid	High	0.25	16	4	4	4	4	4	4
Phenicols	Chloramphenicol	Low	1	32	32	32	.	.	16	16
Quinolones	Ciprofloxacin	High	0.125	8	8	4	4	4	2	2
Streptogramins	Virginiamycin	High	0.03	64	8	NA	.	NA	.	NA
Tetracycline	Tetracycline	Low	0.5	32	4	4	.	.	8	8

[a] non-wild type/resistant is greater than the value stated
'.' – no breakpoint available
'NA' – not tested for this species

Table 13: Breakpoints used for interpretation of *Campylobacter coli* and *Campylobacter jejuni* antibiotic susceptibility data.

					ECOFF[a]		Clinical breakpoints (mg/L)			
			Range (mg/L)		(mg/L)		EUCAST[a]		CLSI[a]	
Class	Drug	Importance	Low	High	*coli*	*jejuni*	*coli*	*jejuni*	*coli*	*jejuni*
Aminoglycosides	Gentamicin	Medium	0.125	16	2	2	.	.	.	.
Aminoglycosides	Streptomycin	Low	0.5	16	4	4	.	.	.	.
Ketolides	Telithromycin	NR	0.5	8	NA	4	NA	.	NA	.
Lincosamides	Clindamycin	Medium	0.0313	32	1	0.5	.	.	.	.
Macrolides	Azithromycin	Low	0.0313	2	0.5	0.25	8	4	.	.
Macrolides	Erythromycin	Low	0.0625	128	8	4	8	4	16	16
Phenicols	Chloramphenicol	Low	2	32	16	16	.	.	.	.
Phenicols	Florfenicol	Low	0.0313	32	4	4	.	.	.	.
Quinolones	Ciprofloxacin	High	0.008	16	0.5	0.5	0.5	0.5	2	2
Quinolones	Nalidixic acid	NR	1	64	32	16	.	.	.	.
Tetracycline	Tetracycline	Low	0.125	64	2	1	2	2	8	8

[a] non-wild type/resistant is greater than the value stated
'.' – no breakpoint available
'NA' – not tested for this species
'NR' – no importance rating

Genetic analysis

DNA extraction and library preparation

Isolates with resistance to high-importance antibiotics or with resistance to 3 or more classes of antibiotics were selected for initial sequencing. For each isolate, bacterial culture was prepared during AST preparation and an aliquot of overnight growth in broth was collected for DNA extraction (*E. coli*, *Salmonella* and *Enterococcus*) or colonies were collected directly from the CSBA (*Campylobacter*). Isolates were stored at –20°C until extraction. For isolates in broth, a 200 µL aliquot was extracted using a MagMAX™-96 DNA Multi-Sample Kit (ThermoFisher: 4413021) with a volume modified protocol: 200 µL multi-sample DNA lysis buffer, 240 µL propan-2-ol, 16 µL of DNA Binding Beads, and 30 µL each of DNA Elution Buffer-1 and DNA Elution Buffer-2. Isolates collected from agar were processed using the same protocol; however, the bacterial pellet was suspended in 400 µL lysis buffer before addition of propanol.

WGS library preparation was performed using a modified Tecan genomics robot and Celero EZ™ DNA-Seq (Tecan 0568) chemistry according to the manufacturer's instructions with reduced volumes.

DNA sequencing and analysis

After QC checks, the samples were sequenced on the Novaseq 6000 using the 2 x 150 base pair paired-end sequencing chemistry at the National Association of Testing Authorities accredited Institute for Immunology and Infectious Diseases, Murdoch Medical Genomics Core facility or on the Illumina Nextseq 500 platform with a high output 2 x 150 kit within the Mudoch University AMRID laboratory. Sequence quality was checked using FastQC (Babraham Bioinformatics 2023). Species identification was performed using Kraken (Wood, Lu, and Langmead 2019) with standard variables. Genomes were assembled using SPAdes v3.14.0 (Bankevich et al. 2012) and AMR genes were identified using AMRFinder+ (version 4.0.3, database version 2024-10-22.1) (Feldgarden et al. 2021). Multilocus sequence types (MLST/ST) for each isolate were identified using the MLST tool (version 2.19.0) based on the pubMLST database (Seemann; Jolley and Maiden 2010). *Salmonella* serotypes were identified using SeqSero2 (Zhang et al. 2019).

Heat maps for the presence and absence of genetic resistance determinants

Resistance gene classes were identified from WGS reads using AMRFinder+. Heatmaps for the presence and absence of resistance to identified classes of antibiotics were created using package pheatmap (Kolde 2019) in R v. 4.4.0 (R Core Team 2024).

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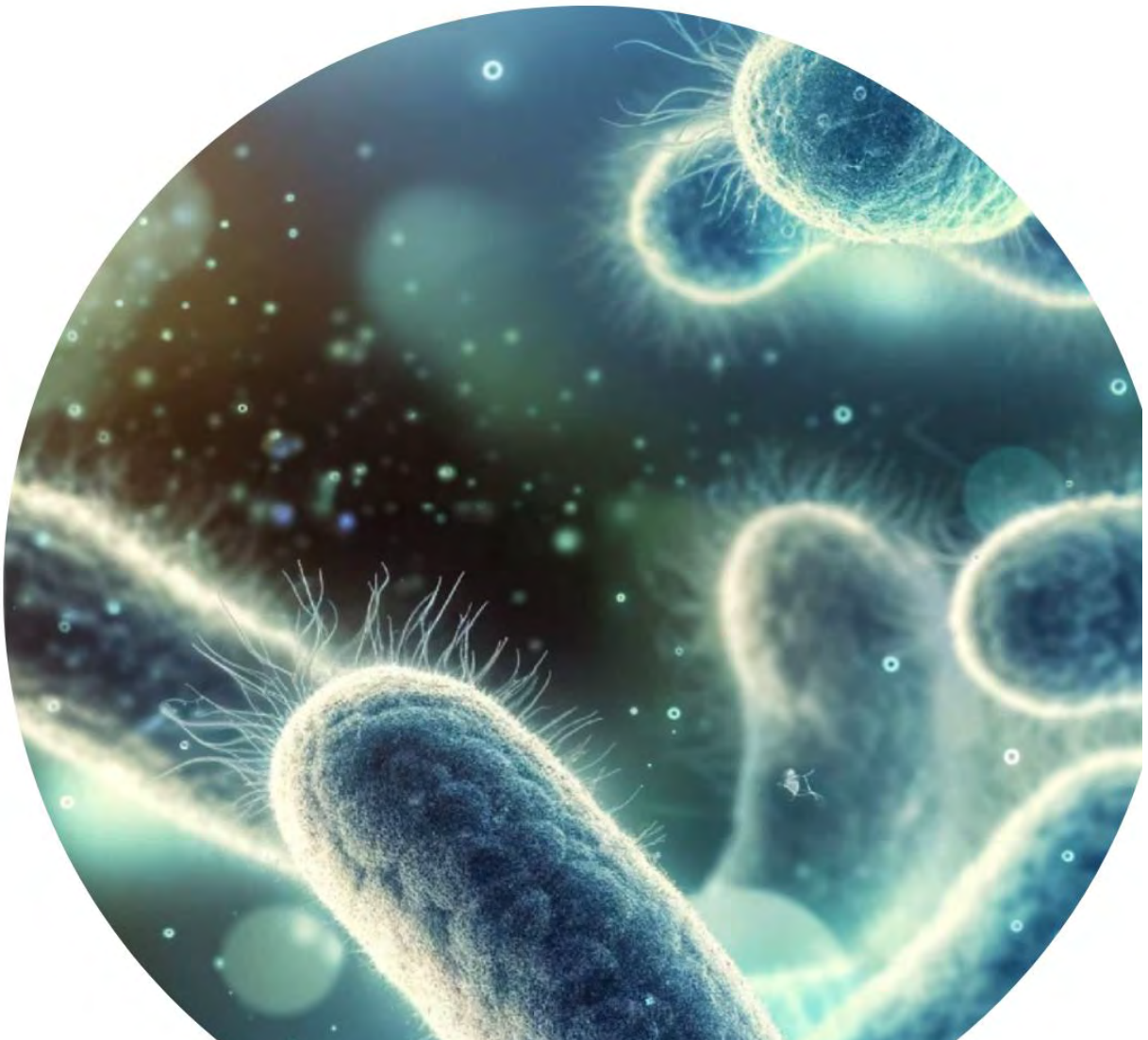
Supplementary tables



Contents

Supplementary Table 1: Prevalence of bacterial species

Prevalence of different bacterial species (n = 2,555 isolates) collected from retail meat with identity confirmed by MALDI-TOF MS prior to AST

	Detection Rate (%) (number isolated/number samples tested)		
	Beef	Chicken meat	Pork
Escherichia coli	64.6 (372/576)	74.2 (299/403)	61.5 (480/780)
Enterococcus faecalis	53.5 (154/288)	64.9 (189/291)	68 (198/291)
Enterococcus faecium	18.4 (53/288)	18.6 (54/291)	9.3 (27/291)
Salmonella	Not tested	8.7 (174/2005)	2.5 (20/809)
Campylobacter coli	Not tested	26.9 (231/860)	Not tested
Campylobacter jejuni	Not tested	35.3 (304/860)	Not tested

Supplementary Table 2: Minimum inhibitory concentrations (MICs) *E. coli* beef

MIC distributions of *E. coli* isolated from retail beef samples (n = 372)

	Antibiotic concentration (mg/L)																	Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.008	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	**ECOFF**	**CLSI**	**EUCAST**
amk								78.5	17.5	4									0	0	0
																			(0,1)	(0,1)	(0,1)
amc										73.1	25.5	0.8	0.3	0.3					1.3	0.5	1.3
																			(0.4,3.1)	(0.1,1.9)	(0.4,3.1)
amp								7.3	33.9	45.7	3.5	0.3		9.4					9.7	9.4	9.7
																			(6.9,13.1)	(6.6,12.8)	(6.9,13.1)
cta		28.5	50.5	18.8	1.6			0.5											0.5	0	0
																			(0.1,1.9)	(0,1)	(0,1)
ctz						21	4.3	0.8		0.5									0.5	0	0
																			(0.1,1.9)	(0,1)	(0,1)
chl									1.9	27.7	64	3.8	0.8	1.9					2.7	2.7	0
																			(1.3,4.9)	(1.3,4.9)	.
cip		99.2			0.8														0.8	0	0
																			(0.2,2.3)	(0,1)	(0,1)
col						99.2	0.5	0.3											0	0	0
																			(0,1)	(0,1)	(0,1)
flo										28.5	57.3	12.9	0.3	1.1					1.3	.	.
																			(0.4,3.1)	.	.
gen						77.7	18	4	0.3										0	0	0
																			(0,1)	(0,1)	(0,1)
mer		57.5	41.4	0.5															0	0	0
																			(0,1)	(0,1)	(0,1)
sme											17.2	25.8	45.2	4	1.1		0.3	6.5	7.8	.	.
																			(5.3,11)	.	.
tet								83.9	2.4	1.1		0.3	2.4	9.9					12.6	12.6	.
																			(9.4,16.4)	(9.4,16.4)	.
tri						34.4	48.1	12.4	0.5	0.3	0.3		4						4.6	4	4.3
																			(2.7,7.2)	(2.3,6.6)	(2.5,6.9)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amk – amikacin, amc – amoxicillin/clavulanate, amp – ampicillin, cta – cefotaxime, ctz – ceftazidime, chl – chloramphenicol, cip – ciprofloxacin, col – colistin, flo – florfenicol, gen – gentamicin, mer – meropenem, sme – sulfamethoxazole, tet – tetracycline, tri – trimethoprim, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 3: MICs *E. coli* chicken meat

MIC distributions of *E. coli* isolated from retail chicken meat samples (n = 299)

	Antibiotic concentration (mg/L)																	Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.008	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	**ECOFF**	**CLSI**	**EUCAST**
amk								78.3	17.7	3.3	0.7								0	0	0.01
																			(0,1.2)	(0,1.2)	(0,1.2)
amc										68.2	29.1	1.7	0.7	0.3					2.7	1	2.7
																			(1.2,5.2)	(0.2,2.9)	(1.2,5.2)
amp								5	31.1	41.1	2	0.3	0.3	20.1					20.7	20.4	20.7
																			(16.3,25.8)	(16,25.4)	(16.3,25.8)
cta		24.7	50.8	20.7	3		0.3	0.3											0.7	0	0.01
																			(0.1,2.4)	(0,1.2)	(0,1.2)
ctz						18.4	4	0.3	0.3	0.3									0.7	0	0.01
																			(0.1,2.4)	(0,1.2)	(0,1.2)
chl									2	33.4	59.9	2	0.7	2					2.7	2.7	.
																			(1.2,5.2)	(1.2,5.2)	.
cip		97.3			0.3	0.3				2									2.7	2	2
																			(1.2,5.2)	(0.7,4.3)	(0.7,4.3)
col						99	1												0	0	0.01
																			(0,1.2)	(0,1.2)	(0,1.2)
flo										28.4	63.9	7		0.7					0.7	.	.
																			(0.1,2.4)	.	.
gen						68.6	27.8	2.7	0.3			0.3	0.3						0.7	0.7	0.7
																			(0.1,2.4)	(0.1,2.4)	(0.1,2.4)
mer		40.5	57.5	1															0	0	0.01
																			(0,1.2)	(0,1.2)	(0,1.2)
sme											15.1	20.1	47.5	3	2.3		0.7	11.4	14.4	.	.
																			(10.6,18.9)	.	.
tet								78.9	3			1	1.3	15.7					18.1	18.1	.
																			(13.9,22.9)	(13.9,22.9)	.
tri						35.5	46.2	10.4	1	0.3			6.7						7	6.7	6.7
																			(4.4,10.5)	(4.1,10.1)	(4.1,10.1)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amk – amikacin, amc – amoxicillin/clavulanate, amp – ampicillin, cta – cefotaxime, ctz – ceftazidime, chl – chloramphenicol, cip – ciprofloxacin, col – colistin, flo – florfenicol, gen – gentamicin, mer- meropenem, sme – sulfamethoxazole, tet – tetracycline, tri – trimethoprim, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 4: MICs *E. coli* pork

MIC distributions of *E. coli* isolated from retail pork samples (n = 480)

	Antibiotic concentration (mg/L)																	Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.008	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	**ECOFF**	**CLSI**	**EUCAST**
amk								75.4	21.5	2.7	0.4								0	0	0.05
																			(0,0.8)	(0,0.8)	(0,0.8)
amc										60.4	37.1	2.1	0.4						2.5	0.4	2.5
																			(1.3,4.3)	(0.1,1.5)	(1.3,4.3)
amp								3.3	22.3	31.7	2.7	0.4		39.6					40	39.6	40
																			(35.6,44.5)	(35.2,44.1)	(35.6,44.5)
cta		25	47.5	22.1	5			0.2			0.2								0.4	0.2	0.2
																			(0.1,1.5)	(0,1.2)	(0,1.2)
ctz						25.8	6	1.3		0.2									0.2	0	0
																			(0,1.2)	(0,0.8)	(0,0.8)
chl									2.1	21.7	56.3	4	4.6	11.5					16	16	.
																			(12.9,19.6)	(12.9,19.6)	.
cip		96.3	0.8	0.4	1.9	0.2				0.4									2.5	0.4	0.4
																			(1.3,4.3)	(0.1,1.5)	(0.1,1.5)
col						99	0.4	0.6											0	0	0
																			(0,0.8)	(0,0.8)	(0,0.8)
flo										20.4	57.5	15.6	1	5.4					6.5	.	.
																			(4.4,9)	.	.
gen						68.8	27.7	2.5	0.4		0.4		0.2						0.6	0.6	0.6
																			(0.1,1.8)	(0.1,1.8)	(0.1,1.8)
mer		54.2	43.1	0.6															0	0	0
																			(0,0.8)	(0,0.8)	(0,0.8)
sme											10.4	19.8	33.3	2.3	2.9	0.2	0.6	30.4	34.2	.	.
																			(29.9,38.6)	.	.
tet								59.4	1.7	0.6	0.6	0.2	4.4	33.1					37.7	37.7	.
																			(33.4,42.2)	(33.4,42.2)	.
tri						32.3	35	11	1	0.2			20.4						20.6	20.4	20.4
																			(17.1,24.5)	(16.9,24.3)	(16.9,24.3)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amk – amikacin, amc – amoxicillin/clavulanate, amp – ampicillin, cta – cefotaxime, ctz – ceftazidime, chl – chloramphenicol, cip – ciprofloxacin, col – colistin, flo – florfenicol, gen – gentamicin, mer – meropenem, sme – sulfamethoxazole, tet – tetracycline, tri – trimethoprim, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 5: *E. coli* genotype – ciprofloxacin

Genotype of ciprofloxacin-resistant *E. coli* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23090194	109	2: fpi_qui	5: ami_eff_fos_fpi_qui	*acrF aph(3'')-Ib aph(6)-Id glpT_E448K gyrA_S83L mdtM sul2 uhpT_E350Q*	Beef
23090109	2952	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaLAP-2 blaTEM-1 glpT_E448K mdtM qnrS1 tet(A)*	Beef
23100279	401	5: bla_fpi_phe_qui_tet	2: eff_fos	*acrF glpT_E448K mdtM*	Beef
23070179	212	1: qui	3: eff_fos_qui	*acrF glpT_E448K gyrA_S83L mdtM*	Chicken meat
23100446		1: qui	3: eff_fos_qui	*acrF glpT_E448K gyrA_S83L mdtM*	Chicken meat
23020235	354	1: qui	5: eff_fmi_fos_nit_qui	*acrF cyaA_S352T emrD glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T uhpT_E350Q*	Chicken meat
23100160	354	2: bla_qui	6: bla_eff_fmi_fos_nit_qui	*acrF blaI cyaA_S352T emrD fosA7 glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T uhpT_E350Q*	Chicken meat
23020307	354	2: bla_qui	6: bla_eff_fmi_fos_nit_qui	*acrF blaTEM-1 cyaA_S352T emrD glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T uhpT_E350Q*	Chicken meat
23020336	354	2: bla_qui	6: bla_eff_fmi_fos_nit_qui	*acrF blaTEM-1 cyaA_S352T emrD glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T uhpT_E350Q*	Chicken meat
23100119	354	2: fpi_qui	5: eff_fmi_fos_nit_qui	*acrF cyaA_S352T emrD fosA7 glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T uhpT_E350Q*	Chicken meat
23020201	354	4: ami_fpi_qui_tet	8: ami_eff_fmi_fos_fpi_nit_qui_tet	*aac(3)-IId acrF cyaA_S352T dfrA17 emrD glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T tet(B) uhpT_E350Q*	Chicken meat
23090142	215	3: bla_qui_tet	4: bla_eff_qui_tet	*acrF blaLAP-2 blaTEM-1 mdtM qnrS1 tet(A)*	Pork
23020154	86	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaLAP-2 blaTEM-1 glpT_E448K mdtM qnrS1 tet(A)*	Pork
23020221	641	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaLAP-2 blaTEM-1 glpT_E448K mdtM qnrS1 tet(A)*	Pork

23020226	641	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaLAP-2 blaTEM-1 glpT_E448K mdtM qnrS1 tet(A)*	Pork
23090165	2705	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaTEM-1 glpT_E448K mdtM qnrS13 tet(A)*	Pork
23020078	7589	3: bla_qui_tet	5: bla_eff_fos_qui_tet	*acrF blaLAP-2 blaTEM-1 glpT_E448K mdtM qnrS1 tet(A)*	Pork
23020258	898	3: bla_qui_tet	8: ami_bla_eff_fos_fpi_qui_sin_tet	*aadA1 acrF blaLAP-2 blaTEM-1 dfrA51 glpT_E448K mdtM qnrS1 sat2 tet(A)*	Pork
23020200	354	4: ami_fpi_qui_tet	8: ami_eff_fmi_fos_fpi_nit_qui_tet	*aac(3)-IId acrF cyaA_S352T dfrA17 emrD glpT_E448K gyrA_D87N gyrA_S83L mdtM nfsA_H11Y parC_E84G parC_S80I parE_I355T tet(B) uhpT_E350Q*	Pork
23020302	131	4: bla_fpi_qui_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 emrD glpT_E448K parE_I529L ptsI_V25I qnrS1 sul3 tet(A) uhpT_E350Q*	Pork
23100328	131	4: bla_fpi_qui_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 emrD glpT_E448K parE_I529L ptsI_V25I qnrS1 sul3 tet(A) uhpT_E350Q*	Pork
23070190	744	4: fpi_phe_qui_tet	7: ami_eff_fos_fpi_phe_qui_tet	*aadA1 aadA2 acrF catA1 cmlA1 glpT_E448K gyrA_D87N gyrA_S83L mdtM parC_A56T parC_S80I sul3 tet(B)*	Pork
23020283	362	5: bla_fpi_phe_qui_tet	11: ami_bla_eff_fmi_fos_fpi_lin_mac_phe_qui_tet	*aadA1 aadA2 acrF blaTEM-1 cyaA_S352T emrD floR fosA4 glpT_E448K lnu(F) mdtM mph(A) qnrS1 sul2 sul3 tet(A) tet(M) uhpT_E350Q*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref-laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: fpi – folate pathway inhibitors, qui – quinolones, ami – aminoglycosides, fos – fosfomycin, eff – efflux pumps, bla – beta-lactams, phe – phenicols, nit – nitrofurans, fmi – fosmidomycin, sin – streptothricin.

Supplementary Table 6: *E. coli* genotype third generation cephalosporin

Genotype of third generation cephalosporin resistant *E. coli* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23070234	58	2: bla_c3g	3: c3g_eff_fos	*acrF ampC_C-42T glpT_E448K mdtM*	Beef
23100440	58	3: bla_c3g_tet	5: ami_c3g_eff_fos_tet	*acrF ampC_C-42T aph(3'')-Ib aph(6)-Id glpT_E448K mdtM tet(B)*	Beef
23070240	58	2: bla_c3g	3: c3g_eff_fos	*acrF ampC_C-42T glpT_E448K mdtM*	Chicken meat
23100469	58	3: bla_c3g_tet	5: ami_c3g_eff_fos_tet	*acrF ampC_C-42T aph(3'')-Ib aph(6)-Id glpT_E448K mdtM tet(B)*	Chicken meat
23020061	6187	2: bla_c3g	3: c3g_eff_fos	*acrF ampC_C-42T glpT_E448K mdtM*	Pork
23020034	58	4: bla_c3g_fpi_tet	6: ami_c3g_eff_fos_fpi_tet	*aadA5 acrF blaCTX-M-1 dfrA17 glpT_E448K mdtM sul2 tet(A)*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: bla – beta-lactams, c3g – 3rd generation cephalosporins, tet – tetracyclines, eff – efflux pumps, fos – fosfomycin, fpi – folate pathway inhibitors, ami – aminoglycosides.

Supplementary Table 7: *E. coli* genotypes multi-class resistance

Genotypes of multi-class resistant *E. coli* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23100214	16353	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 dfrA1 emrD glpT_E448K parE_I529L ptsI_V25I sul1 tet(A) uhpT_E350Q*	Beef
23100472	3714	3: bla_fpi_tet	5: bla_eff_fos_fpi_tet	*acrF blaTEM-1 glpT_E448K mdtM sul2 tet(A)*	Beef
23100500	58	3: bla_fpi_tet	6: ami_bla_ eff_fos_fpi_tet	*aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA7 glpT_E448K mdtM sul2 tet(A)*	Beef
23020072	1665	3: fpi_phe_tet	7: ami_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF cmlA1 dfrA12 glpT_E448K mdtM mef(B) sul3 tet(A)*	Beef
23020070	58	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 dfrA51 floR glpT_E448K mdtM sul3 tet(A)*	Beef
23090162	867	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_lin_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K lnu(F) mdtM sul1 tet(B)*	Beef
23100134	867	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_lin_phe_tet	*aadA1 aadA2 acrF blaI blaTEM-1 cmlA1 dfrA12 glpT_E448K lnu(F) mdtM sul1 tet(B)*	Beef
23100164	2628	4: bla_fpi_phe_tet	9: ami_bla_blo_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 aadA5 acrF blaTEM-1 bleO cmlA1 dfrA12 dfrA17 glpT_E448K mdtM mef(B) sul1 sul3 tet(A)*	Beef
23100190	871	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Beef
23100266	11713	4: bla_fpi_phe_tet	2: eff_fos	*acrF glpT_E448K mdtM*	Beef
23100397	58	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Beef
23100564	7384	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_phe_sin_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM sat2 sul3 tet(A) uhpT_E350Q*	Beef
23090089	10	3: bla_fpi_phe	5: ami_bla_eff_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 mdtM sul3*	Chicken meat
23090115	23	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 catA1 glpT_E448K mdtM sul1*	Chicken meat

23090136	23	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 catA1 glpT_E448K mdtM sul1*	Chicken meat
23100270	58	3: bla_fpi_phe	2: eff_fos	*acrF glpT_E448K mdtM*	Chicken meat
23100614	540	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM cmlA1 glpT_E448K sul3*	Chicken meat
23100616	540	3: bla_fpi_phe	5: ami_bla_eff_fos_phe	*aadA15 acrF blaTEM-1 cmlA1 glpT_E448K*	Chicken meat
23020080	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Chicken meat
23020300	453	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Chicken meat
23020320	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Chicken meat
23020329	16353	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 dfrA1 emrD glpT_E448K parE_I529L ptsI_V25I sul1 tet(A) uhpT_E350Q*	Chicken meat
23070233	16353	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 dfrA1 emrD glpT_E448K parE_I529L ptsI_V25I sul1 tet(A) uhpT_E350Q*	Chicken meat
23070259	16353	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 dfrA1 emrD glpT_E448K parE_I529L ptsI_V25I sul1 tet(A) uhpT_E350Q*	Chicken meat
23090195	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 glpT_E448K mdtM sul2 tet(A)*	Chicken meat
23090209	10	3: bla_fpi_tet	4: bla_eff_fpi_tet	*acrF blaTEM-1 dfrA5 mdtM tet(A)*	Chicken meat
23100392	16356	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_sin_tet	*aadA1 acrF blaTEM-1 dfrA1 glpT_E448K mdtM sat2 tet(B)*	Chicken meat
23100453	16360	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 mdtM sul2 tet(A)*	Chicken meat
23100471	3714	3: bla_fpi_tet	5: bla_eff_fos_fpi_tet	*acrF blaTEM-1 glpT_E448K mdtM sul2 tet(A)*	Chicken meat
23100576	88	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 glpT_E448K mdtM sul2 tet(A)*	Chicken meat
23100619	155	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM dfrA1 glpT_E448K mdtM sul1 tet(A)*	Chicken meat
23100303	141	3: fpi_phe_tet	7: ami_eff_fos_fpi_mti_phe_tet	*acrF aph(3'')-Ib aph(6)-Id emrD floR glpT_E448K marR_S3N sul2 tet(A)*	Chicken meat

23100271	58	4: ami_bla_fpi_tet	2: eff_fos	*acrF glpT_E448K mdtM*	Chicken meat
23090272	2936	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR mdtM sul3 tet(A)*	Chicken meat
23020010	10	3: bla_fpi_phe	5: ami_bla_eff_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 mdtM sul3*	Pork
23020204	8580	3: bla_fpi_phe	7: ami_bla_eff_fos_fpi_mac_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 emrD glpT_E448K mdtM mef(B) sul3*	Pork
23020248	540	3: bla_fpi_phe	7: ami_bla_blo_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 bleO cmlA1 dfrA51 glpT_E448K mdtM sul3*	Pork
23020314	641	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K mdtM sul3*	Pork
23070226	548	3: bla_fpi_phe	5: ami_bla_eff_fpi_phe	*aadA1 aadA2 blaTEM-1 cmlA1 mdtM sul3*	Pork
23090237	10	3: bla_fpi_phe	3: eff_fpi_phe	*acrF cmlA1 mdtM sul3*	Pork
23100362	10	3: bla_fpi_phe	5: ami_bla_eff_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 mdtM sul3*	Pork
23100470	994	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM sul3*	Pork
23100615	540	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K sul3*	Pork
23100632	101	3: bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM sul3*	Pork
23020003	453	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Pork
23020004	877	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 dfrA5 glpT_E448K mdtM sul3 tet(A)*	Pork
23020027	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 dfrA51 glpT_E448K mdtM sul2 tet(A)*	Pork
23020041	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM dfrA12 dfrA51 glpT_E448K mdtM sul3 tet(A) tet(M)*	Pork
23020063	86	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*acrF aph(3'')-Ib aph(6)-Id blaLAP-2 blaTEM-1 dfrA5 glpT_E448K mdtM qnrS1 sul2 tet(A)*	Pork

23020064	10	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA12 mdtM tet(B)*	Pork
23020067	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork
23020083	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 glpT_E448K mdtM sul2 tet(A)*	Pork
23020088	15640	3: bla_fpi_tet	6: ami_bla_eff_fpi_nit_tet	*aadA1 acrF blaTEM mdtM nfsA_W159STOP sul1 tet(B)*	Pork
23020102	131	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA8 emrD glpT_E448K ptsI_V25I sul2 tet(A) uhpT_E350Q*	Pork
23020116	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A) tet(B)*	Pork
23020170	1721	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 mdtM sul2 tet(A)*	Pork
23020174	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Pork
23020177	101	3: bla_fpi_tet	8: ami_bla_blo_eff_fos_fpi_lin_tet	*aad9 aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 bleO dfrA12 glpT_E448K lnu(C) lnu(G) mdtM sul1 tet(A)*	Pork
23020194	75	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 dfrA1 fosA7.5 glpT_E448K mdtM sul1 tet(A)*	Pork
23020206	11417	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 glpT_E448K mdtM sul2 tet(A)*	Pork
23020242	156	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_mti_tet	*aadA1 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 glpT_E448K mdtM soxS_A12S sul3 tet(B)*	Pork
23020265	453	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Pork
23020277	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork
23020325	641	3: bla_fpi_tet	5: bla_eff_fos_fpi_tet	*acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Pork
23020484	16358	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*aadA1 acrF blaTEM-1 dfrA51 mdtM sul1 tet(A)*	Pork
23070143	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork

23070163	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork
23070167	101	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork
23070199	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 glpT_E448K mdtM sul2 tet(A)*	Pork
23070201	131	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA8 emrD glpT_E448K ptsI_V25I sul2 tet(A) uhpT_E350Q*	Pork
23070228	58	3: bla_fpi_tet	4: bla_eff_fos_tet	*acrF blaTEM-1 glpT_E448K mdtM tet(A)*	Pork
23070258	2594	3: bla_fpi_tet	5: ami_bla_eff_fos_fpi	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA5 emrD glpT_E448K mdtM sul2 uhpT_E350Q*	Pork
23090205	16353	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_qui_tet	*aadA1 acrF blaTEM-1 dfrA1 emrD glpT_E448K parE_I529L ptsI_V25I sul1 tet(A) uhpT_E350Q*	Pork
23090241	58	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_mac_tet	*aadA5 acrF aph(3'')-Ib aph(6)-Id blaTEM dfrA17 glpT_E448K mdtM mph(A) sul1 sul2 tet(A)*	Pork
23100224	1122	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*aadA1 aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA12 mdtM sul3 tet(B)*	Pork
23100232	2077	3: bla_fpi_tet	6: bla_eff_fos_fpi_qui_tet	*acrF blaTEM-1 dfrA14 fosA7.5 glpT_E448K mdtM qnrS sul3 tet(A)*	Pork
23100351	10	3: bla_fpi_tet	5: ami_bla_eff_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA14 mdtM sul2 tet(B)*	Pork
23100384	542	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K mdtM sul3 tet(A) tet(M)*	Pork
23100388	16356	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_sin_tet	*aadA1 acrF blaTEM-1 dfrA1 glpT_E448K mdtM sat2 tet(B)*	Pork
23100390	453	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 acrF blaTEM-1 glpT_E448K mdtM sul3 tet(B)*	Pork
23100413	16356	3: bla_fpi_tet	7: ami_bla_eff_fos_fpi_sin_tet	*aadA1 acrF blaTEM-1 dfrA1 glpT_E448K mdtM sat2 tet(B)*	Pork
23100427	10	3: bla_fpi_tet	7: ami_bla_blo_eff_fpi_mac_tet	*aadA5 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 bleO dfrA17 estT mdtM sul1 sul2 tet(A)*	Pork
23100505	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA7 glpT_E448K mdtM sul2 tet(A)*	Pork

23100541	10	3: bla_fpi_tet	7: ami_bla_blo_eff_fpi_mac_tet	*aadA5 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 bleO dfrA17 estT mdtM sul1 sul2 tet(A)*	Pork
23100605	58	3: bla_fpi_tet	6: ami_bla_eff_fos_fpi_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 dfrA12 dfrA51 glpT_E448K mdtM sul3 tet(A) tet(M)*	Pork
23100531	206	3: bla_phe_tet	7: bla_blo_eff_nit_phequi_qui_tet	*acrF blaTEM bleO mdtM nfsA_R203C oqxA oqxB parC_A56T tet(B)*	Pork
23020098	297	3: fpi_phe_tet	7: ami_blo_eff_fos_fpi_phequi_tet	*aadA2 acrF aph(3')-IIa ble bleO dfrA12 glpT_E448K mdtM oqxA oqxB sul1 tet(A)*	Pork
23020229	16357	3: fpi_phe_tet	8: ami_eff_fos_fpi_mac_phe_qui_tet	*aadA1 aadA2 acrF cmlA1 emrD glpT_E448K mdtM mef(B) parC_A56T sul3 tet(A) tet(B)*	Pork
23020253	4442	3: fpi_phe_tet	7: ami_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF cmlA1 glpT_E448K mdtM mef(B) sul3 tet(A)*	Pork
23070242	10	3: fpi_phe_tet	5: ami_eff_fpi_phe_tet	*aadA1 aadA2 acrF aph(3'')-Ib aph(6)-Id cmlA1 dfrA12 mdtM sul1 sul3 tet(A)*	Pork
23090166	794	3: fpi_phe_tet	7: ami_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF cmlA1 dfrA12 floR glpT_E448K mdtM mph(A) sul3 tet(A)*	Pork
23090175	10	3: fpi_phe_tet	5: ami_eff_fpi_phe_tet	*aadA1 aadA2 acrF aph(3'')-Ib aph(6)-Id cmlA1 dfrA12 mdtM sul1 sul3 tet(A)*	Pork
23090260	11417	3: fpi_phe_tet	6: ami_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF cmlA1 dfrA12 glpT_E448K mdtM sul3 tet(A)*	Pork
23100293	453	3: fpi_phe_tet	6: ami_eff_fos_fpi_phe_tet	*acrF aph(3'')-Ib aph(6)-Id floR glpT_E448K mdtM sul2 tet(A)*	Pork
23100325	141	3: fpi_phe_tet	7: ami_eff_fos_fpi_mti_phe_tet	*acrF aph(3'')-Ib aph(6)-Id emrD floR glpT_E448K marR_S3N sul2 tet(A)*	Pork
23100601	117	3: fpi_phe_tet	8: ami_eff_fmi_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF cmlA1 cyaA_S352T emrD glpT_E448K mdtM mef(B) sul3 tet(A)*	Pork
23100426	641	4: ami_bla_fpi_phe	6: ami_bla_eff_fos_fpi_phe	*aac(3)-IId aadA1 aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM sul2 sul3*	Pork
23020016	10	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 dfrA5 mdtM sul3 tet(A)*	Pork
23020036	3519	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork

23020062	16355	4: bla_fpi_phe_tet	9: ami_bla_eff_fmi_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 cyaA_S352T glpT_E448K mdtM mef(B) sul3 tet(B)*	Pork
23020069	58	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 dfrA51 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23020086	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(B)*	Pork
23020124	2522	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23020165	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_phe_sin_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA1 dfrA12 floR mdtM sat2 sul2 sul3 tet(A) tet(M)*	Pork
23020191	871	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23020231	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(A)*	Pork
23020275	131	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_phe_qui_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 emrD floR glpT_E448K parE_I529L ptsI_V25I sul2 tet(A) uhpT_E350Q*	Pork
23020280	34	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(A)*	Pork
23020282	641	4: bla_fpi_phe_tet	5: ami_bla_eff_fos_fpi	*aac(3)-IId aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 dfrA12 glpT_E448K mdtM sul2*	Pork
23020299	7394	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A) uhpT_E350Q*	Pork
23020308	58	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM mef(B) sul3 tet(B)*	Pork
23020322	542	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K mdtM sul3 tet(A)*	Pork
23020324	16362	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork

23070144	1771	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 emrD glpT_E448K mdtM mef(B) sul3 tet(A)*	Pork
23070150	101	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23070168	1141	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR mdtM sul3 tet(A)*	Pork
23070218	101	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF aph(3")-Ib aph(6)-Id blaTEM-1 cmlA1 glpT_E448K mdtM sul2 sul3 tet(A)*	Pork
23070222	1722	4: bla_fpi_phe_tet	8: ami_bla_eff_fmi_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 cyaA_S352T dfrA12 emrD floR glpT_E448K mdtM sul3 tet(A) uhpT_E350Q*	Pork
23070235	871	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23090197	297	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM mef(B) sul2 sul3 tet(B)*	Pork
23090219	10	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM sul3 tet(A)*	Pork
23090250	215	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(A)*	Pork
23090256	10	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF aph(3")-Ib aph(6)-Id blaTEM-1 cmlA1 mdtM sul3 tet(B)*	Pork
23100125	867	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_lin_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 glpT_E448K lnu(F) mdtM sul1 tet(B)*	Pork
23100161	1716	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23100183	16363	4: bla_fpi_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR mdtM sul3 tet(A)*	Pork
23100225	16361	4: bla_fpi_phe_tet	8: ami_bla_blo_eff_fpi_nit_phe_tet	*aadA1 aadA2 acrF blaTEM-1 bleO cmlA1 dfrA51 mdtM nfsA_E223STOP sul3 tet(A)*	Pork
23100231	2041	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM mef(B) sul3 tet(A)*	Pork

23100248	867	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF aph(3'')-Ib aph(6)-Id blaTEM-1 cmlA1 glpT_E448K mdtM sul3 tet(A) tet(B)*	Pork
23100259	3519	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23100277	278	4: bla_fpi_phe_tet	2: eff_fos	*acrF glpT_E448K mdtM*	Pork
23100331	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF aph(3')-Ia blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(A)*	Pork
23100386	3519	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23100404	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sul3 tet(A) tet(M)*	Pork
23100420	10	4: bla_fpi_phe_tet	8: ami_bla_eff_fpi_mac_phe_sin_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 mdtM mef(B) sat2 sul3 tet(A) tet(M)*	Pork
23100467	127	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mti_phe_tet	*acrF aph(3'')-Ib aph(6)-Id blaTEM-1 catA1 emrD glpT_E448K marR_S3N sul2 tet(A)*	Pork
23100483	16359	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_phe_sin_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR mdtM sat2 sul3 tet(A)*	Pork
23100507	10	4: bla_fpi_phe_tet	7: ami_bla_eff_fpi_lin_phe_tet	*aad9 aadA1 aadA8 blaTEM-1 cmlA1 dfrA12 floR lnu(C) mdtM sul3 tet(A)*	Pork
23100524	16354	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 dfrA5 floR glpT_E448K mdtM sul2 sul3 tet(A) tet(M)*	Pork
23100533	3519	4: bla_fpi_phe_tet	7: ami_bla_eff_fos_fpi_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 floR glpT_E448K mdtM sul3 tet(A)*	Pork
23100538	2035	4: bla_fpi_phe_tet	8: ami_bla_eff_fos_fpi_mac_phe_tet	*aadA1 aadA2 acrF blaTEM-1 cmlA1 dfrA12 glpT_E448K mdtM mef(B) sul3 tet(A)*	Pork
23100553	206	4: bla_fpi_phe_tet	10: ami_bla_blo_eff_fpi_lin_phe_pheq ui_qui_tet	*aadA1 aadA2 acrF aph(3'')-Ib aph(3')-IIa aph(6)-Id blaTEM-1 ble cmlA1 dfrA12 lnu(G) mdtM oqxA oqxB parC_A56T sul1 sul3 tet(A)*	Pork

23020263	117	5: ami_bla_fpi_phe_tet	10: ami_bla_blo_eff_fmi_fos_fpi_phe_ phequi_tet	*aac(3)-IVa aadA1 aadA2 acrF aph(3'')-Ib aph(3')-IIa aph(3')-Ia aph(6)-Id blaTEM-1 ble bleO cmlA1 cyaA_S352T emrD glpT_E448K mdtM oqxA oqxB sul2 sul3 tet(A)*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: bla – beta-lactams, fpi – folate pathway inhibitors, tet – tetracyclines, eff – efflux pumps, fos – fosfomycin, ami – aminoglycosides, qui – quinolonies, lin – lincosamides, blo – bleomycin, mac – macrolides, phe – phenicols, sin – streptothricin. This table does not include multi-class resistant isolates with ciprofloxacin or 3rd generation cephalosporin resistance, which are included in Supplementary Tables 5 and 6.

Supplementary Table 8: MICs *E. faecalis* beef

MIC distributions of *E. faecalis* isolated from retail beef samples (n = 154)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	ECOFF	CLSI	EUCAST
amp					40.9	59.1											0	0	0
																	(0,2.4)	(0,2.4)	(0,2.4)
chl						2.6	6.5	88.3	2.6								0	0	.
																	(0,2.4)	(0,2.4)	.
cip		2.6	2.6	35.1	56.5	3.2											0	0	0
																	(0,2.4)	(0,2.4)	(0,2.4)
dap				7.1	34.4	49.4	8.4	0.6									0.6	0.6	.
																	(0,3.6)	(0,3.6)	.
ery			27.3	26.6	33.1	8.4			0.6	3.9							4.5	4.5	.
																	(1.8,9.1)	(1.8,9.1)	.
gen								98.1	1.9								0	.	.
																	(0,2.4)	.	.
lin			1.9	1.9	1.3	76	18.8										0	0	0
																	(0,2.4)	(0,2.4)	(0,2.4)
nit									93.5	5.8				0.6			0.6	0.6	0.6
																	(0,3.6)	(0,3.6)	(0,3.6)
str												96.8	1.9		0.6	0.6	1.3	.	.
																	(0.2,4.6)	.	.
tei			92.9	7.1													0	0	0
																	(0,2.4)	(0,2.4)	(0,2.4)
tet				61.7	15.6		1.3		0.6	1.9	18.8						21.4	21.4	.
																	(15.2,28.8)	(15.2,28.8)	.
van				17.5	43.5	39											0	0	0
																	(0,2.4)	(0,2.4)	(0,2.4)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 9: MICs *E. faecalis* chicken meat

MIC distributions of *E. faecalis* isolated from retail chicken meat samples (n = 189)

Drug	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	ECOFF	CLSI	EUCAST
amp					54	46											0	0	0
																	(0,1.9)	(0,1.9)	(0,1.9)
chl						2.6	6.3	82	7.4		1.6						1.6	1.6	.
																	(0.3,4.6)	(0.3,4.6)	.
cip		0.5	2.1	35.4	59.8	2.1											0	0	0
																	(0,1.9)	(0,1.9)	(0,1.9)
dap			1.1	3.2	26.5	56.1	12.8	0.5									0.5	0.5	.
																	(0,2.9)	(0,2.9)	.
ery			17.5	15.9	32.8	11.1			1.1	21.7							22.8	22.8	.
																	(17,29.4)	(17,29.4)	.
gen								96.8	3.2								0	.	.
																	(0,1.9)	.	.
lin			0.5	2.1	1.6	79.9	14.8	1.1									1.1	1.1	1.1
																	(0.1,3.8)	(0.1,3.8)	(0.1,3.8)
nit									96.3	3.2		0.5					0.5	0.5	0.5
																	(0,2.9)	(0,2.9)	(0,2.9)
str												98.4		0.5		1.1	1.1	.	.
																	(0.1,3.8)	.	.
tei			97.4	2.6													0	0	0
																	(0,1.9)	(0,1.9)	(0,1.9)
tet				41.8	10.1	0.5			1.1	12.2	34.4						47.6	47.6	.
																	(40.3,55)	(40.3,55)	.
van				5.3	59.8	34.9											0	0	0
																	(0,1.9)	(0,1.9)	(0,1.9)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 10: MICs *E. faecalis* pork

MIC distributions of *E. faecalis* isolated from retail pork samples (n = 198)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	ECOFF	CLSI	EUCAST
amp					48	52											0	0	0
																	(0,1.8)	(0,1.8)	(0,1.8)
chl						1.5	7.1	81.8	3.5	1.5	4.5						4.5	6.1	.
																	(2.1,8.5)	(3.2,10.3)	.
cip		1	2	42.4	50.5	4											0	0	0
																	(0,1.8)	(0,1.8)	(0,1.8)
dap				2.5	26.3	52	18.2	1									1	1	.
																	(0.1,3.6)	(0.1,3.6)	.
ery			22.2	23.2	29.8	13.1			0.5	11.1							11.6	11.6	.
																	(7.5,16.9)	(7.5,16.9)	.
gen								99	0.5						0.5		0.5	.	.
																	(0,2.8)	.	.
lin			0.5	0.5	1	70.7	15.2	3									3	3	3
																	(1.1,6.5)	(1.1,6.5)	(1.1,6.5)
nit									96	4							0	0	0
																	(0,1.8)	(0,1.8)	(0,1.8)
str												92.9	1	1	1.5	3.5	5.1	.	.
																	(2.4,9.1)	.	.
tei			93.4	6.6													0	0	0
																	(0,1.8)	(0,1.8)	(0,1.8)
tet				54	13.1			0.5		2	30.3						32.8	32.3	.
																	(26.3,39.8)	(25.9,39.3)	.
van	0.5			9.1	54	36.4											0	0	0
																	(0,1.8)	(0,1.8)	(0,1.8)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 11: *E. faecalis* genotype

Genotype of multi-class or critically important antimicrobial resistant of *E. faecalis* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23110187	40	1: nit	1: lin_str	*lsa(A)*	Beef
23110538	16	2: lip_tet	2: lin_str_tet	*lsa(A) tet(M)*	Beef
23110395	1961	1: lip	1: lin_str	*lsa(A)*	Chicken meat
23110152	502	1: nit	2: lin_str_tet	*lsa(A) tet(O)*	Chicken meat
23110602	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Chicken meat
23110566	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Chicken meat
23110375	1962	1: lip	1: lin_str	*lsa(A)*	Pork
23110402	21	1: lip	1: lin_str	*lsa(A)*	Pork
23110453	47	3: mac_oxa_tet	7: ami_fpi_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(6)-Ia ant(9)-Ia aph(3')-IIIa dfrF erm(B) fexA lsa(A) optrA tet(L) tet(M)*	Pork
23110394	314	3: mac_phe_tet	6: fpi_lin_lin_mac_str_lin_str_phe_tet	*catA dfrG erm(B) lnu(G) lsa(A) tet(L) tet(M)*	Pork
23110030	16	4: ami_mac_phe_tet	7: ami_lin_lin_mac_str_lin_str_phe_sin_tet	*aac(6')-Ie/aph(2'')-Ia ant(6)-Ia aph(3')-IIIa catA erm(B) lnu(B) lsa(A) lsa(E) sat4 spw tet(M)*	Pork
23110183	498	4: ami_mac_phe_tet	7: ami_fpi_lin_mac_str_lin_str_phe_sin_tet	*ant(6)-Ia aph(3')-IIIa catA dfrG erm(B) lsa(A) sat4 tet(L) tet(M)*	Pork

23110157	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork
23110038	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork
23110147	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork
23110068	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork
23110207	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork
23110601	506	4: mac_oxa_phe_tet	8: ami_fpi_ion_lin_mac_str_lin_str_phe_phe_oxa_tet	*ant(9)-Ia aph(3')-IIIa dfrG erm(B) fexA lsa(A) narA narB optrA tet(L) tet(M)*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: nit – nitrofurans, lip – lipopeptides, tet – tetracyclines, str – streptogramins, lin – lincosamides, oxa – oxazolidinone, ion – ionophore, phe – phenicols, fpi – folate pathway inhibitors, sin – streptothricin, mac – macrolides.

Supplementary Table 12: MICs *E. faecium* beef

MIC distributions of *E. faecium* isolated from retail beef samples (n = 53)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	**ECOFF**	**CLSI**	**EUCAST**
amp					50.9	39.6	7.5	1.9									1.9	0	0
																	(0,10.1)	(0,6.7)	(0,6.7)
chl							17	79.2	1.9		1.9						1.9	1.9	.
																	(0,10.1)	(0,10.1)	.
cip			7.5	26.4	17	35.8	9.4	3.8									0	13.2	3.8
																	(0,6.7)	(5.5,25.3)	(0.5,13)
dap				1.9	5.7	39.6	47.2	3.8	1.9								1.9	5.7	.
																	(0,10.1)	(1.2,15.7)	.
ery			17	1.9	7.5	47.2	13.2	7.5	1.9	3.8							13.2	13.2	.
																	(5.5,25.3)	(5.5,25.3)	.
gen								96.2	3.8								0	.	.
																	(0,6.7)	.	.
lin					1.9	54.7	43.4										0	0	0
																	(0,6.7)	(0,6.7)	(0,6.7)
nit										24.5	30.2	11.3	26.4	7.5			7.5	45.3	.
																	(2.1,18.2)	(31.6,59.6)	.
str												90.6	3.8	3.8	1.9		9.4	.	.
																	(3.1,20.7)	.	.
tei			37.7	52.8	9.4												0	0	0
																	(0,6.7)	(0,6.7)	(0,6.7)
tet				88.7				1.9			9.4						11.3	9.4	.
																	(4.3,23)	(3.1,20.7)	.
van			11.3	69.8	5.7	13.2											0	0	0
																	(0,6.7)	(0,6.7)	(0,6.7)
vir		13.2	18.9	35.8	24.5	3.8				3.8							3.8	.	.
																	(0.5,13)	.	.

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, vir – virginiamycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 13: MICs *E. faecium* chicken meat

MIC distributions of *E. faecium* isolated from retail chicken meat samples (n = 54)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
drug	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	**ECOFF**	**CLSI**	**EUCAST**
amp					33.3	53.7	11.1			1.9							1.9	1.9	1.9
																	(0,9.9)	(0,9.9)	(0,9.9)
chl						3.7	22.2	72.2	1.9								0	0	.
																	(0,6.6)	(0,6.6)	.
cip			1.9	13	16.7	48.1	13	5.6	1.9								1.9	20.4	7.4
																	(0,9.9)	(10.6,33.5)	(2.1,17.9)
dap				1.9	7.4	24.1	61.1	5.6									0	5.6	.
																	(0,6.6)	(1.2,15.4)	.
ery			9.3	7.4	24.1	18.5	7.4	3.7		29.6							33.3	33.3	.
																	(21.1,47.5)	(21.1,47.5)	.
gen								98.1	1.9								0	.	.
																	(0,6.6)	.	.
lin					3.7	74.1	22.2										0	0	0
																	(0,6.6)	(0,6.6)	(0,6.6)
nit									3.7	44.4	16.7	9.3	20.4	5.6			5.6	35.2	.
																	(1.2,15.4)	(22.7,49.4)	.
str												87	1.9		7.4	3.7	13	.	.
																	(5.4,24.9)	.	.
tei			51.9	46.3	1.9												0	0	0
																	(0,6.6)	(0,6.6)	(0,6.6)
tet				61.1		1.9					37						37	37	.
																	(24.3,51.3)	(24.3,51.3)	.
van			11.1	68.5	3.7	16.7											0	0	0
																	(0,6.6)	(0,6.6)	(0,6.6)
vir		9.3	22.2	46.3	14.8		3.7		1.9	1.9							3.7	.	.
																	(0.5,12.7)	.	.

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, vir – virginiamycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 14: MICs *E. faecium* pork

MIC distributions of *E. faecium* isolated from retail pork samples (n = 27)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	**ECOFF**	**CLSI**	**EUCAST**
amp					29.6	44.4	18.5	7.4									7.4	0	0
																	(0.9,24.3)	(0,12.8)	(0,12.8)
chl							29.6	70.4									0	0	.
																	(0,12.8)	(0,12.8)	.
cip			3.7	25.9	18.5	37	7.4	3.7	3.7								3.7	14.8	7.4
																	(0.1,19)	(4.2,33.7)	(0.9,24.3)
dap				3.7	11.1	37	48.1										0	0	.
																	(0,12.8)	0,12.8)	.
ery			14.8		14.8	14.8	14.8	7.4		33.3							40.7	40.7	.
																	(22.4,61.2)	(22.4,61.2)	.
gen								100									0	.	.
																	(0,12.8)	.	.
lin					11.1	66.7	22.2										0	0	0
																	(0,12.8)	(0,12.8)	(0,12.8)
nit										40.7	18.5	14.8	22.2	3.7			3.7	40.7	.
																	(0.1,19)	(22.4,61.2)	.
str												74.1		7.4	7.4	11.1	25.9	.	.
																	(11.1,46.3)	.	.
tei			44.4	55.6													0	0	0
																	(0,12.8)	(0,12.8)	(0,12.8)
tet				66.7							33.3						33.3	33.3	.
																	(16.5,54)	(16.5,54)	.
van			18.5	66.7		14.8											0	0	0
																	(0,12.8)	(0,12.8)	(0,12.8)
vir		14.8	18.5	33.3	7.4	7.4	18.5										0	.	.
																	(0,12.8)	.	.

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amp – ampicillin, chl – chloramphenicol, cip – ciprofloxacin, dap – daptomycin, ery – erythromycin, gen – gentamicin, lin – linezolid, nit – nitrofurantoin, str – streptomycin, tei – teicoplanin, tet – tetracyline, van – vancomycin, vir – virginiamycin, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 15: *E. faecium* genotype

Genotype of multi-class or critically important antimicrobial resistant of *E. faecium* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23110396	2685	1: lip	2: ami_mac_str	*aac(6')-I msr(C)*	Beef
23110466	2733	1: nit	3: ami_mac_str_ple	*aac(6')-I eat(A)_T450I msr(C)*	Beef
23110482	2733	1: nit	3: ami_mac_str_ple	*aac(6')-I eat(A)_T450I msr(C)*	Beef
23110036	62	1: nit	4: ami_lip_mac_str_ple	*aac(6')-I dltC_S63C eat(A)_T450I msr(C)*	Beef
23110605	21	1: str	5: ami_ion_lip_mac_str_ple	*aac(6')-I eat(A)_T450I liaR_E75K msr(C) narA narB*	Beef
23110134	32	1: str	5: ami_ion_lip_mac_str_ple	*aac(6')-I eat(A)_T450I liaR_E75K msr(C) narA narB*	Beef
23110549	1258	2: nit_tet	3: ami_mac_str_ple	*aac(6')-I eat(A)_T450I msr(C)*	Beef
23110669	2738	3: ami_mac_tet	4: ami_lin_lin_str_mac_str	*aac(6')-I ant(6)-Ia lnu(B) lsa(E) msr(C) spw*	Beef
23110414	5	3: bla_mac_tet	7: ami_ion_lin_mac_str_lip_mac_str_ple_tet	*aac(6')-I eat(A)_T450I erm(B) liaR_E75K msr(C) narA narB tet(M)*	Beef
23110422	29	1: qui	4: ami_lip_mac_str_ple	*aac(6')-I eat(A)_T450I liaR_E75K msr(C)*	Chicken meat
23110664	54	1: str	5: ami_ion_lip_mac_str_ple	*aac(6')-I eat(A)_T450I liaR_E75K msr(C) narA narB*	Chicken meat
23110454	94	2: mac_nit	3: ami_mac_str_ple	*aac(6')-I eat(A)_T450I msr(C)*	Chicken meat
23110021	2044	2: mac_str	7: ami_avi_lin_mac_str_lip_mac_str_ple_str	*aac(6')-I eat(A)_T450I emtA erm(B) liaR_E75K liaS_E192G msr(C) vat(E)*	Chicken meat
23110668	2737	3: ami_mac_tet	7: ami_ion_lin_mac_str_lip_mac_str_ple_tet	*aac(6')-I ant(6)-Ia eat(A)_T450I erm(B) liaR_E75K msr(C) narA narB tet(S)*	Chicken meat
23110458	2731	3: mac_nit_tet	4: ami_lin_mac_str_mac_str_tet	*aac(6')-I erm(T) msr(C) tet(L) tet(M)*	Chicken meat

23110405	2737	3: mac_nit_tet	4: ami_lin_mac_str_mac_str_tet	*aac(6')-I erm(T) msr(C) tet(L) tet(M)*	Chicken meat
23110386	1287	4: ami_bla_mac_tet	9: ami_bla_lin_lin_mac_str_lin_str_lip_mac_str_ple_tet	*aac(6')-I ant(6)-Ia aph(3')-IIIa eat(A)_T450I erm(B) liaR_E75K liaS_E192G lnu(B) lsa(E) msr(C) pbp5_E629V spw tet(L) tet(M)*	Chicken meat
23110631	21	1: qui	3: ami_lip_mac_str	*aac(6')-I liaR_E75K msr(C)*	Pork
23110616	2730	3: ami_mac_tet	8: ami_ion_lin_mac_str_lip_mac_str_ple_sin_tet	*aac(6')-I ant(6)-Ia aph(3')-IIIa eat(A)_T450I erm(B) liaR_E75K msr(C) narA narB sat4 tet(L) tet(M)*	Pork
23110247	2728	3: ami_mac_tet	5: ami_lin_mac_str_lip_mac_str_tet	*aac(6')-I ant(6)-Ia erm(B) liaR_E75K msr(C) tet(L) tet(M)*	Pork
23110258	2728	3: ami_mac_tet	5: ami_lin_mac_str_lip_mac_str_tet	*aac(6')-I ant(6)-Ia erm(B) liaR_E75K msr(C) tet(L) tet(M)*	Pork
23110476	2734	3: ami_mac_tet	9: ami_ion_lin_lin_mac_str_lin_str_lip_mac_str_ple_tet	*aac(6')-I ant(6)-Ia eat(A)_T450I erm(B) liaR_E75K lnu(B) lsa(E) msr(C) narA narB spw tet(M)*	Pork
23110325	2730	4: ami_bla_mac_tet	8: ami_ion_lin_mac_str_lip_mac_str_ple_sin_tet	*aac(6')-I ant(6)-Ia aph(3')-IIIa eat(A)_T450I erm(B) liaR_E75K msr(C) narA narB sat4 tet(L) tet(M)*	Pork
23110420	5	4: bla_mac_nit_tet	7: ami_ion_lin_mac_str_lip_mac_str_ple_tet	*aac(6')-I eat(A)_T450I erm(B) liaR_E75K msr(C) narA narB tet(M)*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: lip – lipopeptides, ami – aminoglycosides, mac – macrolides, str – streptogramins, ple – pleuromutilin, nit – nitrofurans, ion – ionophore, avi – avilamycin, tet – tetracyclines.

Supplementary Table 16: MICs *Salmonella* spp. chicken meat

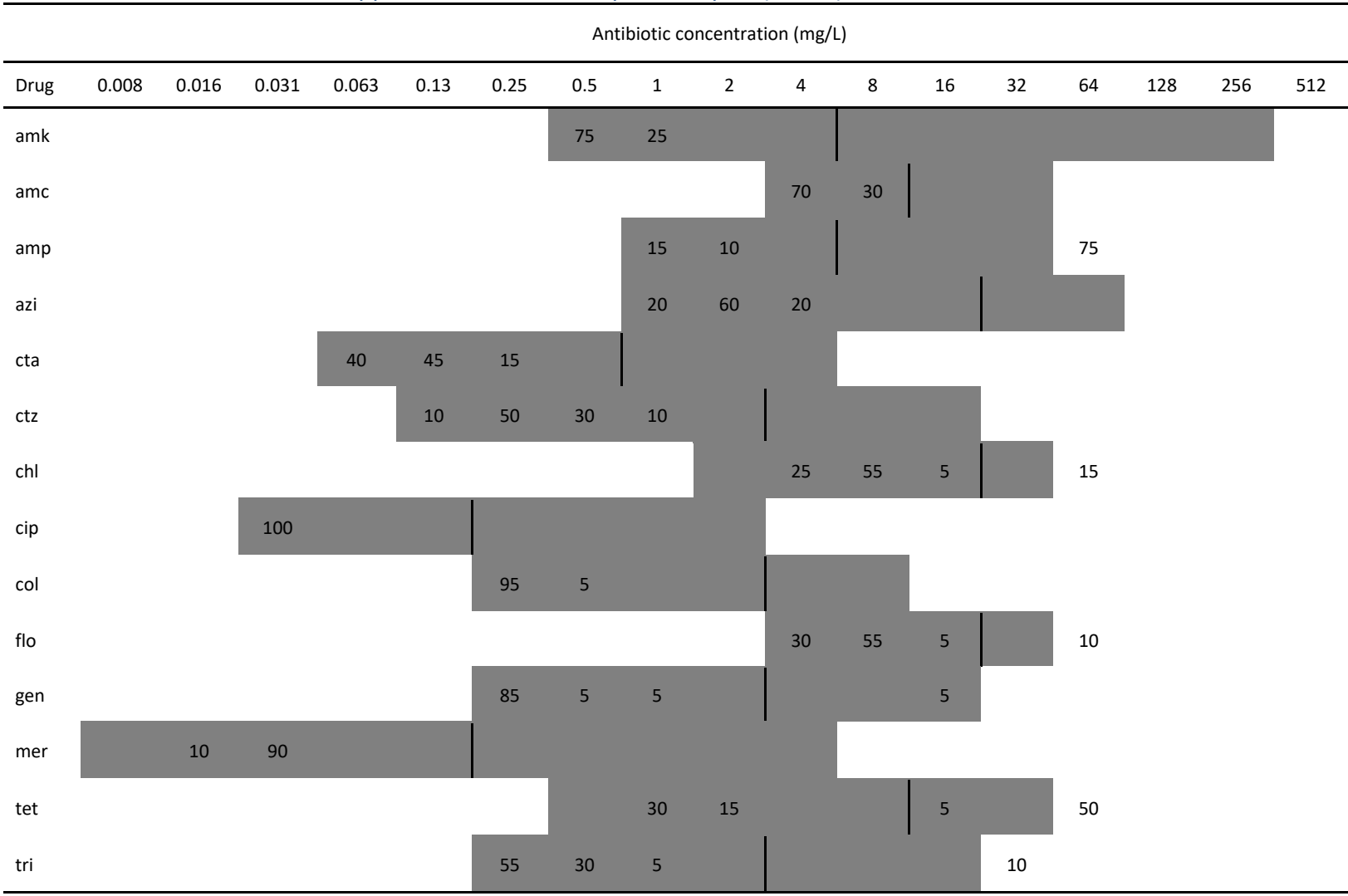
MIC distributions of *Salmonella* spp. isolated from retail chicken meat samples (n = 174)

Drug	Antibiotic concentration (mg/L) 0.008	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	Percentage resistant (%) (95% Confidence Intervals) ECOFF	CLSI	EUCAST
amk							51.7	38.5	8	1.7							0	.	0
																	(0,2.1)	.	(0,2.1)
amc										97.7	2.3						0	.	0
																	(0,2.1)	.	(0,2.1)
amp								50.6	39.7	5.7	0.6			3.4			4	3.4	3.4
																	(1.6,8.1)	(1.3,7.4)	(1.3,7.4)
azi								3.4	92.5	4							0	.	.
																	(0,2.1)	.	.
cta				36.8	48.9	13.8	0.6										0	0	0
																	(0,2.1)	(0,2.1)	(0,2.1)
ctz					23	43.7	31.6	1.7									0	.	0
																	(0,2.1)	.	(0,2.1)
chl									1.1	19	77	2.9					0	0	.
																	(0,2.1)	(0,2.1)	.
cip			100														0	0	0
																	(0,2.1)	(0,2.1)	(0,2.1)
col						90.2	8.6	1.1									0	.	0
																	(0,2.1)	.	(0,2.1)
flo										34.5	63.2	2.3					0	.	.
																	(0,2.1)	.	.
gen						93.7	5.2	1.1									0	.	0
																	(0,2.1)	.	(0,2.1)
mer		6.3	93.1	0.6													0	0	0
																	(0,2.1)	(0,2.1)	(0,2.1)
tet							1.1	62.1	31	1.1				4.6			4.6	4.6	.
																	(2,8.9)	(2,8.9)	.
tri						27	64.4	8	0.6								0	.	0
																	(0,2.1)	.	(0,2.1)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amk – amikacin, amc – amoxicillin/clavulanate, amp – ampicillin, azi – azithromycin, cta – cefotaxime, ctz – ceftazidime, chl – chloramphenicol, cip – ciprofloxacin, col – colistin, flo – florfenicol, gen – gentamicin, mer – meropenem, tet – tetracycline, tri – trimethoprim, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 17: MICs *Salmonella* spp. pork

MIC distributions of *Salmonella* spp. isolated from retail pork samples (n = 20)



Drug	Percentage resistant (%) (95% Confidence Intervals) ECOFF	CLSI	EUCAST
amk	0 (0,16.8)	.	0 (0,16.8)
amc	0 (0,16.8)	.	0 (0,16.8)
amp	75 (50.9,91.3)	75 (50.9,91.3)	75 (50.9,91.3)
azi	0 (0,16.8)	.	.
cta	0 (0,16.8)	0 (0,16.8)	0 (0,16.8)
ctz	0 (0,16.8)	.	0 (0,16.8)
chl	15 (3.2,37.9)	15 (3.2,37.9)	.
cip	0 (0,16.8)	0 (0,16.8)	0 (0,16.8)
col	0 (0,16.8)	.	0 (0,16.8)
flo	10 (1.2,31.7)	.	.
gen	5 (0.1,24.9)	.	5 (0.1,24.9)
mer	0 (0,16.8)	0 (0,16.8)	0 (0,16.8)
tet	55 (31.5,76.9)	55 (31.5,76.9)	.
tri	10 (1.2,31.7)	.	10 (1.2,31.7)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: amk – amikacin, amc – amoxicillin/clavulanate, amp – ampicillin, azi – azithromycin, cta – cefotaxime, ctz – ceftazidime, chl – chloramphenicol, cip – ciprofloxacin, col – colistin, flo – florfenicol, gen – gentamicin, mer – meropenem, tet – tetracycline, tri – trimethoprim, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%).

Supplementary Table 18: *Salmonella* spp. genotype

Genotypes of multi-class resistant *Salmonella* spp. isolated from retail pork meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23100809	515	3: bla_fpi_phe	5: ami_bla_eff_fpi_phe	*aadA1 aadA2 blaTEM-1 cmlA1 dfrA12 mdsA mdsB sul3*	Pork
23100755	19	3: bla_phe_tet	6: ami_bla_eff_fpi_phe_tet	*aph(3'')-Ib aph(6)-Id blaTEM-1 floR mdsA mdsB sul2 tet(A)*	Pork
23100668	34	5: ami_bla_fpi_phe_tet	8: ami_bla_blo_eff_fpi_lin_phe_tet	*aac(3)-IV aadA1 aadA2 aph(3'')-Ib aph(3')-IIa aph(3')-Ia aph(4)-Ia aph(6)-Id blaTEM-1 bleO cmlA1 dfrA12 floR lnu(G) mdsA mdsB sul1 sul2 tet(A) tet(B) tet(H)*	Pork

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity – the original source of the isolate. Abbreviations: bla – beta-lactams, fpi – folate pathway inhibitors, phe – phenicols, eff – efflux pumps, ami – aminoglycosides, tet – tetracyclines, blo – bleomycin, lin – lincosamides.

Supplementary Table 19: MICs *C. coli* chicken meat

MIC distributions of *Campylobacter coli* isolated from retail chicken meat samples (n = 207)

	Antibiotic concentration (mg/L)															Percentage resistant (%) (95% Confidence Intervals)		
Drug	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	ECOFF	CLSI	EUCAST
azi		48.8	33.3	15.9	0.5		0.5		1							1.4 (0.3,4.2)	.	0 (0,1.8)
chl								88.9	8.7	1.9	0.5					0 (0,1.8)	.	.
cip				91.3	6.8	0.5			1.4							1.4 (0.3,4.2)	1.4 (0.3,4.2)	1.4 (0.3,4.2)
cli		16.4	23.7	34.3	19.3	4.8	0.5		0.5				0.5			1 (0.1,3.4)	.	.
ery			4.8	7.2	22.7	22.7	18.4	21.7	1.4		0.5				0.5	1 (0.1,3.4)	0.5 (0,2.7)	1 (0.1,3.4)
flo	1	1	1	1.9	3.9	32.9	37.2	17.9	2.9	0.5						0.5 (0,2.7)	.	.
gen					90.8	7.7	1			0.5						0.5 (0,2.7)	.	.
nal							1.9	8.7	52.7	25.1	8.2	1.9	1	0.5		1.4 (0.3,4.2)	.	.
str							90.3	8.7	0.5			0.5				0.5 (0,2.7)	.	.
tet						94.7	1.4	2.4				1.4				1.4 (0.3,4.2)	1.4 (0.3,4.2)	1.4 (0.3,4.2)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: azi – azithromycin, chl – chloramphenicol, cip – ciprofloxacin, cli – clindamycin, ery – erythromycin, flo – florfenicol, gen – gentamicin, nal – nalidixic acid, str – streptomycin, tet – tetracycline, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0%)

Supplementary Table 20: MICs *C. jejuni* chicken meat

MIC distributions of *Campylobacter jejuni* isolated from retail chicken meat samples (n = 225)

	Antibiotic concentration (mg/L)																Percentage resistant (%) (95% Confidence Intervals)		
drug	0.016	0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	2048	ECOFF	CLSI	EUCAST
azi		64.4	20	15.1					0.4								0.4	.	0.4
																	(0,2.5)	.	(0,1.6)
chl								88	9.3	1.8	0.9						0	.	.
																	(0,1.6)	.	.
cip				83.1	0.4	0.4		1.8	4.9	8.4	0.9						16	14.2	16
																	(11.5,21.5)	(9.9,19.5)	(11.5,21.5)
cli		42.7	24.9	15.1	16.4		0.4						0.4				0.9	.	.
																	(0.1,3.2)	.	.
ery			3.6	2.2	20.9	46.2	23.6	3.1							0.4		0.4	0.4	0.4
																	(0,2.5)	(0,2.5)	(0,2.5)
flo	0.4			0.4	1.3	12.4	54.7	27.1	3.6								0	.	.
																	(0,1.6)	.	.
gen					72.9	27.1											0	.	.
																	(0,1.6)	.	.
nal							1.8	1.8	35.6	44	3.6	5.3	1.8	6.2			13.3	.	.
																	(9.2,18.5)	.	.
str							97.8	1.8	0.4								0	.	.
																	(0,1.6)	.	.
tel						56.9	35.1	5.8	2.2								0	.	.
																	(0,1.6)	.	.
tet						83.1	0.4		0.4	0.9	2.7	4.4	8				16.4	15.1	16.4
																	(11.9,21.9)	(10.7,20.5)	(11.9,21.9)

Values in the right-hand columns indicate the percentage of microbiologically or clinically resistant isolates based on ECOFF, CLSI or EUCAST clinical breakpoints. Dark grey shaded areas indicate the range of dilutions tested for each antibiotic. Numbers within the shaded areas indicate the percentage of isolates that grew at the indicated antibiotic concentration. Numbers to the right of the dark grey shaded areas indicate the percentage of isolates that grew above all concentrations tested. Vertical lines indicate the ECOFF breakpoint. Abbreviations: azi – azithromycin, chl – chloramphenicol, cip – ciprofloxacin, cli – clindamycin, ery – erythromycin, flo – florfenicol, gen – gentamicin, nal – nalidixic acid, str – streptomycin, tel – telithromycin, tet – tetracycline, '.' – no breakpoint available. Colours indicate levels of resistance: Rare (< 0.1%), Very low (> 0.1–1.0%), Low (> 1.0–10.0%), Moderate (> 10.0–20.0%), High (> 20.0–50.0%), Very high (> 50.0–70.0%), Extremely high (> 70.0).

Supplementary Table 21: *C. coli* genotype

Genotypes of multi-class or critically important antimicrobial resistant of *C. coli* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23040007	6775	2: qui_tet	1: bla	*blaOXA-193*	Chicken meat
24020030	6184	1: qui	3: bla_mac_qui	*50S_L22_A103V blaOXA-193 gyrA_T86I*	Chicken meat
24030002	1181	5: ami_mac_phe_qui_tet	1: bla	*blaOXA-193*	Chicken meat

Phenotypes were identified according to CLSI microbroth dilution guidelines using RASP and ECOFFs. Genotype was identified using AMRFinder+ after sequence assembly using SPAdes. Key: Ref – laboratory-allocated identification number for each isolate, Genotype – the number of and classes of antimicrobials with an associated gene found, Phenotype – the number and class of antimicrobials with a resistant phenotype, Genes – all the AMR associated genes identified, ST – multi-locus sequence type, Commodity - the original source of the isolate. Abbreviations: qui – quinolones, bla – beta-lactams, mac – macrolides, tet – tetracyclines, ami – aminoglycosides, phe – phenicols.

Supplementary Table 22: *C. jejuni* genotype

Genotype of multi-class or critically important antimicrobial resistant of *C. jejuni* isolated from retail meat products

Ref	ST	Phenotype	Genotype	Genes	Commodity
23120068	7323	1: qui	3: bla_mac_qui	*gyrA_T86I blaOXA-193 rplV_A103V*	Chicken meat
24010032	7323	1: qui	3: bla_mac_qui	*blaOXA-193 blaOXA-61_G-57T gyrA_T86I rplV_A103V*	Chicken meat
23120209	7323	1: qui	3: bla_mac_qui	*blaOXA-193 blaOXA-61_G-57T gyrA_T86I rplV_A103V*	Chicken meat
24010038	7323	1: qui	3: bla_mac_qui	*blaOXA-193 blaOXA-61_G-57T gyrA_T86I rplV_A103V*	Chicken meat
23120208	7323	1: qui	3: bla_mac_qui	*blaOXA-193 blaOXA-61_G-57T gyrA_T86I rplV_A103V*	Chicken meat

24010034	7323	1: qui	3: bla_mac_qui	*blaOXA-193 blaOXA-61_G-57T gyrA_T86I rplV_A103V*	Chicken meat
23040055	305	2: qui_tet	3: mac_qui_tet	*L22_A103V gyrA_T86I tet(O)*	Chicken meat
23060009	10130	2: qui_tet	3: bla_qui_tet	*blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23120203	1078	2: qui_tet	3: mac_qui_tet	*rplV_A103V gyrA_T86I tet(O)*	Chicken meat
23120025	2895	2: qui_tet	4: bla_mac_qui_tet	*rplV_A103V blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23120023	2895	2: qui_tet	4: bla_mac_qui_tet	*rplV_A103V blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23120063	2398	2: qui_tet	2: qui_tet	*gyrA_T86I tet(O)*	Chicken meat
24020091	50	2: qui_tet	2: bla_tet	*blaOXA-591 tet(O)*	Chicken meat
23070400	305	2: qui_tet	3: bla_qui_tet	*blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23070595	305	2: qui_tet	3: bla_qui_tet	*blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23070602	10130	2: qui_tet	3: bla_qui_tet	*blaOXA-193 gyrA_T86I tet(O)*	Chicken meat
23120006	1078	2: qui_tet	3: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat
24030032	1078	2: qui_tet	3: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat
23120041	1078	2: qui_tet	3: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat
23120039	1078	2: qui_tet	3: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat

23120061	1078	2: qui_tet	4: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O)*	Chicken meat
23120155	1078	2: qui_tet	3: mac_qui_tet	*gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat
24020113	2895	2: qui_tet	4: bla_mac_qui_tet	*blaOXA-193 gyrA_T86I rplV_A103V tet(O)*	Chicken meat
24030021	1078	2: qui_tet	4: bla_mac_qui_tet	*blaOXA gyrA_T86I rplV_A103V tet(O/M/O)*	Chicken meat
23120007	2895	2: qui_tet	4: bla_mac_qui_tet	*blaOXA-193 gyrA_T86I rplV_A103V tet(O)*	Chicken meat
24030028	2895	2: qui_tet	4: bla_mac_qui_tet	*blaOXA-193 gyrA_T86I rplV_A103V tet(O)*	Chicken meat
24020092	2895	3: mac_qui_tet	4: bla_mac_qui_tet	*blaOXA-193 gyrA_T86I rplV_A103V tet(O)*	Chicken meat

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