



Australian Government
Department of Health

Review of published and grey literature on the presence of Antimicrobial resistance in food in Australia and New Zealand



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EXECUTIVE SUMMARY

Antibiotics are used to treat and prevent infectious bacterial diseases in humans and in domestic and food-producing animals. If bacteria become resistant to antimicrobials, then antibiotics become ineffective. While antimicrobial resistance (AMR) is to some extent a natural phenomenon, certain human actions accelerate this process of increasing resistance. The single most powerful contributor to resistance is the global unrestrained use of antibiotics. This includes their underuse, overuse and misuse in both human and animal health (food animals and companion animals), and in agriculture. The World Health Organization (WHO) has described AMR as one of the key global health issues facing our generation. The global nature of the problem means that no one country can act in isolation. Increasing international travel, medical tourism and global trade provide the opportunities for resistance to spread across all borders. Both the Australian and New Zealand Governments have recognised and responded to the global challenge of AMR through several initiatives for assessment and improved antimicrobial use. In 2015, the Australian Government released the first *National Antimicrobial Resistance Strategy 2015-2019* to guide a national response to the threat of antibiotic misuse and resistance. Similarly, in 2017 the New Zealand Government set out their objectives, vision and goals for managing antimicrobials, and published the *New Zealand Antimicrobial Resistance Action Plan*.

The aim of this study was to review published and grey literature on the presence and extent of AMR in food in Australia and New Zealand for the period 1999 to early 2018. This report provides an overview of available evidence for AMR presence in the food production, processing and retail sectors of red meat, pork, poultry meat, dairy, egg, seafood and horticultural products.

Summary tables were prepared to assess and compare the status of available AMR knowledge in each food sector for both Australia and New Zealand. Following comparison of the different food sector and country AMR data, a metric to approximately rank the availability of AMR data was established, where the status of available AMR knowledge was designated Substantial (+++); Moderate (++); Limited (+); or None (-). The review revealed that surveys of AMR in food close to the point of consumption (e.g. foods sampled at retail) were relatively few compared with surveys on-farm or at primary processing. Consequently, the assessments are for an overall measure of AMR data availability along the food chain.

Available AMR literature and data for Australian red meat (particularly beef), pork and chicken meat were assessed and designated as Substantial (+++). In these Australian food sectors, AMR prevalence data for animal pathogen, sentinel indicator and zoonotic foodborne pathogen bacteria are largely available. In comparison, AMR data for New Zealand dairy, red meat, pork and chicken meat sectors, and the Australian dairy sector are ranked as being of Moderate (++) completeness. Limited (+) AMR data are currently available for the food sectors of horticulture (in both Australia and New Zealand), eggs (Australia) and seafood (Australia). A ranking of None (-) was assigned to eggs and seafood in New Zealand due to the absence of any available AMR knowledge for these sectors.

In order to optimally address the knowledge gaps that currently exist in both Australia and New Zealand, support national public health objectives and support Australia and New Zealand's food export industries, recommendations for future action on AMR in food systems have been prepared. The following recommendations are presented in order of the priority considered necessary to deliver the most substantial impact and to comply with standards for globally harmonised surveillance.

Recommendation 1: A senior governance body (e.g. ASTAG) should develop the findings of this report and ensure that food AMR surveillance is included fully within the design and implementation of both Australian and New Zealand national objectives for integrated active surveillance for AMR, including,

Australian National Antimicrobial Resistance Strategy 2015-2019

Objective 3. Develop nationally coordinated One Health surveillance of antimicrobial resistance and antimicrobial usage.

New Zealand Antimicrobial Resistance Action Plan

Objective 2. Surveillance and research – Strengthen the knowledge and evidence base about antimicrobial resistance through surveillance and research.

In the event that full implementation of Recommendation 1 is not practical, the Recommendations 2-4 below are intended to address the most substantial AMR knowledge gaps associated with specific food industry sectors identified in this review.

Recommendations for the implementation of food sector-specific pilot studies appropriate for the provision of harmonised surveillance data

Recommendation 2: As necessary, design and implement targeted pilot surveys for AMR in the specific industry sectors for which very limited or no AMR data are currently available. These food industry sectors are horticulture, eggs and seafood in both Australia and New Zealand.

Recommendation 3: As necessary, design and implement targeted survey approaches to address particular AMR knowledge gaps identified in the Australian dairy sector and New Zealand dairy, red meat, pork and poultry meat sectors.

Recommendations 2 & 3 must involve a developmental process that maintains an emphasis on defining what possible determinations are likely as a result of the surveillance activities and therefore appropriately consider factors relevant to a set of pre-defined and explicit objectives.

Recommendation 4: Focus on the development and application of genomic technologies for efficiency gains and precision in food systems AMR surveillance. Consideration is to be given to them having the capacity to be implemented affordably and on a scale that addresses the complexity of the distribution of AMR in the food supply.

GLOSSARY

AGISAR - WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance

AIHW - Australian Institute of Health and Welfare

AMR - Antimicrobial resistance

APL - Australian Pork Limited

APVMA - Australian Pesticides and Veterinary Medicines Authority

ARG(s) - Antimicrobial resistance gene(s)

AST - Antimicrobial susceptibility testing

ASTAG - Australian Strategic and Technical Advisory Group on AMR

AVA - Australian Veterinary Association

*bla*_{CTX-M} - CTX-M (Cefotaxime Munich) type β -lactamase gene

CIJIG - Commonwealth Interdepartmental Joint Expert Technical Advisory Committee on Antibiotic Resistance

CLSI - Clinical and Laboratory Standards Institute

DAFF - Australian Government Department of Agriculture, Forestry and Fisheries

DCT - Dry cow therapy

DNA - Deoxyribonucleic acid

ECOFF(s) - Epidemiological cut-off value(s)

EFSA - European Food Safety Authority

ESBL(s) - Extended-spectrum β -lactamase(s)

ESC(s) - Extended-spectrum cephalosporin(s)

ESR - Institute of Environmental Science and Research Limited

ETEC - Enterotoxigenic Escherichia coli

EUCAST - European Committee on Antimicrobial Susceptibility Testing

FAO - Food and Agriculture Organisation of the United Nations

FSANZ - Food Standards Australia and New Zealand

JETACAR - Joint Expert Technical Advisory Committee on Antibiotic Resistance

MDR - Multidrug-resistant

MIC - Minimum inhibitory concentration

MIC50 - Minimum inhibitory concentration required to inhibit 50% of tested organisms

MIC90 - Minimum inhibitory concentration required to inhibit 90% of tested organisms

MLA - Meat & Livestock Australia

MPI - New Zealand Ministry for Primary Industries

MRL - Maximum residue limit

MRSA - Methicillin-resistant *Staphylococcus aureus*

NARMS - National Antimicrobial Resistance Monitoring System

NCCLS - National Committee for Clinical Laboratory Standards (renamed as CLSI after 2005)

NSW - New South Wales

NTIS - National Technical Information Service (United States Department of Commerce)

NZ - New Zealand

OIE - World Organisation for Animal Health

PCR - Polymerase chain reaction

PIANZ - Poultry Industry Association of New Zealand

Psa - *Pseudomonas syringae* pv. *actinidiae*

rDNA - Ribosomal deoxyribonucleic acid

RRDP - Rural Research and Development for Profit (Australian Government Department of Agriculture and Water Resources)

rRNA - Ribosomal ribonucleic acid

SCAA - Shared class antimicrobial agents

spp. - Species

ST(s) - Sequence types(s)

STEC - Shiga toxin-producing *Escherchia coli*

UK - United Kingdom

USA - United States of America

USDA - United States Department of Agriculture

VRE - Vancomycin-resistant enterococci

WHO - World Health Organization

BACKGROUND

Antibiotics are used to treat, control and prevent infectious bacterial diseases in humans and in domestic and food-producing animals. If bacteria become resistant to antimicrobials, then antibiotics become ineffective.

Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses and parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard medical treatments become ineffective and infections persist and may spread to others. Health care professionals and veterinarians are left with limited or in some instances, no available treatment options.

While AMR is to some extent a natural phenomenon, certain human actions accelerate this process of increasing resistance. The single most powerful contributor to resistance is the global unrestrained use of antibiotics. This includes their underuse, overuse and misuse in both human and animal health (food animals and companion animals), and in agriculture¹.

In animals, as a result of AMR, veterinarians may also have only limited treatment options and/or may have to change treatments leading to higher costs². Infectious microorganisms carrying AMR can reduce animal health, welfare, biosecurity and production outcomes. Carriage of resistant bacteria by animals can result in the spread of these bacteria, and the transfer of genetic material responsible for resistance, to people who come into contact with them. Carriage by food-producing animals may pose a risk via foodborne transmission³

The World Health Organization (WHO) has described AMR as one of the key global health issues facing our generation. The global nature of the problem means that no one country can act in isolation. Increasing international travel, medical tourism and global trade provide the opportunities for resistance to spread across borders⁴. Both the Australian and New Zealand Governments have recognised and responded to the global challenge of AMR through several initiatives for assessment and improved antimicrobial use.

Australian context

Twenty years ago, in 1998, Australia established a Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). JETACAR comprised experts from the areas of human health, veterinary medicine and primary industry. It was tasked with assessing the scientific evidence of a link between the use of antibiotics in food-producing animals, the

¹ Chan, M. Combat drug resistance: no action today means no cure tomorrow. World Health Day 2011, 6 April 2011. World Health Organization. Available from: http://www.who.int/mediacentre/news/statements/2011/whd_20110407/en/

² Shaban RZ, Simon GI, Trott DJ, Turnidge J & Jordan D 2014, Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia, report to the Department of Agriculture. <http://www.agriculture.gov.au/SiteCollectionDocuments/animal-plant/animal-health/amria.doc>

³ Barrett J. Airborne Bacteria in CAFOs: Transfer of Resistance from Animals to Humans. Environmental Health Perspectives 2005; 113(2): A116–A117.

⁴ Commonwealth of Australia 2015 Responding to the threat of Antimicrobial Resistance - Australia's First National Antimicrobial Resistance Strategy 2015-2019. <https://www.amr.gov.au/resources/national-amr-strategy>

emergence and selection of antibiotic-resistant bacteria and their spread to humans, and to recommend future risk management strategies.

The 1999 JETACAR report made 22 recommendations that fell into five main categories:

- regulatory controls aimed at ensuring responsible use of antibiotics in humans and food-producing animals;
- monitoring and surveillance of the use of antibiotics and changes in antibiotic resistance patterns;
- infection prevention strategies and hygienic measures to reduce the need for antibiotics;
- education, including prudent-use codes of practice; and
- further research into antibiotic use and alternatives to antibiotics.

In 2014, the Australian Government Department of Agriculture commissioned a report, *Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia*.^{Error! Bookmark not defined.} The Report recommends that national surveillance of antimicrobial usage and AMR in animals and agriculture requires the co-operation of Commonwealth and State Departments, including Agriculture, Primary Industries and Health portfolios, as well as academic and industry stakeholders at both governance and operational levels. For continued success and efficiency, programs must be integrated with existing and planned surveillance activities for humans and operate under a 'One Health' umbrella.

Following review of progress and continuing effectiveness of the JETACAR recommendations, in June 2015, the Australian Government released the first *National Antimicrobial Resistance Strategy 2015-2019*⁴ (the Strategy) to guide the response to the threat of antibiotic misuse and resistance. The Strategy was developed in partnership with industry and government, and will guide action by governments, health professionals, veterinarians, farmers and communities to reduce the emergence of resistant bacteria. It focuses predominantly on bacterial resistance and the rapid development of resistance to antibiotics as the area of greatest concern.

On 10 November 2016, the then Minister for Health launched the *Implementation Plan: National Antimicrobial Resistance Strategy 2015-2019*⁵ (Implementation Plan), which identifies the proposed approach to addressing each of the key priorities identified within the Strategy.

New Zealand context

Similar to Australia, initiatives to monitor and manage AMR in New Zealand have been underway for some time. The New Zealand Ministry for Primary Industries (MPI) and its predecessors convened expert panels to consider AMR in animals and plants in 1999 and 2004, and has undertaken activities related to AMR surveillance and antimicrobial use in

⁵ Implementation Plan: Australia's First National Antimicrobial Resistance Strategy 2015-2019.
<https://www.amr.gov.au/resources/national-amr-implementation-plan>

food and agriculture.⁶ The New Zealand Ministry of Health has also undertaken activities focussed on managing AMR in humans.⁷

In 2017 the New Zealand Government set out their objectives, vision and goals for managing antimicrobials, and published the New Zealand Antimicrobial Resistance Action Plan (Ministry of Health and Ministry for Primary Industries, 2017b, a). Just prior to this, the New Zealand Veterinary Association set the aspirational goal “By 2030, NZ Inc. will not need antibiotics for the maintenance of the health and welfare of animals” (New Zealand Veterinary Association, 2015).

New Zealand’s AMR action plan does not set out actions specifically targeting AMR in food but provides for national surveillance of AMR and antimicrobial consumption (Objective 2), and for strategies to ensure antimicrobials are used appropriately in animal health and agriculture (Objective 4). In accordance with Objective 4 of the New Zealand action plan, the MPI has published guidance on the prudent use of antimicrobials in relation to animals and plants (New Zealand Ministry for Primary Industries, 2017).

A 2017 editorial considering antimicrobial use and AMR in New Zealand noted, “both antimicrobial usage and the occurrence of AMR in animals are relatively low compared to the rest of the world” (Guardabassi, 2017). Yet a review focussing on extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae published in the same journal edition revealed a lack of data on the presence and prevalence of these ESBL genes in bacteria from ruminant food animals, and more widely from New Zealand foods and the environment (Toombs-Ruane *et al.*, 2017). Data on antimicrobial use in humans and animals are readily available for New Zealand (Hillerton *et al.*, 2017) but data on AMR among bacteria carried by food-producing animals are not (Guardabassi, 2017). Animals entering the food chain are not routinely tested for AMR (Toombs-Ruane *et al.*, 2017).

In New Zealand food animals most antibiotics are used in the poultry, pig and dairy industries (Hillerton & Allison, 2015). Antibiotics are used among New Zealand animals for therapeutic purposes (i.e. treatment of an existing medical issue) and preventative purposes (i.e. to control a disease when it is likely to occur or has started to occur). However, since 2000 antibiotics are not prescribed at sub-therapeutic doses for the purpose of promoting animal growth in New Zealand (Manson *et al.*, 2004).

Antimicrobial use guidelines and classification in Australia and New Zealand

The classification of different antimicrobials is an important approach to assist in managing antimicrobial resistance, ensuring that all antimicrobials, especially critically important antimicrobials, are used prudently in both human and veterinary medicine. Internationally, the WHO publishes the list of *Critically Important Antimicrobials for Human Medicine* (World Health Organization, 2017) and in Australia, the Australian Strategic and Technical Advisory Group on AMR (ASTAG) has published guidance titled *Importance Ratings and Summary of*

⁶ New Zealand Food Safety – Antimicrobial resistance. (<https://www.mpi.govt.nz/processing/agricultural-compounds-and-vet-medicines/antimicrobial-resistance/>, accessed 21 June 2018)

⁷ New Zealand Ministry of Health – Antimicrobial resistance. (<https://www.health.govt.nz/our-work/diseases-and-conditions/antimicrobial-resistance/>, accessed 21 June 2018)

Antibacterial Uses in Humans in Australia (ASTAG, 2018). The purpose of the Antibacterial Importance Ratings is to provide guidance to clinicians and the pharmaceutical industry about the importance of antibacterial agents available for human use in Australia as well as those agents not used in human health, but that have the potential to select for cross resistance to other antibacterials. ASTAG uses the importance ratings of High, Medium and Low to categorise the severity of impact anticipated from the emergence of resistance to particular antimicrobials (ASTAG, 2018). For example, if an antibacterial is rated as 'High', ASTAG would consider that the severity of impact caused by bacteria resistant to that antibacterial is high, as there are few or no treatment alternatives to such infections.

The Australian Veterinary Association (AVA) has further used the ASTAG *Importance Ratings* to develop guidelines titled *Veterinary Use of Antibiotics Highly Important to Human Health* for application with food animals including pigs, poultry, cattle, sheep and aquaculture (Australian Veterinary Association, 2017). The ASTAG importance ratings Low, Medium and High are aligned with first-line (empirical therapy), second-line (if first-line agents are not available due to treatment failure or on the basis of the results of culture and susceptibility testing), third-line (last resort option) and prohibited drugs. In 2018, a revised ASTAG ratings document was released which lists both human and veterinary antibiotics (ASTAG, 2018).

Similarly, the risk management of antimicrobial resistance due to non-human use is also promoted through first-, second- and third-line best practice guidelines prepared by the New Zealand Veterinary Association. These are available for the dairy and red meat industries (New Zealand Veterinary Association, 2018b, a).

Antimicrobial susceptibility testing guidelines for Australia and New Zealand

For the interpretation of AMR in the selected studies, the WHO definition was applied⁸:

“Antimicrobial resistance is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. Resistant microorganisms (including bacteria, fungi, viruses and parasites) are able to withstand attack by antimicrobial drugs, such as antibacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others”.

Antimicrobial susceptibility testing (AST) refers to *in vitro* methods used to determine the susceptibility of a bacterium to an antimicrobial agent. AST assists both human and veterinary clinicians to determine the most appropriate antimicrobial agents to treat microbial infections. AST is also an important tool to monitor the emergence and spread of antimicrobial resistance (AMR). The following brief introduction to AST and identification of more extensive resources for AST guidance has been summarised from the Australia and New Zealand Standard Diagnostic Procedures (ANZDP; 2014)⁹.

⁸ <http://www.who.int/mediacentre/factsheets/fs194/en/>

⁹ Australia and New Zealand Standard Diagnostic Procedures, July 2014. Available at [Australia and New Zealand Standard Diagnostic Procedures, July 2014](#)

To enable data from AMR surveillance to be compared and interpreted reliably, it is important that laboratories use standardized procedures for AST. The ANZSDP provides information on the principles and practices of AST, with an emphasis on the preferred methods for Australia and New Zealand. AST methods involve culturing a sample to obtain a pure bacterial isolate and testing to determine which antimicrobial agents inhibit the growth of, or kill the pathogen¹⁰. The methods may use broth dilution, agar dilution or disk diffusion methods (methods which are also incorporated in an increasing number of automated platforms for AST). AST testing methodology for Australia and New Zealand (outlined in the ANZSDP) follows the recommendations of the World Organisation for Animal Health (OIE) for use of established and validated methods¹¹; including the disk diffusion method, the broth dilution method and the agar dilution method. The ANZSDP recommends conforming to the Clinical and Laboratory Standards Institute (CLSI) standards^{Error! Bookmark not defined.} for the above methods. Implementation of CLSI standards allows harmonised interpretation of AST and is consistent with the recommendations of Australia's JETACAR report.

Classification of antimicrobial-resistant bacteria in food systems

There are many ways to present the information gathered through this study. For the purposes of this review, the information has been initially separated by each food sector. For each food sector, AMR data relating to specific groups or species of bacteria are separated into those that are pathogenic to animals, those that naturally inhabit animals (commensals) and those that are pathogenic to humans. The latter are called zoonotic pathogens in this report.

Some bacteria, such as *Campylobacter* spp. and *Salmonella* spp., are known foodborne pathogens and are classified as zoonotic bacteria in this report. Others, such as *Escherichia coli*, *Staphylococcus* spp. and the enterococci, form part of the natural microbiota of humans and animals, so are primarily classified as commensals. Despite this classification, it must be recognised that particular genotypes or pathotypes of these commensal species may also cause human and animal infections. Problems primarily arise when these commensal bacteria are resistant to an antimicrobial treatment. Examples include ESBL-producing *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Indeed, much of the information about AMR among commensal bacteria inhabiting animals comes from testing in veterinary laboratories as part of investigating the cause of animal infections.

It must also be recognised that within these commensal bacterial genera there are certain known human pathogenic strains. For example, *E. coli* are natural inhabitants of the gut microbiome but some strains, particularly those able to produce shiga toxins (shiga toxin-producing *E. coli*, STEC), are important foodborne pathogens. *E. coli* O157 is an example of a

¹⁰ CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard third edition. CLSI document VET01-A4. 2013.

¹¹ OIE Terrestrial Manual Guideline 2.1. Laboratory methodologies for bacterial antimicrobial susceptibility testing. 2012.
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/GUIDE_3.1_ANTIMICROBIAL.pdf

pathogenic *E. coli* associated with severe morbidity and high mortality rates among humans. The *E. coli* isolates assessed for AMR are not always tested for the presence of markers for human pathogenicity, but if this information is available, known pathogenic strains are discussed with other zoonotic bacteria.

Based on this approach, bacterial species-level data on AMR are presented as follows:

Animal pathogens: The causative agents of respiratory disease (e.g. *Histophilus somni*, *Mannheimia haemolytica* and *Pasteurella multocida*)

Both *E. coli* and enterococci (i.e., *E. faecium* and *E. faecalis*) are also defined as ‘indicator bacteria’ for AMR because “*most resistance phenotypes present in the animal populations are usually also present in these species; these bacteria are deemed to suffer similar selective pressure and exposure to resistance determinants that other micro-organisms present in the gut flora.*”¹²

For the purpose of clarity concerning specific terminology the following definitions, developed in the recent review of AMR in retail food in the UK¹³, are used in this report.

Foodborne pathogens (adapted from EFSA definition)¹⁴: “*These are pathogenic (disease-causing) micro-organisms such as bacteria (...). Humans get foodborne infections usually through the consumption of food or drinking water contaminated by these bacteria. Infection can also occur through direct contact with food-producing animals or contaminated environment. Human-to-human transmission through faecal-oral route can also occur (e.g., secondary transmission from primary cases). They enter the body through the gastrointestinal tract where the first symptoms often occur. Many of these micro-organisms are commonly found in the intestines of healthy food-producing animals. The risks of contamination are present from farm to fork and require prevention and control throughout the food chain*”.

Commensal bacteria (EFSA definition)¹⁵: “*Are those bacteria that live in or upon the (human or the animal) host without causing disease. Mostly, this co-existence is of mutual benefit. However, many commensals can cause disease if they enter body sites that are normally sterile or when the host’s immune defence is impaired*”.

Indicator bacteria (EFSA definition)¹⁶: “*Those micro-organisms that are used to represent Gram-positive and Gram-negative bacteria present in the gut flora of humans and animals. EFSA recommends the use of *E. coli* (Gram-negative) and Enterococci (i.e., *E. faecium* and *E. faecalis*) as indicators for Gram-negative and Gram-positive bacteria, respectively. The reasoning provided for the selection of these bacteria as indicators is that most resistance*

¹² EFSA definition

¹³ A systematic review to assess the significance of the food chain in the context of antimicrobial resistance (AMR) with particular reference to pork and poultry meat, dairy products, seafood and fresh produce on retail sale in the UK (2016). <https://www.food.gov.uk/sites/default/files/media/document/amr-systematic-review-final-report-2016.pdf>

¹⁴ <http://www.efsa.europa.eu/en/topics/topic/foodbornezoonoticdiseases>

¹⁵ EFSA. EFSA approaches to risk assessment in the area of antimicrobial resistance, with an emphasis on commensal microorganisms. EFSA journal. 2011. 9(10):29.

phenotypes present in the animal populations are usually also present in these species; these bacteria are deemed to suffer similar selective pressure and exposure to resistance determinants that other micro-organisms present in the gut flora". Indicator bacteria are deemed more suitable for the assessment of selective pressure caused by antimicrobial therapy than foodborne pathogens in livestock species due to being ubiquitous in the gut flora.

AIMS AND OBJECTIVES

The Australian Department of Health Food and Nutrition Policy Section of the Preventive Health Policy Branch has committed to action under Priority 3.7 of the Implementation Plan - specifically to; *undertake a literature review to determine the extent to which AMR is present in food, the extent to which food is a route of transmission of AMR, and to identify gaps to inform decision making about the extent of surveillance required and future work*. This is a commitment towards Objective 3 of the Strategy: to develop nationally coordinated One Health surveillance of AMR and antimicrobial usage. It requires a co-ordinated response in all sectors where antimicrobials are used, including in the human health, food and agricultural sectors.

The aim of this study was to review published and grey literature on the presence of AMR in food in Australia and New Zealand. This report provides an overview of the extent to which AMR is understood to be present in food in Australia and New Zealand and identifies gaps in current knowledge. This report considers the following primary food products:

- red meat;
- pork;
- poultry meat;
- dairy;
- egg;
- seafood; and
- horticultural products.

The findings of the literature review will inform understanding of the presence of AMR in the food chain and the nature and extent of risks this may pose to human health. This knowledge will in turn guide future decision making in this area.

METHODS

Scope of research

The requirements for the literature review and report scope were to investigate the presence and frequency of AMR in known foodborne pathogens and commensal bacteria to specific critically important antimicrobials in food in both Australia and New Zealand that could pose a health risk to consumers. There were no specific limitations to the resistant bacterial strains to be included. The literature review scope was however limited to the Australian and New Zealand context as it is considered important to hold a specific review for this region due to the particular nature of Australia and New Zealand's tight biosecurity and food regulation systems.

The scope was limited to published and grey literature for research and surveillance conducted in Australia and New Zealand from 1998 onwards, enabling the capture of research undertaken since the 1999 JETACAR report (1999).

Eligibility criteria

Only studies that considered the following were considered for inclusion in this review.

- red meat, pork, poultry, eggs, milk and milk products, fish and shellfish and horticulture,
- at production, processing and retail level
- produced in Australia and New Zealand (i.e. not imported)

Original scientific articles, literature, grey literature, systematic reviews, scientific opinions and surveillance reports published since 1998 until April 2018 were considered for the purpose of this review.

Studies that assessed AMR prevalence, transmission of resistant bacteria or genetic determinants to humans in/from the following sources: companion animals, horses or exotic pets, direct contact with wildlife; healthcare settings (nosocomial infections; unless the primary cause was a foodborne pathogen of animal or horticultural produce origin) veterinary practice or humans, were not considered eligible.

Study screening process

This study applied a rapid systematic review methodology¹⁶ to meet the six month project timetable. A set of potentially relevant studies were compiled from several sources: 1) forward and backward citation searches on one relevant food animal AMR reference for each country Australia or New Zealand; 2) an updated search of a recent relevant report on food AMR for each country and 3) known relevant studies provided by the review consortium or other known experts. Articles identified by this method, spanning the period from 1998 to present, were considered a 'validation set'. The common key words of these studies were then used to compile the literature search to be tested in PubMed, ensuring that all validation set articles were found by this strategy. Following validation, the search strategies were applied to the various relevant literature and grey literature databases and sources nominated (see Appendix 1 for further details). Two or more reviewers screened titles and abstracts for inclusion and any discrepancies in included references were resolved by discussion or through a third reviewer.

Additional details of the literature search data sources and strategy, including engagement with food AMR stakeholders in Australia and New Zealand for AMR grey literature discovery (Appendix 3), is provided in the APPENDICES section of this report.

¹⁶ Ganann, R., D. Ciliska, and H. Thomas, Expediting systematic reviews: methods and implications of rapid reviews. *Implement Sci*, 2010. 5: p. 56; Polisena, J., et al., Rapid review programs to support health care and policy decision making: a descriptive analysis of processes and methods. *Syst Rev*, 2015. 4: p. 26; Tricco, A.C., et al., A scoping review of rapid review methods. *BMC Med*, 2015. 13: p. 224.

ANTIMICROBIAL RESISTANCE IN RED MEAT AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

The Australian red meat industry includes beef cattle, sheep and goats. Meat and Livestock Australia produce fact sheets outlining the performance and characteristics of the three red meat production systems annually (Meat & Livestock Australia, 2017). A summary of Australia's red meat production systems is provided below. Whilst the majority of goatmeat is harvested from semi-wild rangeland goats and therefore numbers are unknown, Australia accounts for approximately 2% (25 million head) of the global cattle herd and 6% (67 million head) of the global sheep flock. In 2016-17, Australia produced 2.07 million tonnes cwt of beef and veal, 670,000 tonnes cwt of sheepmeat and 31,000 tonnes cwt goatmeat. Beef production occurs predominantly in Queensland (49.5%) and along with New South Wales (NSW) and Victoria comprise >85% of production. Cattle may spend between 50 and 120 days in any of Australia's 450 accredited feedlots and in 2016-17, 39% of all adult cattle slaughtered were marketed as feedlot cattle. The majority of lamb and mutton production occurs in Victoria (43%) with NSW contributing >20% to sheepmeat production. Australia exports 68% of its total beef and veal, 57% of lamb, 92% of mutton and 90% of total goat production ranking it as the world's largest exporter of beef, veal and goatmeat, and the second largest exporter of sheepmeat in 2015. The gross value of production in 2016-17 was \$17 billion, of which beef and veal contributed \$12.7 billion and sheepmeat \$3.9 billion. Beef exports were valued at \$8.3 billion and included \$1.2 billion from live cattle exports. Sheepmeat and goatmeat exports totalled \$2.66 billion and \$250 million, respectively. Australians consume an average of 27.6kg of beef and veal and 10.6kg of lamb and mutton per capita per annum which is 50% and 110% more than the global average for beef and veal and lamb and mutton, respectively.

Australia has a predominance of extensive livestock grazing systems which take advantage of low stocking densities and low antimicrobial usage. Consequently, livestock produced under such grazing systems have very low rates of infectious disease and reports into the development, prevalence and impact of AMR seldom occur. The use of feedlots to intensively finish red meat animals prior to slaughter results in substantial increases in stocking densities and provides increased opportunity for the spread of infectious disease (Meat & Livestock Australia, 2006). Bovine respiratory disease is the most common infectious disease observed in cattle with foot issues and gastrointestinal problems also common. Increased vaccination rates over recent years has assisted in reducing the presence of bovine respiratory disease in feedlot cattle and gastrointestinal infections in calves. Treatment of disease in cattle typically occurs through the use of common antimicrobials such as penicillins, tetracyclines and sulfonamides. The use of antimicrobials of high or critical importance to human medicine in cattle is low with ceftiofur and virginiamycin representing the two uses relevant to human health. Cautious use of ceftiofur in feedlots is encouraged to reduce the likelihood of ESBL resistance developing. Consistent with judicious use principles suggested for ceftiofur, the Australian Lot Feeders' Association in conjunction with Meat & Livestock Australia have developed antimicrobial stewardship

guidelines for the Australian cattle feedlot industry. The guidelines are being rolled out in 2018 and aim to preserve the effectiveness of antimicrobials for human and animal health.

A search of the literature identified a number of studies that examined the prevalence of AMR at the production, processing or retail level. The studies generally reflect the internationally accepted approach of assessing AMR within red meat animal production systems by conducting surveys for the prevalence of resistance in enteric commensal bacteria and zoonotic pathogens such as *E. coli*, *Enterococcus* and *Salmonella*, respectively. As antimicrobial use in red meat production systems occurs primarily to enhance animal health and welfare, smaller studies detailing the assessment of AMR in animal pathogens are also of relevance. This approach is consistent with the JETACAR recommendations for a strategy for AMR surveillance in Australian animals (Anon., 2003; JETACAR, 1999) that proposed a list of organisms and antimicrobials as national priorities for antimicrobial resistance surveillance. As *Campylobacter* is presently not considered a food safety or antimicrobial resistance issue in red meat production, the list of bacteria and antimicrobials can be grouped as animal pathogens (gram +ve or -ve), commensals (*E. coli* and *Enterococcus*) and zoonotic pathogens (*Salmonella*) as shown in Table 1.

Table 1: Organisms and antimicrobials proposed as national priorities for animal antimicrobial resistance surveillance (JETACAR, 1999).

Antimicrobial class	Animal Pathogens		Commensals		Zoonotic pathogens
	Gram -ve	Gram +ve	<i>E. coli</i>	<i>Enterococcus</i>	<i>Salmonella</i>
Aminoglycosides	✓	✓	✓	✓	✓
Amphenicols	✓	✓	✓		✓
β-lactams	✓	✓	✓	✓	✓
Cephalosporins	✓		✓		✓
Glycopeptides		✓		✓	
Lincosamides		✓			
Macrolides	✓	✓		✓	
Quinolones	✓	✓	✓	✓	✓
Streptogramins				✓	
Sulfonamides	✓	✓	✓		✓
Tetracyclines	✓	✓	✓	✓	✓

Histophilus somni, *Mannheimia haemolytica* and *Pasteurella multocoda*

The prevalence of AMR in causative agents of bovine respiratory disease has been investigated as part of small, geographically constrained studies. Goldspink *et al.* (2015) reported on the antimicrobial susceptibility of 53 *Histophilus somni* isolates that were associated with bovine respiratory disease in mostly feedlot cattle from NSW and Queensland. Using minimum inhibitory concentration (MIC) methods for ceftiofur, tetracycline, enrofloxacin, tilmicosin, florfenicol and tulathromycin, all isolates were considered pan-susceptible except for one tetracycline-resistant isolate. Similarly, an earlier study of AMR in *Mannheimia haemolytica* and *Pasteurella multocoda* isolates from diseased cattle in south east Queensland demonstrated that 49/50 were susceptible to tilmicosin (Stephens *et al.*, 1993). *M. haemolytica* is also a causative agent of clinical mastitis in sheep. A study investigating an outbreak of mastitis in a poll dorset flock from Victoria isolated 17 *M. haemolytica* across a four-month period. Resistance to neomycin, streptomycin and sulphafurazole was detected in some of the isolates, but they were all susceptible to penicillin, ampicillin, ceftiofur, amoxycillin/clavulanic acid, ciprofloxacin, tetracycline, erythromycin and trimethoprim (Omaleki *et al.*, 2016). The treatment of clinical mastitis in dairy cattle herds provides opportunity for the development of AMR which may ultimately enter the beef cattle supply chain. AMR associated with bovine mastitis will be reviewed in the dairy cattle section of this review.

Salmonella

Abraham *et al.* (2014b) investigated the prevalence of AMR in a collection of 165 *S. enterica* sourced between 2007 and 2011 from confirmed cases of salmonellosis in livestock. The study included 21 isolates from beef cattle and 85 from dairy cattle and reported AMR presence in 33% and 32% of the isolates, respectively. Typhimurium and Bovismorbificans were the serotypes most frequently associated with AMR from beef or dairy cattle, however there was no statistical association between serotype and the presence of AMR. The study also reported that class 1 integrons (mobile genetic elements capable of acquiring multiple AMR genes) were present in two beef cattle and five dairy cattle isolates. Although the study did demonstrate an absence of resistance to critical antimicrobials, the presence of class 1 integrons provides a potential mechanism by which new antimicrobial resistance genes (ARG) can be rapidly acquired and disseminated.

An investigation into bovine salmonellosis in dairy and dairy beef calves examined the presence of AMR in 76 *Salmonella* isolates, 14 of which were from dairy beef calves (Izzo *et al.*, 2011). In this study *Salmonella* isolates from dairy beef calves were more likely ($p < 0.05$) to be resistant to ampicillin, kanamycin, sulfamethoxazole/trimethoprim and tetracycline than isolates from dairy calves. Resistance to 3rd generation cephalosporins was not identified in dairy beef calves, however the study was the first to report the presence of a ceftiofur-resistant *Salmonella* in a dairy calf. Resistance to 3rd generation cephalosporins was also the focus of the study by Sparham *et al.* (2017) that reported on 31 cefotaxime-resistant isolates of human and animal origin. Investigations into the animal isolates determined that they were mostly bovine in origin and they clustered geographically in a dairy-producing region of Victoria, Australia. This finding, along with the close relatedness of

locally acquired cases of cefotaxime-resistant *Salmonella* suggest they arose from a point source and raise concerns about the possible risks of off-label use of ceftiofur in dairy cattle. These data indicate the potential for emergence of resistance to an antimicrobial agent with a high ASTAG importance rating in dairy cattle herds. Since older dairy cows and calves enter the beef supply chain, this evidence for the development of *Salmonella* AMR is notable (and for completeness has also been reviewed below in the section *Australia - AMR in dairy animal zoonotic pathogens*).

E. coli

The presence of AMR in clinical *E. coli* isolates was assessed as part of the first national Australian veterinary AMR survey (Abraham et al., 2015). In this study 26% of bovine isolates qualified as MDR, primarily as a result of ampicillin, streptomycin, trimethoprim/sulfamethoxazole and tetracycline resistance. It also reported the first detection of extended-spectrum cephalosporin (ESC) resistance in two isolates of *E. coli* from calves (Abraham et al., 2015). The sequence types of *E. coli* detailed in the report had not previously been identified in Australian food-producing animals or as a cause of human infection. The authors propose that introduction may have occurred through human carriers entering the country or migratory wild birds (Abraham et al., 2015).

AMR in red meat animal commensals

E. coli and *Enterococcus* spp.

Unlike studies that assess AMR in clinical isolates, studies that assess AMR in healthy animals or at retail typically require active collection of samples. Consequently, they occur at low frequency and indeed for some Australian food systems are yet to occur at a scale that enables point prevalence comparisons with international datasets. Nevertheless there has been substantial effort in the assessment of AMR in bacteria from beef cattle with initial data available from the late 1970s, when the then Animal Health Committee examined *E. coli* from livestock (Barton et al., 2003). From 2003 to 2004 the then Australian Government Department of Agriculture, Forestry and Fisheries (DAFF) funded a pilot AMR surveillance program that focused on *E. coli* and *Enterococcus* in healthy grass-fed, feedlot and dairy cattle (DAFF, 2007). The study assessed 194 *E. coli* and 158 *Enterococcus* comprising *E. faecium* (n=21) and *E. faecalis* (n=17). Using predominantly CLSI breakpoints, the *E. coli* isolates demonstrated low levels of resistance to florfenicol (1%) and tetracycline (3%). Similarly *Enterococcus* isolates were generally pan-susceptible to all antimicrobials tested with the only notable observation occurring in 2 of 21 (9.5%) of *E. faecium* isolates that expressed resistance to erythromycin and virginiamycin (DAFF, 2007).

In 2013, Meat & Livestock Australia (MLA) funded the largest investigation into the prevalence of AMR in healthy Australian beef cattle at slaughter. The survey collected faecal samples from grass- and grain-fed beef cattle (n=910), dairy cattle (n=290) and veal calves (n=300) and evaluated the prevalence of AMR in 800 *E. coli* and 800 *Enterococcus* (96 *E. faecalis* and 120 *E. faecium*) isolates (Barlow et al., 2015, 2017). Overall, the rates of AMR observed in *E. coli* were low (Table 2) with resistance to fluoroquinolones or 3rd generation cephalosporins not observed. Furthermore, resistance to three or more classes of antimicrobials were detected infrequently and the resistances observed were typically to antimicrobials of limited importance to human medicine (Barlow et al., 2015). In *E. faecalis*

and *E. faecium* isolates resistance to flavomycin (80.2%) and lincomycin (85.4 – 94.2%) was routinely observed though once again, resistance to antimicrobials considered critically or highly important to human medicine was not observed (Barlow *et al.*, 2017). Importantly, regardless of the bacteria-antimicrobial combination assessed, there appeared to be minimal evidence that specific production practices are responsible for disproportionate contributions to AMR development. The low levels of resistance observed in both the Department of Agriculture, Canberra and MLA funded studies are supported further by the findings of the 2007 to 2008 survey of AMR bacteria in Australian food. In that study 81% of *E. coli* were pan-susceptible to all antimicrobials tested and when resistance was observed in the *E. coli* or *Enterococcus* isolates assessed it was generally limited to older antimicrobials of limited human clinical significance (Barlow & Gobius, 2008).

Genetic based studies

In addition to AMR studies that target a particular animal pathogen, commensal or zoonotic pathogen there are studies that attempt to detail the AMR load of a production system or environment without bias towards particular bacteria. Increasingly, the importance of studies that assess the molecular diversity underpinning the development and transfer of AMR (i.e. resistome¹⁷) is being recognised. Resistome studies are yet to be completed for red meat animals or their associated environments, however there are AMR studies that have focused on the role of mobile genetic elements known as integrons. In 2008 and 2009, Barlow *et al.* (2008, 2009) reported on the prevalence and composition of class 1 and class 2 integrons, both at and through the slaughter process using an unbiased detection and isolation procedure. The studies detailed the presence of integrons in more than 10 genera, many of which would never be a focus of conventional AMR surveys. Analysis of the gene cassettes within the integrons determined that most were likely to carry genes that encode resistance to older antimicrobials of limited human clinical significance such as chloramphenicol, trimethoprim and aminoglycosides.

Furthermore, integrons could be recovered from grass-fed, grain-fed and certified organically produced cattle at similar rates suggesting that their presence may be independent of production system practices. The distribution of integrons in *E. coli* isolates recovered from 304 animal clinical cases corroborate the findings from studies on healthy animals, with integrons most likely to harbour genes encoding resistance to trimethoprim and aminoglycosides (Dawes *et al.*, 2010). Overall, the findings are of importance as they demonstrate that production practices and regulations relating to the use of antimicrobials in beef cattle production systems is not encouraging the development of integrons that carry resistance genes to critical or highly important human antimicrobials. Nevertheless it

¹⁷ The resistome is defined as the collection of all antibiotic resistance genes in both pathogenic and non-pathogenic bacteria. The resistome includes antibiotic resistance genes present in pathogenic bacteria, antibiotic-producing bacteria, cryptic resistance genes (very low or absent gene expression) and precursor genes (which may evolve to full resistance under antibiotic selection pressure).

Wright GD. 2007. *The antibiotic resistome: the nexus of chemical and genetic diversity*. Nature Reviews Microbiology. 5: 175–186. doi:10.1038/nrmicro1614.

signals the need for ongoing prudent use of antimicrobials to ensure that development of integrons does not occur within beef production systems.

Table 2: Antimicrobial resistance patterns of *E. coli* and *Salmonella* from beef cattle, dairy cattle and veal calf faecal samples (Barlow et al., 2015).

Antimicrobial Resistance Patterns ^a	<i>E. coli</i> ^b			<i>Salmonella</i> ^b			
	Beef (N=469)	Dairy (N=155)	Veal (N=176)	Beef (N=106)	Dairy (N=75)	Veal (N=36)	Major serotypes present
ALL SENSITIVE	432 (92.1)c	150 (96.8)	164 (93.2)	97 (91.5)	75 (100)	36 (100)	Typhimurium
AMP		1 (0.6)	1 (0.6)				
STR	1 (0.2)		1 (0.6)	1 (0.9)			Adelaide
TET	30 (6.4)		1 (0.6)				
AMP FAZ			1 (0.6)				
AMP TET		1 (0.6)					
FAZ TET	1 (0.2)						
STR TET	4 (0.9)	1 (0.6)	1 (0.6)				
TET SXT	1 (0.2)						
AMP STR TET			1 (0.6)				
AMP STR SXT				1 (0.9)			Meleagridis

Antimicrobial Resistance Patterns ^a	<i>E. coli</i> ^b			<i>Salmonella</i> ^b			
	Beef (N=469)	Dairy (N=155)	Veal (N=176)	Beef (N=106)	Dairy (N=75)	Veal (N=36)	Major serotypes present
AMP TET SXT				1 (0.9)			Dublin
AUG2 AMP FAZ			1 (0.6)				
AMP STR TET SXT		2 (1.3)	1 (0.6)	6 (5.7)			Typhimurium (3), Orion (2), Anatum (1)
GEN STR TET SXT			1 (0.6)				
AUG2 AMP FAZ TET			1 (0.6)				
AMP KAN STR TET SXT			2 (1.1)				

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

b Total *E. coli* (N=800) and total *Salmonella* (N=217) were isolated from beef cattle, dairy cattle and veal cattle faeces and then tested for AMR.

c Figures in parentheses are percent.

AMR in red meat zoonotic pathogens

E. coli

In studies that determine the prevalence of AMR in *E. coli* from healthy animals there is often little consideration given to the virulence markers that each isolate may possess. Consequently surveys of AMR in pathotypes of *E. coli* are scarce and rarely reported. Lajhar *et al.* (2017) reported the AMR profiles of 78 *E. coli* isolates belonging to the O26 serogroup, the majority of which belonged to the enterohaemorrhagic *E. coli* (EHEC) or atypical enteropathogenic *E. coli* (aEPEC) pathotypes. Low levels of AMR were observed in the cattle O26 isolates with resistance to any antimicrobial occurring in <9% of isolates. The results correlate well with the findings from surveys on generic *E. coli* from healthy animals confirming that such a focus is appropriate for AMR prevalence studies.

Salmonella

The prevalence of MDR *Salmonella* in healthy red meat animals at slaughter and subsequently in the products that are produced from those animals has been highlighted of late due to a push in the United States to have particular serotypes of MDR *Salmonella* classified as adulterants in some red meat products. In the period from May 2000 until the last annual report in 2009, the Australian *Salmonella* Reference Centre reported on the antimicrobial susceptibility of 1,977 *Salmonella* isolates from cattle. Across almost a decade of reporting, no resistance to fluoroquinolones was observed and only a single isolate was shown to be resistant to 3rd generation cephalosporins. These findings are matched by those of Barlow *et al.* (2015) who reported on the prevalence of 217 *Salmonella* isolates recovered from 1500 beef cattle, dairy cattle and veal calves. The report described a low prevalence of overall AMR, low rates of MDR and an absence of resistance to antimicrobials of high or critical importance to human health (Table 2).

Clostridium difficile

Cattle have been identified as potential reservoirs of a major enteropathogen of human health, *Clostridium difficile*. Increasing reports of genetic overlap between animal and human isolates have facilitated a need to further understand the AMR profiles of these groups of isolates. In 2016, Knight and Riley reported that pan-susceptibility was observed to vancomycin, metronidazole, rifaximin, amoxicillin/clavulanate, meropenem and piperacillin/tazobactam in a collection of 171 *C. difficile* isolates comprising 31 neonatal calf and 91 human clinical samples (Moono *et al.*, 2016). Multidrug resistance was observed in 25% of human *C. difficile* but was not observed in any of the animal isolates suggesting that resistance may be a function of the use of antimicrobials in the human healthcare system as opposed to use in food production systems.

NEW ZEALAND

Industry background

New Zealand red meat animals are also predominantly farmed on extensive pastures, resulting in low stocking densities and low antimicrobial usage. Feedlots are used for overwintering in some areas, and also for producing grain-finished beef.

Provisional data for 2017 show 3.6 million beef cattle, which are predominantly raised on outdoor pastures, often with sheep (Beef + Lamb NZ, 2018). Beef is also produced from cull dairy cows, very young (bobby) calves and young bulls. For the year ending 30 September 2017, 2.4 million cattle and 1.7 million calves were processed at export plants and abattoirs, and 633,000 tonnes of beef and veal were produced with the majority being exported (Beef + Lamb NZ, 2018).

Sheep are raised outdoors for meat and wool. For the year ending 30 September 2017, 19.5 million lambs and 3.7 million sheep were processed at export plants and abattoirs, producing 362,000 tonnes of lamb and 94,000 tonnes of mutton (Beef + Lamb NZ, 2018). Most of this is exported.

The deer and goat industries are comparatively smaller. Data for 2017 show approximately 850,000 deer were being farmed for venison, velvet and other deer products, and approximately 300,000 were processed at export plants and abattoirs (Beef + Lamb NZ, 2018). Goats are farmed for meat, milk and fibre. The largest populations are located in the Waikato, where New Zealand's only goat milk drying plant is located.¹⁸ For the year ended September 2017, approximately 90,000 export graded goats were processed to produce 1,100 tonnes of meat.

Estimates for New Zealand red meat consumption are lower than those reported for Australia, with per capita consumptions of 17 kg of beef and veal, and 6.3 kg of lamb and mutton for the year ending September 2017 (Beef + Lamb NZ, 2018).

The red meat industry promotes the responsible use of antimicrobials.¹⁹ Of the available information on AMR among red meat animals in New Zealand, most data are from cattle. Dairy animals are discussed separately (see next section).

AMR in red meat animal pathogens

No data were identified.

AMR in red meat animal commensals

Escherichia coli

A set of *E. coli* isolates was obtained from swabs of dressed carcasses of very young calves as part of a 2009/10 survey of AMR bacteria present in food and food animals (Heffernan et al., 2011). Of the 300 *E. coli* isolates, approximately half (48%) were susceptible to all 17 of the antimicrobials tested, although more than 40% of the isolates demonstrated resistance to streptomycin, sulfamethoxazole or tetracycline. There was no resistance to the 3rd generation cephalosporins cefotaxime and ceftiofur, the fluoroquinolone ciprofloxacin nor the aminoglycoside gentamicin. There was notable resistance to ampicillin (24%) and trimethoprim (13%). None of the *E. coli* isolates produced ESBL or AmpC β -lactamase.

¹⁸ [The New Zealand Dairy Goat Co-operative](#) (accessed 21 June 2018).

¹⁹ [Antimicrobial use in NZ beef and sheep farming](#) (accessed 21 June 2018).

Enterococcus spp.

E. faecalis (n=185) and *E. faecium* (n=92) were isolated from swabs of dressed carcasses of very young calves during a 2009/10 survey (Heffernan et al., 2011). No resistance to ampicillin, vancomycin or a high concentration of gentamicin was reported among either species. Of the *E. faecalis* isolates, 42% were susceptible to all the antimicrobials tested, but 36% were resistant to a high concentration of streptomycin and 55% were resistant to tetracycline. *E. faecalis* is intrinsically resistant to quinupristin/dalfopristin. Tetracycline resistance was also notable among the *E. faecium* isolates (62%), along with ciprofloxacin resistance (79%) and quinupristin/dalfopristin resistance (26%). Only 5.4% of *E. faecium* isolates were fully susceptible to all antimicrobials tested.

AMR in red meat zoonotic pathogens

Campylobacter spp.

Data on AMR among *Campylobacter* spp. isolated from the faeces and offal of beef cattle and sheep, and from carcass swabs of very young calves, are available. These indicate AMR is low among *Campylobacter* spp. but the numbers of isolates tested were low:

- *C. jejuni* (n=34) and *C. coli* (n=5) were isolated from beef cattle faeces collected in Canterbury during 2001, plus an additional five *C. jejuni* isolates and one *C. coli* isolate were obtained from samples of beef offal (liver, kidney, heart) (Harrow et al., 2004). All 45 isolates were susceptible to erythromycin, ciprofloxacin, nalidixic acid and tetracycline.
- In the same Canterbury study (Harrow et al., 2004), 28 *Campylobacter* spp. isolated from sheep faeces (15 *C. jejuni* and 13 *C. coli*) and an additional 24 *C. jejuni* isolates from sheep offal were all susceptible to erythromycin, ciprofloxacin, nalidixic acid and tetracycline.
- 93% of 56 isolates of *Campylobacter* spp. (49 *C. jejuni* and 7 *C. coli*) from dressed carcasses of very young calves swabbed during a 2009/10 survey were susceptible to all seven antimicrobials tested (Heffernan et al., 2011). Notably, no resistance to erythromycin, ciprofloxacin or nalidixic acid was detected. Streptomycin resistance was observed among *C. jejuni* (8.2% resistant).

Salmonella spp.

Salmonella spp. were also isolated in the aforementioned survey of very young calves (Heffernan et al., 2011). However, as only 19 isolates were isolated, the prevalence of AMR cannot be considered conclusive.

A 2010 report (Broughton et al., 2010) summarises AMR among 268 *Salmonella* described as being from a “bovine” source. These isolates were selected from non-human *Salmonella* isolates received by ESR between 2002 and 2007. They were submitted by diagnostic veterinary laboratories, the national surveillance programme for processed meats and commercial laboratories that refer isolates from food and environmental sources. Almost one-third of these isolates were not susceptible (i.e. demonstrated resistance or intermediate susceptibility) to streptomycin. The proportions not susceptible to the other antibiotics were lower: Sulfonamides (13%), tetracycline (12%) and <3% for ampicillin and trimethoprim.

“Ovine” isolates were also tested (n=342). The only notable result was for streptomycin (25% not susceptible).

ESR continues to periodically test a sample of non-typhoidal *Salmonella* isolates from animals, foods and the environment for AMR.²⁰ Detailed data on animal source to support this current review are not readily available.

E. coli O26

E. coli O26 is known to be pathogenic to humans. *E. coli* O26 isolates from bovines (n=120), collected during the period 1985 to 2016, were whole genome sequenced and analysed for the presence of eight classes of antibiotic resistance genes (Browne et al., Unpublished). The isolates may have been from samples from dairy cows, beef cows or calves. Genes for β -lactam, phenicol, quinolone and trimethoprim resistance were not detected, and only one (0.8%) isolate harboured the macrolide-resistance gene. The genes for sulphonamide, tetracycline and aminoglycoside resistance were detected in 9%, 11% and 25% of the isolates, respectively.

ANTIMICROBIAL RESISTANCE IN DAIRY AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

Commercial dairy production in Australia is predominantly bovine, with a lesser degree of milk production from other animal species, which include sheep, goat, camel and buffalo. In 2016-17, there were 1.5 million dairy cows in Australia, producing over 9000 million litres of milk (Dairy Australia, 2018a). During this same period, 37% of milk products were exported, worth \$3 billion (Dairy Australia, 2018a). Dairying in Australia is predominantly in coastal areas, relying on natural rainfall for pasture growth (Dairy Australia, 2018b). Unlike dairying in the colder climates of the Northern Hemisphere, dairy cows in Australia are principally pasture-based, entering dairy sheds primarily for milking. Therefore the majority of the cows' life is spent on pasture, which will influence the nature of the microorganisms to which they are exposed. As commercial production is mainly bovine, research on antibiotic-resistant microorganisms in the dairy industry also predominantly focuses on dairy cows.

The position of the Australian Veterinary Association (AVA) is that antimicrobials should be used as little as possible, but sufficiently enough to treat infection (Australian Veterinary Association, 2017). In order to preserve the use of antimicrobials highly important for human health, the AVA have advocated for the responsible use of antimicrobials in veterinary medicine. The preliminary choice of antimicrobials should ideally be selected from the list of “first line” antimicrobial agents as indicated by the AVA (Table 3). Second line antimicrobials are indicated in cases of treatment failure and third line antimicrobials are used only as a last resort (Australian Veterinary Association, 2017). Further to this, the AVA suggest alternatives to antimicrobial use where possible through improved animal

²⁰ Public Health Surveillance. *Salmonella*. <https://surv.esr.cri.nz/antimicrobial/salmonella.php>

husbandry, farm management, vaccination, and infection prevention and control (Australian Veterinary Association, 2017). If antimicrobials are used, steps need to be taken to ensure that antimicrobial residues do not end up in the food chain (Australian Veterinary Association, 2017; Dairy Australia, 2012). A program has been organised by Dairy Australia which detailed 10 steps for dairy farmers to employ to minimise the risk of antimicrobial residues in dairy calves that are sent for slaughter (Dairy Australia, 2012).

Table 3: Antimicrobial agents indicated for the treatment of cattle in Australia, divided into first line, second line and third line, as recommended by the Australian Veterinary Association (Australian Veterinary Association, 2017).

First line	Second line	Third line	Use Prohibited
Ampicillin/Amoxicillin	Amoxicillin-clavulanate	Ceftiofur	Fluoroquinolones
Erythromycin	Cefuroxime	Polymixin B (may be used first line as topical treatment for individual animals)	Gentamicin
Oxytetracycline	Cloxacillin	Virginiamycin	Chloramphenicol
Sulphonamides	Apramycin		Nitrofurans
Oleandomycin	Lincomycin		
Tilmicosin	Trimethoprim-		
Tylosin	Sulphonamides		
Penicillin	Tulathromycin		
Florfenicol			
Framycetin			
Neomycin			
Streptomycin			

The contribution of the dairy farm environment as the preliminary step in the farm to retail chain has not been examined in great detail in Australia. Limited investigations have been reported regarding the presence of antibiotic-resistant microorganisms on dairy farms. A broad Australian study that looked at the presence of various pathogens in the environment of seven bovine, caprine and ovine farms characterised the presence and antibiotic resistance of *Salmonella* on the farms (McAuley et al., 2017). *Salmonella* was only detected on one of the bovine farms in the soil, feed, farm water sources, raw milk and milk filter, from which the serotypes *S. Orion*, *S. Zanzibar* and *S. Infantis* were obtained. None of the 12 resulting unique *Salmonella* isolates were resistant to any of the 17 aminoglycosides, β -lactams, carbapenems, cephalosporins, amphenicols, quinolones, sulfonamides or tetracyclines tested. Information on the antibiotic resistances present in other genera in dairy farm environments is lacking. The following examination of the literature is based on antibiotic resistance investigations of food and animal-derived microorganisms.

AMR in dairy environment pathogens

Listeria monocytogenes

Listeria monocytogenes is another microorganism of concern in dairy products as it can cause the serious illness listeriosis. Although *L. monocytogenes* is generally reduced by milk pasteurisation to concentrations that are less likely to present a problem to human health (Farber, 1989), post-pasteurisation contamination may occur, providing entry of *Listeria* into final product. An assessment of the antimicrobial resistance of *L. monocytogenes* in Australian food sources included 52 dairy products (Wilson et al., 2018). The isolates were all susceptible to tetracycline and penicillin G; however, resistance was observed to ciprofloxacin in two of the dairy food isolates, with one of these isolates also having erythromycin resistance (Wilson et al., 2018). In one of the isolates, the fluoroquinolone efflux protein (*fepA*) regulator, *fepR*, had a single-point mutation that was identified as the potential cause of the ciprofloxacin resistance. The erythromycin-resistant isolate was found to have the *ermB* gene (Wilson et al., 2018).

AMR in dairy animal pathogens

Mannheimia haemolytica

An investigation of a clinical outbreak of mastitis in sheep found that *Mannheimia haemolytica* was the causative agent (Omaleki et al., 2016). Milk from 21 mastitic sheep and three dead sheep yielded 16 isolates of *M. haemolytica*. All of the isolates were susceptible to ampicillin, amoxicillin/clavulanic acid, ceftiofur, ciprofloxacin, erythromycin, tetracycline and trimethoprim. Extensive resistance was seen to neomycin (44%), streptomycin (56%) and sulphafurazole (68%), and all isolates were resistant to novobiocin. Multiple drug resistance was seen in four of the isolates to all four of these antibiotics.

AMR in dairy animal commensals

Enterococcus

Enterococci have various intrinsic resistances to antibiotics, which is also influenced by species (McAuley, 2017), so it is important that species are defined when considering antibiotic resistance in enterococci. *E. faecalis* was the predominant species isolated from raw milk obtained from dairy factories (McAuley, 2017; McAuley & Craven, 2005). The population of *E. faecalis* (n=60) exhibited resistance to ampicillin (1.7%), chloramphenicol (1.7%), ciprofloxacin (1.7%), erythromycin (11.7%), gentamicin (6.7%), streptomycin (31.7%) and tetracycline (46.7%) but not to penicillin or vancomycin. A predominant phenotype in 26.7% of the *E. faecalis* was the combination of resistance to streptomycin and tetracycline, with four isolates having the multiple drug resistance patterns to chloramphenicol, erythromycin and tetracycline, erythromycin, streptomycin and tetracycline, or gentamicin, streptomycin and tetracycline. Genetic analysis using polymerase chain reaction (PCR) determined that chloramphenicol resistance was conveyed by the *cat* gene, erythromycin resistance was conveyed by the *ermB* gene, and tetracycline resistance was conveyed by the *tetL*, *tetM* or *tetS* genes, with *tetM* predominating. In addition to raw milk, butter (n=14), Cheddar cheese (n=7) and spray-dried milk powder (n=10) products contained a range of enterococci species which were screened but negative for vancomycin resistance (McAuley & Craven, 2005).

Both *E. faecalis* (n=9) and *E. faecium* (n=25) from dairy cow faeces were susceptible to the glycopeptides, tigecycline, daptamycin, linezolid, penicillins and chloramphenicol tested in a survey of cattle populations at slaughter (Barlow et al., 2017). These two species both showed some resistance (<20% of isolates) to erythromycin and tetracycline. *E. faecalis* additionally had a similar amount of resistance to kanamycin and streptomycin (Barlow et al., 2017), but the levels of resistance to streptomycin and tetracycline were much lower than those seen for *E. faecalis* from raw milk (McAuley & Craven, 2005). The former study emphasized that all of the enterococci assessed were susceptible to the medically important antimicrobials linezolid, daptomycin, tigecycline and vancomycin (Barlow et al., 2017).

E. coli

A pilot surveillance program of *E. coli* from dairy cattle caecal specimens (n=65) found that there was no antimicrobial resistance to 10 antimicrobials (ampicillin, chloramphenicol, ceftiofur, cefotaxime, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, tetracycline and trimethoprim/sulphamethoxazole) (DAFF, 2007). A separate study of AMR in *E. coli* isolated from dairy cow faeces focused on four antibiotics that were important in human medicine and had widespread use in the dairy industry (Jordan et al., 2005). That study yielded more than 10,000 *E. coli* isolates and found resistance to sulfamethoxazole, tetracycline, ampicillin and gentamicin in 7.3%, 3.6%, 2.2% and 0.09% of isolates, respectively (Jordan et al., 2005). The larger number of isolates obtained in the latter study may have provided greater sensitivity to detect a lower prevalence of antibiotic resistance.

A more recent study of *E. coli* isolated from dairy cow faeces looking at a broader range of antibiotics (n=16) found that 96.8% of the isolates (n=155) were susceptible to amoxicillin/clavulanic acid, cefazolin, cefotaxime, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, meropenem, nalidixic acid, streptomycin, tetracycline and trimethoprim/sulfamethoxazole (Barlow et al., 2015). The antibiotic resistances that were detected were similar to the study of Jordan et al. (2005), with resistance to ampicillin (1.9%), tetracycline (2.6%) and trimethoprim/sulfamethoxazole (1.3%) (Barlow et al., 2015). Additionally, the more recent study found substantial resistance to florfenicol (58.7%), and multiple drug resistance to the combinations of ampicillin and tetracycline, streptomycin and tetracycline, and ampicillin, streptomycin, tetracycline and trimethoprim/sulfamethoxazole in 0.6%, 0.6% and 1.3% of the isolates, respectively (Barlow et al., 2015). However, resistance to fluoroquinolones, and 3rd and 4th generation cephalosporins, which are of greater importance in human medicine, was not detected in *E. coli* isolated from dairy cow faeces (Barlow et al., 2015).

AMR in dairy animal zoonotic pathogens

Salmonella enterica

Faeces were the main reservoir investigated for the presence of *Salmonella*, which is not unexpected given that *Salmonella* is an enteric pathogen. The detection of antibiotic-resistant *Salmonella* varied between studies, which may have been influenced by the health status of the animals. In a study of *S. enterica* (predominantly *S. Typhimurium*, followed by *S. Bovismorbificans*) isolated from dairy cow faeces obtained at abattoirs, AST against 17 antibiotics only detected resistance to florfenicol in 34.7% of the isolates, with no resistance to cephalosporins and fluoroquinolones (Barlow et al., 2015). Abraham et al. (2014b)

conducted an investigation of AMR in dairy cattle salmonellosis isolates, identifying a greater range of *Salmonella* serotypes and antibiotic resistances than observed by Barlow et al. (2015), however the overall susceptibility (69.4%) to 19 antibiotics assessed was similar to the study of Barlow et al. (2015). The salmonellosis study showed predominant resistance to sulfafurazole in 17.6% of isolates, and the major serotypes were *S. Typhimurium*, *S. Bovismorbificans*, *S. Dublin* and *Salmonella* Subspecies I, serotype 4,5 (Abraham et al., 2014b). *Salmonella* MDR patterns included resistance to ampicillin and sulfafurazole, with additional resistance to either tetracycline or trimethoprim (and one or two other less common resistances). In 3.5% of isolates, MDR to five antibiotics (ampicillin, sulfafurazole, trimethoprim, tetracycline and neomycin) was observed (Abraham et al., 2014b). The genes conferring these resistances were *sul1* and *sul2* (sulphonamide), *dhfrV* (trimethoprim), *blaTEM* (β -lactam), *tetA* and *tetB* (tetracycline), and *aphA1* (aminoglycoside) (Abraham et al., 2014b).

Izzo et al. (2011) compared diarrhoea from dairy calves and dairy beef calves, where dairy beef calves were steers and heifers that originated from the dairy industry, in part, and were being finished off for the beef market. Only one *Salmonella* serotype was isolated from each of the majority of farms, with the predominant serotypes being *S. Dublin* in dairy calves and *S. Newport* in dairy beef calves. All of the *Salmonella* isolates (n=76) were susceptible to nalidixic acid and amikacin. Although 75.8% of the dairy isolates were susceptible to all 12 antibiotics tested, this reduced to 57.1% among the dairy beef isolates, indicating that AMR appeared to be more prevalent in the dairy beef calves. The following proportions of isolates showed resistance to ampicillin (dairy isolates, 12.9%; dairy beef isolates, 42.9%), combination sulphonamides (dairy isolates, 16.1%; dairy beef isolates, 42.9%), tetracycline (dairy isolates, 4.8%; dairy beef isolates, 42.9%), sulfamethoxazole/trimethoprim (dairy isolates, 4.8%; dairy beef isolates, 42.9%), neomycin (dairy isolates, 8.1%; dairy beef isolates, 35.7%), ceftiofur (dairy isolates, 1.6%; dairy beef isolates, 0%), kanamycin (dairy isolates, 4.8%; dairy beef isolates, 28.6%), apramycin (dairy isolates, 4.8%; dairy beef isolates, 7.1%), amoxicillin/clavulanic acid (dairy isolates, 1.6%; dairy beef isolates, 0%) and streptomycin (dairy isolates, 21% %; dairy beef isolates, 42.9%), indicating that antibiotic resistance appeared to be more prevalent in the dairy beef calves. Multiple drug resistance was also more prevalent in the dairy beef isolates (18.8%) compared to the dairy isolates (8.1%). A *S. Anatum* isolate from dairy beef showed MDR to eight antibiotics (ampicillin, sulphonamides combination, tetracycline, sulfamethoxazole/trimethoprim, neomycin, kanamycin, apramycin and streptomycin). The greater prevalence of antibiotic resistance and MDR in the dairy beef herds is potentially concerning and may reflect differences in practice between dairy farms and dairy beef farms. However, Izzo et al. (2011) pointed out that further work was recommended, given the small number of dairy beef farms (n=8) involved in the study.

Human and bovine isolates of *Salmonella* Typhimurium DT44 were characterised at the *Salmonella* Reference Laboratory at the Microbiological Diagnostic Unit Public Health Laboratory at the University of Melbourne (Sparham et al., 2017). The origin of the bovine isolates was unknown but it was suspected that they originated from a dairy-producing region of Victoria. The 18 bovine isolates were resistant to cefotaxime (CTX) and showed potential ESBL production, which was possibly due to a CTX-M-type β -lactamase encoded by *blaCTX-M-9*. All of the isolates were susceptible to the fluoroquinolones ciprofloxacin and

norfloxacin, as well as gentamicin, despite the presence of the antibiotic resistance gene for gentamicin (*aadB*). Of note these bovine isolates were closely related to isolates from humans and were thought to potentially be the source of the human infections, indicating that resistance to 3rd generation cephalosporins in animal populations could foreshadow transmission of antibiotic-resistant infections to the human population (Sparham *et al.*, 2017).

S. aureus

A broad survey of cow, goat and sheep dairy farm environments found *S. aureus* (n=13) on all three farm types in the milk and milk filters (McMillan *et al.*, 2016). The isolates were assessed for resistance to ceftriaxone, ciprofloxacin, erythromycin, oxacillin, penicillin, tetracycline and vancomycin and were sensitive to all antibiotics except for penicillin, to which one bovine isolate was resistant. The bovine isolate was found to carry the *blaZ* gene for penicillin resistance (McMillan *et al.*, 2016).

In a reverse scenario to the potential transmission of antibiotic-resistant *Salmonella* from cattle to the human population (Sparham *et al.*, 2017), an investigation of MRSA in a dairy cow was thought to potentially come from humans (Abraham *et al.*, 2017b). The MRSA isolate was obtained from the milk of a cow with an elevated Bulk Milk Cell Count and sub-clinical mastitis. The isolate was also penicillin-resistant but was susceptible to chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, rifampicin, tetracycline and trimethoprim. Whole genome sequencing of the isolate revealed that it had the staphylococcal cassette chromosome encoding *mecA*, which encodes for methicillin resistance, as well as the genes *blaZ* and *norA* (quinolone resistance). The bovine isolate clustered with sequence type (ST) ST1-IV isolates from humans and it was suspected that reverse zoonotic transmission had occurred (Abraham *et al.*, 2017b).

NEW ZEALAND

Industry background

Like Australia, dairy production in New Zealand is largely based on pasture-fed cattle. Cows' milk production has doubled over the last 20 years, from 10,339 million litres of milk processed in 1996/97 to 20,702 million litres in 2016/17 (LIC/DairyNZ, 2017). Production typically peaks in October and is minimal through the winter months of June and July. There are no official data on the volume of dairy products produced in New Zealand from cows' milk. A large proportion of cows' milk is used to produce milk powder, fluid milk, butter and cheese. New Zealand bovine dairy herds are decreasing in number but increasing in the number of animals per herd (LIC/DairyNZ, 2017). Overall, the total number of dairy cows has increased to approximately five million, and productivity (as measured by milk solid production per cow and per hectare) has also increased. Stocking densities are slowly increasing, e.g. from an average of 2.42 cows/ha in 1996/97 to 2.81 in 2016/17.

There are no consolidated data on milk production from non-bovine species. The Dairy Goat Co-operative, who manufacture goat milk powder products, show 72 supplying shareholders.²¹ There are also dairy goat farms that produce their own fluid milk and/or

²¹ [Dairy Goat Co-operative](#) (accessed 21 June 2018).

other products (e.g. cheeses, yoghurt) for sale to the public, or who supply milk to dairy product manufacturers. Dairy goats may be housed or raised outdoors. There are a few herds of milking buffalo in New Zealand. It appears that the milk from these animals is mainly used for producing yoghurt and cheese.

Within the dairy industry, the greatest use of antibiotics is for the treatment, control and prevention of mastitis (Hillerton & Allison, 2015). Because most new infections of the mammary gland occur before the next calving during the non-lactating period, a common approach is to infuse the udder with a long-acting antibiotic after the last milking of the season (dry cow therapy, DCT). In New Zealand, prophylactic antimicrobial DCT was not routinely used until the introduction of Somatic Cell Count penalties in 1993/94 when it became an economical option.²² DCT is considered to be effective for curing existing infections and preventing new infections. Concern about the implications of blanket DCT (i.e. used for all dry cows in a herd) on the development of AMR, has led to the recommendation in the DairyNZ prudent use guidelines of alternative approaches in the New Zealand dairy industry, such as the use of internal teat sealants (ITS) dependent on the infection status of the herd.²³

AMR research among New Zealand dairy animals has largely focussed on mastitis-causing bacteria, considered to be dairy animal pathogens in this section. *Staphylococcus aureus* is the most important mastitis-causing pathogen that is able to spread from cow to cow, and *Streptococcus uberis* is the most common environmental pathogen isolated from bovine mastitis cases (Burgess & French, 2017). There are other causative agents of bovine mastitis in New Zealand, including *Streptococcus dysgalactiae*, other *Staphylococcus* species (e.g. *Staphylococcus chromogenes*, *Staphylococcus hyicus*), coliforms (e.g. *Escherichia coli* and *Klebsiella* spp.), *Trueperella pyogenes* (previously called *Arcanobacterium pyogenes*), *Enterococcus* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Serratia* spp. and *Nocardia* spp.

²² Woolford, M., and Lacy-Hulbert, S.J. (1996). Mastitis research in New Zealand, Volume Proceedings of the 2nd Pan Pacific Veterinary Conference: Cattle Sessions - incorporating the 13th Annual Seminar of the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association, (VetLearn Foundation). Lacy-Hulbert, J., Blackwell, M., and McDougall, S. (2011). SmartSAMM - The smart approach to minimising mastitis, Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference, (VetLearn Foundation).

²³ <https://www.dairynz.co.nz/about-us/investment/summaries-and-reports/prudent-use-of-antimicrobials-for-mastitis-pilot-study-dry-cow-mastitis-rd1442/>. McDougall, S., and Compton, C. (2010). Controlling mastitis in pasture based systems, Volume Proceedings of the 3rd AVA/NZVA Pan Pacific Veterinary Conference, (Australian Veterinary Association).

While humans may be infected by some of these mastitis-causing bacteria, such as *S. uberis* and *T. pyogenes*, they are not important foodborne pathogens and information from these has been included among that of other dairy animal pathogens.²⁴ Some of the mastitis-causing bacteria listed above are not commensals, but are found in the environment.

A survey and review of antimicrobial use on dairy farms revealed that penicillins (particularly the semisynthetic antimicrobials cloxacillin, ampicillin and amoxicillin) were the most commonly prescribed antimicrobials (Bryan & Hea, 2017). Their use for DCT is common.

Two cephalosporin-based products are also administered for DCT, containing cephapirin benzathine and cephalonium (MPI, 2016). Both active ingredients are 1st generation cephalosporins which are considered by the WHO to be “highly important”. Increased sales of 3rd and 4th generation cephalosporins (particularly ceftiofur) for treatment of lactating cows has also been noted (MPI, 2016).

AMR in dairy animal pathogens

Streptococcus

Early work evaluated AMR among streptococci isolates from 36,000 milk samples examined at a Waikato Animal Health Laboratory during the period 1976-95 (Carman & Gardner, 1997). Most milk samples were submitted by veterinarians whose clients were having problems with clinical mastitis or high bacterial/somatic cell counts. Some were submitted for evaluation of antibiotic treatment when cows were being dried off. All *Streptococcus* spp. had high (up to 100%) natural resistance to streptomycin. In contrast there was little or no resistance to the other antibiotics tested.

Another large study also found high levels of resistance of *S. uberis* (n=3,429) and *S. dysgalactiae* (n=1,045) to streptomycin and another aminoglycoside, neomycin (Petrovski et al., 2011).²⁵ The proportion of susceptible isolates was as low as 2%. This study collated mastitis testing data from all milk samples submitted to five commercial veterinary laboratories between 2003 and 2006. To improve the quality of the study, only isolates from samples meeting the following criteria were evaluated: (i) only a single bacteria species was isolated from the milk sample, (ii) >1,000 sensitivity tests were available for that bacterial species, and (iii) analysis included at least one antimicrobial that had been tested against >500 isolates.

Nine other antimicrobial agents were tested against these *S. uberis* and *S. dysgalactiae* isolates. Results were reported as the percentage susceptible, with the remainder being a mixture of isolates with intermediate and full resistance. For both streptococci species,

²⁴ *Streptococcus dysgalactiae* is divided into two subspecies (LSPN Website, accessed 25 April 2018). *Streptococcus dysgalactiae* subsp. *equisimilis* are pathogenic to humans, while *Streptococcus dysgalactiae* subsp. *dysgalactiae* are animal pathogens. However, these subspecies are often referred to in the literature as *S. dysgalactiae* and *S. equisimilis* Jensen A & Kilian M (2012) Delineation of *Streptococcus dysgalactiae*, its subspecies, and its clinical and phylogenetic relationship to *Streptococcus pyogenes*. J Clin Microbiol 50: 113-126.

²⁵ Note that for this study, the number of isolates of each species tested against each antibiotic varies. See Petrovski et al. (2011) for the specific denominators.

>90% of isolates were susceptible to the β -lactams, except for ampicillin, which was closer to 80%. Susceptibility to erythromycin and lincomycin was >85% for *S. uberis*, and was 84% and 69% for *S. dysgalactiae*, respectively. Almost all (97%) of *S. uberis* were susceptible to tetracycline but this figure was only 11% for *S. dysgalactiae*.

While this is a relatively large data set, there was no record of whether the milk samples were from subclinical or clinical mastitis cases; the samples were submitted by veterinarians so are more likely to be intractable cases rather than the true pattern of mastitis cases in New Zealand. Also, farms with a comprehensive mastitis management programme were more likely to be over-represented (the data will be influenced by multiple samples from single farms).

A later study (2006/07) also tested AMR among *S. uberis* (n=106) and *S. dysgalactiae* (n=41) isolates from cows' milk samples received by commercial laboratories as part of mastitis investigations (Petrovski *et al.*, 2015). It is not clear whether the 2006 isolates overlapped with those tested in the study of Petrovski *et al.* (2011). Again, a high level of resistance to streptomycin and neomycin was reported for *S. uberis* ($\leq 0.9\%$ susceptible). Of the smaller set of *S. dysgalactiae* isolates, 70% were susceptible to streptomycin but all were resistant to neomycin. Resistance towards most of the other antibiotics was low (>90% susceptible). Some notable exceptions were for *S. uberis* resistance to lincomycin (40% susceptible) and enrofloxacin (62% susceptible), and *S. dysgalactiae* resistance to tetracycline, oxytetracycline or enrofloxacin (2%, 12% or 32% susceptible, respectively).

Sensitivity to neomycin and the related aminoglycoside framycetin was low (39% and 53% sensitive, respectively) among *S. uberis* isolates from milk samples taken during a similar period, 2004-08, although the number of isolates tested was low (61, 59) (Gibson *et al.*, 2010). The milk samples were submitted to different laboratories to those reported by Petrovski *et al.* (2011). Approximately 2,000 *S. uberis* isolates were tested for resistance to seven other antibiotics, of which between 92% (oxacillin) and 100% (amoxicillin and clavulanic acid) were sensitive.

Resistance to penicillins was low (1%) or absent among isolates of *S. dysgalactiae* (n=64) and *S. uberis* (n=102) from milk drawn from cows with clinical and subclinical mastitis (McDougall *et al.*, 2014). Some resistance to trimethoprim/sulfamethoxazole was measured (17% of *S. dysgalactiae* and 13% of *S. uberis*). MIC values were calculated for a range of other antibiotics, but accepted interpretive criteria were available for these organisms so the proportion resistant was not calculated. The time period for this study was not reported but the available information suggests the results from these isolates had not been reported elsewhere. The authors noted that some isolates specified as *S. uberis* may have been *Enterococcus* spp. since the phenotypic tests used do not adequately differentiate these from each other.

A 1998 paper reports the antimicrobial susceptibility of streptococci isolates from colostrum samples from recently calved heifers (Salmon *et al.*, 1998). The proportion of isolates considered resistant to each antimicrobial was not reported since, at the time, interpretive criteria specific for mastitis-causing bacteria were not available for most of the antibiotics

tested²⁶. Instead, the MIC was ascertained for each isolate, with all antimicrobials tested at concentrations ranging from 0.06 to 64.0 µg/ml. All *S. dysgalactiae* isolates (n=15) had MIC values ≤1 µg/ml. The MIC₉₀ for all *S. uberis* (n=85) isolates was ≤2.0 µg/ml, but MIC values of 16.0 µg/ml were reported for cloxacillin, ceftiofur and novobiocin. For the third group comprising multiple *Streptococcus* spp. (n=31) the range of MICs for all tested antibiotics included values >64 µg/ml, however the MIC₉₀ values were all ≤2 µg/ml.

S. aureus

An early study compiled AMR data for *S. aureus* isolates from 36,000 milk samples submitted to a Waikato animal health laboratory during the period 1976-95 (Carman & Gardner, 1997). For the year 1976, 65% of *S. aureus* isolates were resistant to penicillin but a small decline was observed over the remainder of the study period. The authors speculated that this was probably a result of reduced use of this antibiotic. Resistance to streptomycin was <10%. When excluding all isolates of intermediate resistance (by disk diffusion assay) there was no evidence of resistance to cephalothin, nafcillin, oxacillin (cloxacillin) or tetracycline. There was a possible rising trend of resistance to lincomycin.

A later study of *S. aureus* (2003-06) suggests that resistance to penicillin continued to decrease (Petrovski *et al.*, 2011). However, the results are not directly comparable as they are reported as the percentage susceptible with remainder of isolates having intermediate or full resistance. For penicillin, 73% were susceptible, which was comparable to ampicillin (also 73%)^{Error! Bookmark not defined.}. The percentage susceptible to the other β-lactams tested was >97%. Seventy-two percent of *S. aureus* isolates were susceptible to streptomycin but it is not known whether this indicates increased resistance to this antibiotic compared to the earlier study. Some resistance to erythromycin and lincomycin was demonstrated (75% and 66% susceptible, respectively).

Of *S. aureus* isolates (n=107) from cows' milk samples tested in commercial laboratories as part of mastitis investigations during 2006/07, 79% were susceptible to penicillin (Petrovski *et al.*, 2015). It is not clear whether the 2006 isolates overlapped with those tested in the study of Petrovski *et al.* (2011). The same proportions were also resistant to amoxicillin and ampicillin. Resistance to streptomycin was very low (99% susceptible) and was not measured for any other antimicrobial, including erythromycin and lincomycin.

Another study has evaluated the AMR of *S. aureus* isolates from milk samples taken during a similar period, 2004-08 (Gibson *et al.*, 2010). The milk samples were submitted to different laboratories to those reported by Petrovski *et al.* (Petrovski *et al.*, 2011). In agreement with Petrovski *et al.* (Petrovski *et al.*, 2015; Petrovski *et al.*, 2011), 75% of the 2,423 *S. aureus* isolates tested for penicillin resistance were sensitive. In contrast to Petrovski *et al.* (Petrovski *et al.*, 2011), all isolates tested for erythromycin resistance (n=2,243) were sensitive. Sensitivity to seven other antibiotics was >97%. The percentage susceptible to streptomycin (51%) was lower than that reported in Petrovski *et al.* (Petrovski *et al.*, 2015; Petrovski *et al.*, 2011).

²⁶ The antibiotics tested separately in this study were penicillin, cloxacillin, cephalixin, ceftiofur, novobiocin, enrofloxacin, erythromycin and pirlimycin. A combination of penicillin/novobiocin was tested in the range 0.06 µg of penicillin plus 0.13 µg of novobiocin/ml to 64.0 µg of penicillin plus 128.0 µg of novobiocin/ml.

Penicillin resistance was reported as 28% among another 364 *S. aureus* milk isolates, and was 27% for ampicillin (McDougall *et al.*, 2014). The time period for this study was not reported but the available information suggests the results from these isolates had not been reported elsewhere. These isolates were from subclinically (n=159) and clinically (n=104) affected cows sampled during a research project. Based on MIC values, resistance to amoxicillin/clavulanic acid, ampicillin, cloxacillin and penicillin was lower among *S. aureus* isolates from clinical mastitis cases compared to those from subclinical cases. For example, the likelihood of an isolate being defined as resistant to penicillin was 2.5 times greater for isolates from subclinical than clinical cases. The authors noted that further studies were required to determine if prior exposure to antibiotics was one reason behind this finding.

Also published during 2014 was a study of the effectiveness of penethamate hydriodide as a treatment for mastitis (Steele & McDougall, 2014). As part of this work, 55 *S. aureus* isolates from 92 mastitic cows (three Waikato herds) were tested for AMR. The β -lactamase structural gene (*blaZ*) was detected in 24/55 (43.6%) of these isolates and 24/53 (45%) were resistant to penicillin by zone diffusion (one *blaZ*-negative isolate was also resistant). No resistance to cephalothin, novobiocin or tetracycline was observed; one isolate was oxacillin-resistant.

A 1998 paper reports the AMR of 79 *S. aureus* isolates from colostrum samples from recently calved heifers from 11 farms (Salmon *et al.*, 1998). Over the period 1976-95 *S. aureus* moved from being the major cause of mastitis (66% of samples in 1976), to being approximately equal to environmental *Streptococcus* (*S. uberis*, *S. dysgalactiae*). As indicated above, the proportion of isolates considered resistant to each antimicrobial is not reported due to an absence of interpretive criteria specific for mastitis-causing bacteria. Instead, the MIC was ascertained for each isolate, with all antimicrobials tested at concentrations ranging from 0.06 to 64.0 $\mu\text{g/ml}$. The MIC₅₀ values were $\leq 1.0 \mu\text{g/ml}$ for all antibiotics tested, the MIC₉₀ values were $\leq 4.0 \mu\text{g/ml}$ and the MIC range included 64 $\mu\text{g/ml}$ for all antibiotics with the exception of ceftiofur and enrofloxacin. The MIC₅₀ and MIC₉₀ values for penicillin of 0.5 and 4.0 $\mu\text{g/ml}$, respectively, indicates a relatively high proportion were resistant to penicillin, using the CLSI cut-off values as indicated in McDougall *et al.* (2014).

MIC values are also available for 115 *S. aureus* isolated from milk of mastitis infected cows during the period 2002/03 (Situmbeko, 2004). All samples were from North Island farms (Hamilton and Palmerston North) and were submitted for testing by farmers or veterinarians. The MIC values exceeded the highest concentrations tested for ampicillin (MIC $\geq 2 \mu\text{g/ml}$ for 32% of isolates), dihydrosteptomycin (MIC $\geq 8 \mu\text{g/ml}$ for 55% of isolates), and penicillin (MIC $\geq 4 \mu\text{g/ml}$ for 23% of isolates).

Based on CLSI interpretive criteria, there were 39 isolates with borderline penicillin MIC readings (range 0.0625 to 0.25 $\mu\text{g/ml}$) so were tested for the production of β -lactamase enzyme using nitrocefin discs. One isolate tested positive. In total, 39 (34%) isolates were resistant to penicillin based on these criteria, including the one borderline β -lactamase-positive isolate. A later study further analysing these results reports 44/115 (38%) as being penicillin-resistant but does not explain this discrepancy (Grinberg *et al.*, 2005). Grinberg *et al.* (2005) identified clonal populations of *S. aureus* on farms that did not import stock,

which meant that there was a higher probability of *S. aureus* on these farms having the same resistance status.

MRSA

No MRSA were detected in an early study of colostrum milk (Salmon *et al.*, 1998) nor in a survey of milk samples from mastitic cows (Situmbeko, 2004).

A single MRSA isolate has been identified from cows' milk. Of 1,022 *S. aureus* isolates obtained from a private veterinary diagnostic laboratory network, seven were oxacillin-resistant and five were confirmed as MRSA and characterised (Grinberg *et al.*, 2008). One MRSA was from a sample of cows' milk, but the total number of *S. aureus* isolates from cows' milk was not reported. The MRSA isolate from cow's milk was identified from a mixed culture with methicillin-susceptible *S. aureus*. This isolate was resistant to erythromycin and ciprofloxacin and exhibited inducible resistance to clindamycin (according to interpretative criteria for isolates from humans). The other four MRSA were from dogs and a cat. These five isolates, plus two additional MRSA isolates from nasal swabs of veterinarians, all belonged to the British epidemic MRSA 15 strain (EMRSA-15).

Staphylococcus spp. other than S. aureus

Coagulase-negative staphylococci (n=285), isolated from milk samples submitted for mastitis testing to five commercial veterinary laboratories between 2003 and 2006, were largely susceptible to nine antibiotics (Petrovski *et al.*, 2011)^{Error! Bookmark not defined.}. Greater resistance was noted for the β -lactams ampicillin (71% susceptible) and penicillin (55% susceptible).

Staphylococcus spp. isolates (n=175 non-*S. aureus* isolates) from colostrum samples from recently calved heifers have been tested for AMR (Salmon *et al.*, 1998). As for the *Streptococcus* spp. isolates reported previously, the proportion of isolates considered resistant to each antimicrobial is not reported due to an absence of interpretive criteria specific for mastitis-causing bacteria. Instead, the MIC was ascertained for each isolate, with all antimicrobials tested at concentrations ranging from 0.06 to 64.0 $\mu\text{g/ml}$. The MIC₅₀ and MIC₉₀ values were mostly ≤ 1.0 for the antibiotics tested. The data indicate relatively more resistance to cloxacillin, penicillin, novobiocin, erythromycin and pirlimycin.

Enterococcus

AMR was variable among 173 enterococci isolates tested against 11 antimicrobial agents (Petrovski *et al.*, 2011)^{Error! Bookmark not defined.}. These enterococci were isolated from milk samples submitted to five commercial veterinary laboratories between 2003 and 2006 for mastitis testing. Results are reported as percentage susceptible. Particularly low values were reported against some of the β -lactams (e.g. oxacillin, 49%) and the aminoglycosides neomycin (27% susceptible) and streptomycin (12% susceptible).

Trueperella pyogenes

Most (>84%) of the 234 *T. pyogenes* isolates from milk samples submitted to five commercial veterinary laboratories between 2003 and 2006 for mastitis testing were susceptible to nine antimicrobial agents (Petrovski *et al.*, 2011). There was comparatively more resistance to the aminoglycosides neomycin (60% of isolates were susceptible) and streptomycin (73% susceptible).

In another study, 34/35 *T. pyogenes* isolates from the uteruses of post-partum dairy cows were susceptible to nine antimicrobials at concentrations of 4 µg/ml or less (de Boer *et al.*, 2015). The antimicrobials tested were ampicillin, cloxacillin, ticarcillin/clavulanic acid, ceftiofur, cefuroxime, cephalixin, enrofloxacin, clindamycin and oxytetracycline. One *T. pyogenes* isolate was resistant to oxytetracycline at 16 µg/ml and another at 128 µg/ml (the highest concentration tested).

AMR in dairy animal commensals

E. coli

All 209 *E. coli* isolates from the uteri of post-partum dairy cows demonstrated low (<7%) or no resistance to ampicillin, ticarcillin/clavulanic acid, ceftiofur, cefuroxime, cephalixin, enrofloxacin and oxytetracycline (de Boer *et al.*, 2015).

AMR in dairy animal zoonotic pathogens

Campylobacter spp.

Samples of dairy cattle faeces collected from the Canterbury region during 2001 yielded 36 thermotolerant *Campylobacter* isolates; 31 *C. jejuni* and 5 *C. coli* (Harrow *et al.*, 2004). All 36 isolates were susceptible to erythromycin, ciprofloxacin, nalidixic acid and tetracycline. This data set is limited in scope and in the number of isolates tested, but suggests low AMR among *Campylobacter* spp.

ANTIMICROBIAL RESISTANCE IN PORK AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

Based on 2012/13 figures, the Australian pig industry comprises approximately 240,000 breeding sows distributed across 575 enterprises, with 75% of production occurring in Queensland (23%), NSW (24%) and Victoria (24%). Of the remaining states, South Australia has the largest proportion of sows (17%) (Anonymous, 2017). Moving annual total figures from February 2018 (Australian Pork Limited, 2018), showed that 412,363 tonnes of pork meat per annum was produced for domestic consumption (an increase of 6% from February 2017 figures), contributing approximately \$5.2 billion to the Australian economy. Australians have increased their consumption of fresh pork by 25% since 2010. Australia's export market for pork products is comparatively small, worth \$146 million per annum as at February 2018 but exports have increased 14.2% since February 2017. The Australian Pork Industry is also competing with frozen and cooked imported product (February 2018 moving annual total of 166,000 tonnes valued at \$680 million per annum).

Due to restrictions on the importation of live vaccines, a large component of the Australian pig industry is still reliant on *shared class antimicrobial agents* (SCAA; i.e. those registered for use in both human and animal health) to treat and control endemic bacterial diseases (mainly respiratory and gastrointestinal diseases). Nevertheless, adoption of management strategies to reduce disease incidence such as use of autogenous vaccines, dietary additives (e.g. probiotics, acidifiers), all in/all out vs continuous flow production systems, biosecurity

simple husbandry strategies (e.g. no cross fostering), are strongly advocated (Abraham *et al.*, 2017a). SCAAs registered for use in pigs in Australia (or allowed to be used off-label if registered for use in another food-animal species) are shown in Table 4 (Australian Veterinary Association, 2015). These are divided, as recommended by the Australian Veterinary Association, into first-line (empirical therapy), second-line (if first line agents are not available due to treatment failure or on the basis of the results of culture and susceptibility testing), third-line (last resort option) and prohibited drugs. The only third-line agent that can be legally used in pigs in Australia is ceftiofur, however, such use is off-label (ceftiofur is only registered for use for respiratory disease in cattle). Off label use in food-producing animals in Australia is governed by legislation that can differ between states, but generally, off-label use is only recommended when there are no alternative registered drugs for the given food-animal species, and must take into account additional withholding periods.

Table 4: Antimicrobial agents registered for use or available off-label for the treatment of pigs in Australia, divided into first line, second line and third line, as recommended by the Australian Veterinary Association Australian Veterinary Association (2015).

First line	Second line	Third line	Use Prohibited
Amoxicillin Erythromycin Chlortetracycline Oxytetracycline Sulphonamides Kitasamycin Tilmicosin Tylosin Penicillin Florfenicol Neomycin	Amoxicillin-clavulanate Apramycin Lincomycin Trimethoprim-Sulphonamides Tiamulin Tulathromycin Spectinomycin	Ceftiofur (off-label)	Fluoroquinolones Gentamicin Chloramphenicol Nitrofurans Colistin

A survey of antimicrobial use by the Australian pig industry found the main indications for antimicrobial agent use were for *Mycoplasma*, *Lawsonia* and ETEC infections (Jordan *et al.*, 2009). The survey was undertaken for 151 piggeries, representing over 50% of large operations in Australia at the time and was completed by specialist pig veterinarians on behalf of their clients. The overall results confirmed reliance of the industry on antimicrobial agents with a low ASTAG importance rating (tetracyclines, penicillins and sulphonamides), although it was noted that the ESC ceftiofur was used off-label on 25% of farms. However, it could not be determined how widespread ceftiofur use was on any of the farms. No virginiamycin use was reported on any farm.

The Australian industry has been implementing low antimicrobial use production models (Australian Pork Limited, 2013), driven by the need to reduce costs of production and continue to ensure high animal welfare standards together with the development of

market-driven quality assurance programmes²⁷. The industry has established an antimicrobial stewardship framework with other livestock industries (Australian Pork Limited, 2017), which includes an approved medications checklist that is regularly audited by quality assurance programs such as Australian Pork Industry Quality Assurance Program (APIQ; <http://www.apiq.com.au/>). Following publication of the JETACAR report (JETACAR, 1999) and the call for primary industry research bodies to support research on AMR in animals, the Australian Pork industry was one of the first of the animal industries to respond and initiate projects focused specifically on understanding AMR risks in the pig industry (Australian Pork Limited, 2013). One of the main aims of the Co-operative Research Centre for High Integrity Australian Pork and the new research entity Australasian Pork Research Institute Limited (APRIL) is finding credible alternatives to shared class antimicrobial agents for endemic pig diseases such as pleuropneumonia.

AMR in pig pathogens

E. coli

Stephens (2003) reported resistance patterns for haemolytic enterotoxigenic *E. coli* (ETEC) isolated from pigs at the Toowoomba Regional Veterinary Laboratory in Queensland (1999 vs 2001). All of the isolates from 2001 (n = 34) were resistant to tetracycline, with high rates of resistance reported to ampicillin (23.5%), apramycin (35.3%), neomycin (44.1%) and trimethoprim-sulphamethoxazole (47.1%), but no resistance to ESCs. Interestingly, for some antibiotics, the proportion of isolates resistant was lower in 2001 compared with isolates from 1999, e.g. (ampicillin resistance decreased from 35.3% to 23.5%).

Bettelheim *et al.* (2003) undertook an AMR survey of STEC and non-STEC isolated from a range of animal species as well as humans. A total of 47 porcine strains were included, which encompassed 27 clinical isolates from diagnostic samples submitted for culture and susceptibility testing and 20 commensal isolates from healthy pigs (ranging from the 1970s to 1990). Among the collection of non-STEC isolates from pigs, resistance to amoxicillin, sulphonamide and tetracycline was common.

This study was followed up by a detailed characterization of multidrug-resistant (MDR) porcine ETEC isolates obtained from submissions to Veterinary Diagnostic Laboratories in 2006 (Smith *et al.*, 2010). Among porcine ETEC isolates (n = 117), no resistance was identified to fluoroquinolones or ESCs and there was widespread resistance to lower ASTAG importance rating drugs including sulfamethoxazole-trimethoprim and aminoglycosides (including cross-resistance to gentamicin, which cannot be used in food-producing animals in Australia, however the *aac(3)-IV* resistance gene, which imparts cross resistance to both apramycin and gentamicin, was commonly identified). Antimicrobial resistance appeared to be more significant in serotype O141 ETEC strains than the more common O149 strains.

A 2015 study of AMR in a collection of 324 *E. coli* isolates from putative clinical cases of infection in food-producing animals demonstrated the first detection of resistance to ESCs (bovine isolates, 1%; porcine isolates, 3%) and fluoroquinolones (porcine isolates, 1%) in Australia. Apart from a single porcine ETEC isolate carrying *bla*_{CMY-2} the remaining isolates

²⁷ See <http://www.abc.net.au/news/rural/2017-11-21/edwina-beveridge-mark-schipp-pigs/9174406>

belonged to globally disseminated fluoroquinolone- and ESC-resistant *E.coli* clonal lineages considered to be largely commensal with some potential for pathogenicity, such as ST10, and carried *bla*_{CTX-M-14} and *bla*_{CTX-M-9} (Abraham *et al.*, 2015). *E. coli* ST10 is a common *E. coli* clonal lineage in both animals and humans that can acquire antimicrobial resistance, but contains few virulence genes and is only rarely associated with extraintestinal infection (Reid *et al.*, 2017).

A comprehensive survey of pre- and post-weaning diarrhoea was undertaken in 22 pig herds in southeast Australia as part of a recent Pork Cooperative Research Centre investigation into identifying risk factors for ETEC infection (Van Breda *et al.*, 2017). Sampling of pre- and post-weaning piglets isolated 325 *E. coli* isolates (a mixture of both haemolytic and non-haemolytic *E. coli*). A follow-up study characterizing the presence of AMR in the isolate collection found that 6.1% of isolates were resistant to one or more ESCs (resistance was more common in non-ETEC, non-diarrhoea samples) (van Breda *et al.*, 2018). This study screened the 325 isolates for resistance to 29 antimicrobial agents in total, 17 of which were significant in veterinary medicine and 27 of which were significant in human medicine. Resistance to multiple low and medium ASTAG important rating antimicrobial agents was common. In the 20 isolates resistant to ESCs, genes encoding ESBLs not previously reported in Australian pigs were identified including *bla*_{CTX-M-1}, -15, -27 and *bla*_{SHV-12}, in addition to the previously described *bla*_{CTX-M-14} and *bla*_{CMY-2-like} genes. A single isolate was resistant to colistin, but did not carry the *mcr-1* gene.

All six of these ESC genes had been previously identified in Australian human sepsis isolates from NSW (Ginn *et al.*, 2013; Zong *et al.*, 2008). The *bla*_{CTX-M-1} gene, which is commonly identified in pigs in many international studies, is only rarely associated with ESC-resistant isolates from human infections and has not been reported in recent Australian Group on Antimicrobial Resistance AMR surveillance reports as a significant cause of 3rd generation cephalosporin resistance in human sepsis isolates (Jan Bell, personal communication).

Actinobacillus pleuropneumoniae, *Pasteurella multocida*, *Bordetella bronchiseptica* and *Haemophilus parasuis*

Recently Dayao *et al.* (2014) examined the AMR profiles of 71 *Actinobacillus pleuropneumoniae*, 51 *Pasteurella multocida* and 18 *Bordetella bronchiseptica* isolates originating from cases of porcine respiratory disease in Australia. *A. pleuropneumoniae* isolates were resistant to erythromycin (89%), tetracycline (75%), ampicillin (8.5%), penicillin (8.5%) and tilmicosin (25%). *P. multocida* isolates showed resistance to co-trimoxazole (2%), florfenicol (2%), ampicillin (4%), penicillin (4%), erythromycin (14%) and tetracycline (28%). However, all of the *B. bronchiseptica* isolates were resistance to β -lactams (ampicillin, ceftiofur and penicillin); and some were resistant to erythromycin (94%), florfenicol (6%), tilmicosin (22%) and tetracycline (39%). In addition, 27.8% of *B. bronchiseptica* isolates, 9.1% of *A. pleuropneumoniae* isolates, and 4.8% of *P. multocida* isolates were multidrug-resistant. All isolates were susceptible to tulathromycin. One *P. multocida* isolate was resistant to all antimicrobials except for ceftiofur, tilmicosin and tulathromycin. This particular *P. multocida* isolate and one *B. bronchiseptica* isolate were the only isolates to show florfenicol resistance. This study revealed that whilst the majority of Australian pig respiratory pathogens were susceptible to the majority of currently registered antimicrobials used in the pig industry, low levels of

multidrug resistance to older drug classes were reported. No resistance to newly registered drug classes, such as tulathromycin and ceftiofur was observed.

In a follow-up paper identifying known resistance genes in the isolate collection, *bla*_{ROB-1} was associated with β -lactam resistance and *tetB* was identified in 76% of tetracycline-resistant isolates. No macrolide resistance genes were identified (a total of eight known resistance genes were screened by PCR), suggesting that whole genome sequencing may be required to identify the genetic basis of macrolide resistance in the isolates (Dayao *et al.*, 2016). A whole genome sequencing study of representative drug-resistant isolates of *H. parasuis*, *A. pleuropneumoniae*, *P. multocida* and *B. bronchiseptica* identified a nucleotide transition at position 2059 (A to G) in the six copies of the 23S rRNA gene in the *H. parasuis* genome, but the basis of phenotypic macrolide resistance in the other isolates remained unexplained (Dayao *et al.*, 2016).

AMR in pig commensal bacteria

Barton *et al.* (2003) summarised available information on AMR in animals in Australia. Even in early studies using questionable methodology, *E. coli* isolates originating from pigs were identified as being commonly MDR to low ASTAG importance rating antimicrobial agents (most commonly tetracycline, ampicillin, sulphonamides and trimethoprim), whilst no observed resistance to high importance drugs was observed.

In one of the first publications following the JETACAR report that focused on AMR in pork products, Hart *et al.* (2004) investigated AMR in enterococci, *E. coli* and thermophilic *Campylobacter* isolated from pork carcasses and retail pork products in South Australia and pork carcasses from NSW. Substantial rates of resistance (>50%) were observed among *E. coli* isolates to antimicrobial agents given a low ASTAG importance rating (tetracycline, sulphonamides and ampicillin), with intermediate rates of resistance (20-50%) also identified to aminoglycosides with a low ASTAG importance rating (streptomycin and neomycin). No resistance to high (ciprofloxacin) and medium ASTAG importance rating antimicrobial agents (gentamicin, augmentin) were identified, however, none of the isolates were tested for resistance to ESCs. A very high proportion of *Campylobacter* were resistant to erythromycin (low ASTAG rating, but first line treatment for serious *Campylobacter* infections in humans), but none were resistant to fluoroquinolones. Similarly, none of the enterococci were resistant to avoparcin and virginiamycin (the isolates were obtained four years after the withdrawal of avoparcin in Australia) and most were susceptible to high and low level vancomycin, apart from representatives of species that had some intrinsic resistance to the latter. Overall, resistance patterns fitted proposed drug use patterns within the industry, though at the time, no detailed surveys of antimicrobial use in the pig industry were available for verification.

In 2003/04, the then Australian Government DAFF undertook the first pilot surveys of antimicrobial resistance in commensal organisms isolated from samples obtained from the gut of healthy Australian animals at slaughter (DAFF, 2007). Amongst *E. coli* from pigs (n=182), over 30% of isolates were resistant to ampicillin, chloramphenicol, florfenicol, tetracycline and trimethoprim/sulfamethoxazole, with resistance to multiple low ASTAG importance rating drugs a common feature. Nevertheless, no resistance to high ASTAG importance rating antimicrobial agents (fluoroquinolones, ESCs) was identified. However,

pig isolates were noted for their MDR phenotype, with some isolates found to be resistant to up to six antimicrobial agents.

A pilot survey of raw foods, commissioned by the Food Regulation Standing Committee, isolated and tested *E. coli*, *Enterococcus* spp., *Salmonella* spp. and *Campylobacter* spp. from retail pork chops for AMR (Barlow & Gobius, 2008). No *Salmonella* or *Campylobacter* were isolated. A total of 92 *E. coli* isolates were obtained. High rates of resistance to tetracycline (44.5%) and ampicillin (28.2%), and moderate rates of resistance to streptomycin (17.4%), chloramphenicol (13%) and trimethoprim/sulfamethoxazole (13%) were observed. Low rates of resistance to florfenicol (8.7%), amoxicillin/clavulanic acid (3.3%), cefazolin (3.3%), kanamycin (3.3%) and gentamicin (1.1%) were also reported, but no resistance to high ASTAG importance rating antimicrobials was detected. Twenty two percent of isolates were resistant to three or more antimicrobials, with the most common resistance patterns comprising combinations of ampicillin, tetracycline, sulphonamide/trimethoprim, phenicol and early generation aminoglycoside resistance. The majority of the 178 isolates of *Enterococcus* spp. attained were susceptible to the antimicrobials tested, although 22% of the isolates were resistant to one or more antimicrobials.

An Australian Pork Limited (APL) funded project, which was directly commissioned to examine AMR in the industry in response to the JETACAR report, used a mass screening approach to examine antimicrobial resistance in commensal *E. coli* isolated from faecal samples from healthy slaughter age pigs from 72 of the 151 previously surveyed (Hart *et al.*, 2004) Australian farms. The antimicrobial agents investigated were ceftiofur, gentamicin, florfenicol and ampicillin (Smith *et al.*, 2016). From 5003 isolates, the prevalence of ceftiofur-resistant colonies was estimated as being very low (1.8%), however, no extended-spectrum or AmpC β -lactamase genes were identified, indicating the potential existence of additional ESC resistance mechanisms in a small number of commensal *E. coli* isolates. Resistance to the other agents tested varied widely between farms with very large confidence intervals, indicating large heterogeneity in antimicrobial use and associated resistance in commensal *E. coli* on pig farms in Australia, as previously reported by Jordan *et al.* (2009).

A comprehensive analysis of AMR in 192 enterococci isolates obtained from the intestinal tracts of slaughter age pigs in Australia (74.5% of the isolates belonged to *E. faecalis* or *E. faecium*) was reported by Fard *et al.* (2011). The majority of isolates showed resistance (and contained resistance genes imparting resistance) to many low ASTAG importance rating antimicrobial agents used in Australian pig production, namely flavophospholipol, tetracycline, tiamulin and tylosin, with just over half the isolates resistant to virginiamycin. However, no resistance to high ASTAG importance rating antimicrobial agents that are clinically significant in the treatment of nosocomial *Enterococcus* spp. infections was observed. A notable difference in comparison to similar international studies was the absence of ampicillin and high-level gentamicin resistance in the *Enterococcus* spp. isolate collection.

Obeng *et al.* (2012) compared a collection of *Campylobacter* spp. isolates from poultry with *Campylobacter coli* isolates previously obtained from pig faecal samples from 39 piggeries in Australia isolated in the late 1990s and again in 2003/4 in APL-funded studies (Barton & Peng, 2005). Of note, 8.3% of the *C. coli* isolates from pigs were resistant to ciprofloxacin

with the suggestion made in the article that this could indicate illegal (off-label) use of fluoroquinolones on these pig farms. This article provided no other evidence for this assertion and no further investigation was undertaken. However, it is noted that the *Campylobacter* multidrug-efflux gene *cmeB*, which can impart cross-resistance to macrolides and fluoroquinolones, was found in a high proportion of porcine isolates, so possible co-selection may be evident, with macrolides such as tylosin in common use on Australian piggeries (Jordan *et al.*, 2009).

A recent pilot AMR surveillance study sponsored by APL was undertaken on samples obtained from the gut of healthy pigs at slaughter originating from 19 farms distributed throughout Australia (Kidsley *et al.*, 2018). A total of 201 *E. coli* and 69 *Salmonella* spp. isolated were investigated to determine frequencies of resistance to 11 antimicrobial agents. The highest frequencies of non-susceptibility among respective isolates of *E. coli* and *Salmonella* spp. were to ampicillin (60.2% and 20.3%), tetracycline (68.2% and 26.1%), chloramphenicol (47.8% and 7.3%) and trimethoprim/sulfamethoxazole (33.8% and 11.6%). Fifty-one percent of *E. coli* and 21.7% of *Salmonella* spp. isolates were classified as MDR. No isolates were resistant to ESCs. Interestingly, two isolates from the same farm were resistant to fluoroquinolones (MICs of >4µg/ml), and belonged to the diverse host range *E. coli* sequence type ST10, which has been frequently isolated from both humans and animals. If these results are compared with the pilot DAFF sponsored AMR survey conducted in 2003/04, there has been little change in the resistance profiles and MDR status generated for both *E. coli* and *Salmonella* among pig isolates over 10 years (Ndi & Barton, 2011a).

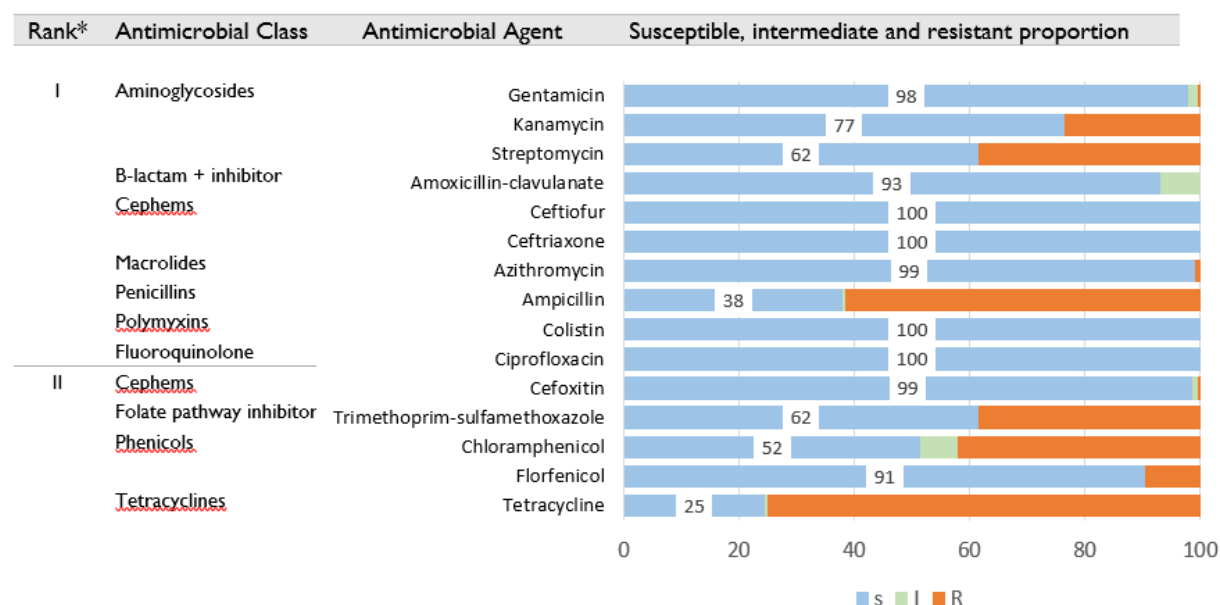
A recent paper undertook an investigation of 60 fresh pork rib samples from local meat supplies in Victoria for carriage of 3rd generation cephalosporin-resistant bacteria (McLellan *et al.*, 2018). Following rinsing in buffered peptone water, samples were grown in Trypticase Soy broth, followed by an enrichment step in TSB containing ceftriaxone (0.25 mg/L) and vancomycin (8 mg/L). Samples were then plated onto chromogenic agar to detect organisms resistant to ESCs. No *E. coli* or *Salmonella* resistant to ESCs were identified from the pork rib samples; the only organisms identified were environmental bacteria intrinsically resistant to 3rd generation cephalosporins including *Acinetobacter baumannii* and *Pseudomonas* species, and Enterobacteriaceae with intrinsic chromosomal AmpC overexpression.

The *bla*_{CTX-M-1} gene was identified in ESC-resistant commensal *E. coli* isolates obtained from faecal swabs from a single piggery in Australia with a history of ceftiofur use to control scours in individual piglets (Abraham *et al.*, 2018). A longitudinal study conducted over four years demonstrated high carriage rates of ESC-resistant *E. coli* in all age groups of pigs in year 1 (86.6%) and 2 (83.3%), compared to 3 (22%) and 4 (8.5%). The *bla*_{CTX-M-1} gene was mapped to an IncI1-ST3 plasmid encoding co-resistance to aminoglycosides and trimethoprim-sulphonamides and was found to have disseminated into 22 commensal *E. coli* clonal lineages. Major STs included ST10, ST5440, ST453, ST2514 and ST23. This study demonstrates the persistence of ceftiofur resistance long after cessation of direct antimicrobial selection pressure. Nevertheless, it is also important to document that ceftiofur-resistant *E. coli* represented only a small fraction of total *E. coli* populations in the faecal sample in finisher pigs (approximately 1 in 40,000 in most samples) (Singh, 2015).

In 2016, the Department of Agriculture and Water Resources funded and commissioned APL to undertake a national AMR surveillance proof-of-concept study in slaughter age pigs (Bell *et al.*, 2018). A systematic-random method of sampling was used to obtain 200 caecal samples from pigs representing 31 farms distributed throughout Australia. The objectives were to estimate the prevalence of resistance against specified antimicrobials amongst *E. coli*, *Salmonella* spp., *Enterococcus* spp., and *Campylobacter* spp. isolated from the randomly selected gut samples of Australian finisher pigs at slaughter. The MICs were interpreted according to CLSI veterinary and/or human clinical breakpoints or the European Committee for Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values (ECOFFs).

In keeping with previous surveys (discussed above) non-susceptibility (i.e. isolates classified as either intermediate or resistant according to clinical breakpoints) to tetracycline (75%), ampicillin (62%) and streptomycin (38%) among the 200 *E. coli* isolates was high. None of the *E. coli* isolates showed non-susceptibility to ceftiofur and no isolate had an ESBL phenotype (ceftriaxone MIC > 1 mg/L). Similarly, none of the *E. coli* isolates showed non-susceptibility to ciprofloxacin or colistin, although four isolates had MICs above the ciprofloxacin ECOFF (non-wild type) but these were all below the susceptible clinical breakpoint. Florfenicol and gentamicin non-susceptibility among *E. coli* was less than 10% and 2%, respectively (Table 5). Forty-six percent of *E. coli* were classified as MDR (exhibiting non-susceptibility to at least one agent in ≥ 3 antimicrobial classes).

Table 5 Antimicrobial resistance pattern for *Escherichia coli* (n=200), proportion susceptible, intermediate and resistant.



* Rank of antimicrobial agents based on World Health Organization's categorisations of critical importance in human medicine: (Collignon *et al.*, 2016) Rank I, Critically important; Rank II, Highly important.

Eight enterococcal species were recovered, three of which contributed to 93.8% of all isolates (*E. faecium*, 57.5%; *E. hirae*, 24.7%; *E. faecalis*, 11.6%). None of the enterococci isolates were resistant to vancomycin and linezolid. The observed resistance to quinupristin-dalfopristin was unexpected; virginiamycin use in pigs was banned in Australia over 13 years

ago. Surveillance data from other countries have documented *Enterococcus* isolates returning to full susceptibility to quinupristin-dalfopristin quite rapidly following removal of virginiamycin. It was suspected that there was an issue with the elevated resistance to quinupristin-dalfopristin since the percentage of non-wild type for virginiamycin was low (28.6%). Repeat quinupristin-dalfopristin MIC testing on a subset of isolates using broth microdilution confirmed the original result, indicating a possible break-point issue for the classification of non-susceptible phenotypes.

AMR in pig zoonotic pathogens

Salmonella spp.

An analysis of 32 *Salmonella* isolates from pigs with clinical infections did not identify any isolates resistant to high ASTAG rating antimicrobials (Abraham *et al.*, 2014b). However, some isolates from pigs were resistant to multiple antimicrobial agents including ampicillin, tetracycline, trimethoprim-sulphonamide, aminoglycosides and chloramphenicol.

Monophasic variants of *S. Typhimurium* with the signature serotype 1,4,[5],12:i:- have risen to international prominence as causes of salmonellosis in humans, with pigs implicated as a possible reservoir, and isolates are often identified as MDR (Weaver, 2017).

Salmonella 1,4,[5],12:i:- was first identified in Australian piggeries in 2015 (Hamilton *et al.*, 2015). A longitudinal study of *Salmonella* shedding among different age groups of pigs in five pig herds in Australia found the isolates to be highly clonal and concluded that a highly related *Salmonella* 1,4,[5],12:i:- population may be circulating within the Australian pig industry and possibly other Australian domestic livestock industries and people (Weaver *et al.*, 2017). *Salmonella* 1,4,[5],12:i:- strains from Australian pigs resembled those of the European clone strains, perhaps having been recently introduced from overseas, and some of these pig isolates shared the same genetic profile as isolates from domestic human salmonellosis cases (Weaver *et al.*, 2017). High level shedding among finisher pigs confirms the public health significance of this clonal lineage, although importantly, none of the isolates were resistant to high ASTAG rating antimicrobials, or antimicrobials commonly used to treat invasive salmonellosis in humans (Weaver *et al.*, 2017).

Whole genome sequence analysis of a single *Salmonella* 1,4,[5],12:i:- strain isolated from a faecal sample from an Australian pig identified a highly similar chromosomal backbone compared to European *Salmonella* 1,4,[5],12:i:- strains from pigs. However, the Australian isolate also harboured a novel large conjugative plasmid that contained arrays of antimicrobial resistance and heavy metal resistance genes with a number of integrons and transposons (Dyall-Smith *et al.*, 2017). Further detailed sequence analysis of this plasmid identified *bla*_{TEM}, *strA*, *strB*, *aadA1*, *aadA2*, *aphA2*, *sul3*, *dfrA12*, *cmlA*, and two copies of *tetA*, encoding resistance to ampicillin, streptomycin, spectinomycin, kanamycin, sulfonamide, chloramphenicol, and tetracycline, respectively (Billman-Jacobe *et al.*, 2018). Heavy metal resistance genes (copper and silver nitrate) were also identified on the plasmid and although their expression appeared to be regulated by complex environmental conditions, the findings indicated that heavy metals, and particularly copper sulphate, which is often incorporated into pig diets, may be significant co-selectors of AMR.

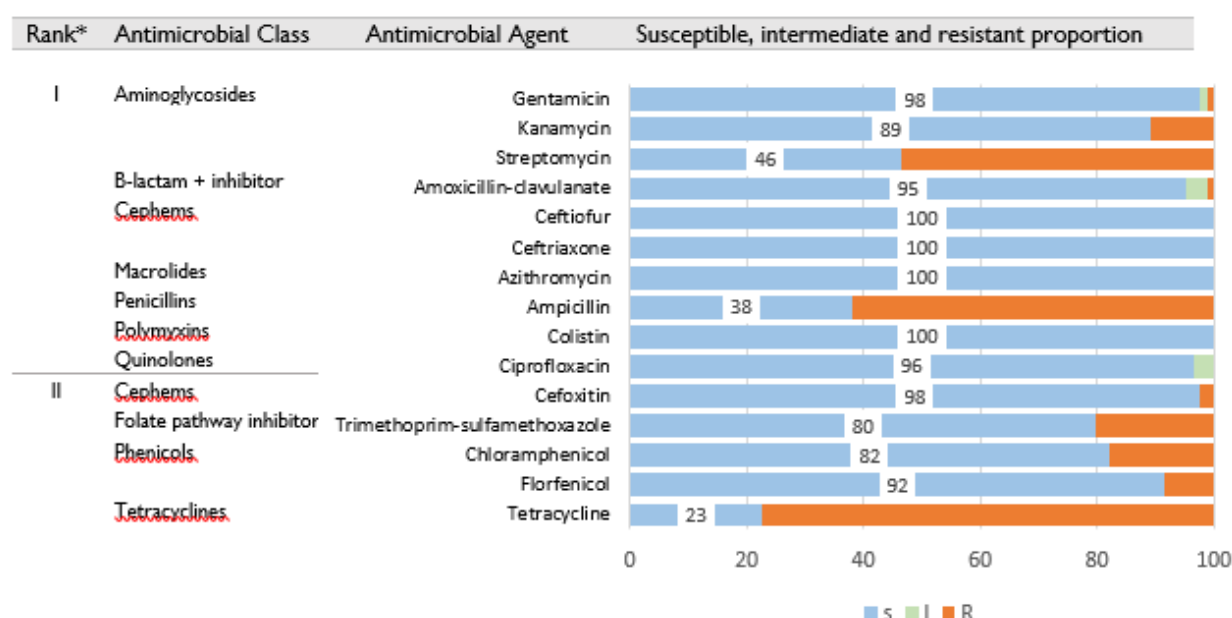
In the 2016 national AMR surveillance proof-of-concept study in slaughter age pigs (Bell *et al.*, 2018), no *Salmonella* spp. were recovered from pigs originating from ten (32%) farms.

Among the 84 *Salmonella* isolates obtained, resistance frequencies were proportionally similar to those obtained for commensal *E. coli* (described above) albeit slightly lower (Table 6). Three isolates had MICs above the ciprofloxacin ECOFF (non-wild type) but these were all below the susceptible clinical breakpoint. A total of 23% of the *Salmonella* spp. isolates were classified as MDR (exhibiting non-susceptibility to at least one agent in ≥ 3 antimicrobial classes).

Campylobacter spp.

Campylobacter spp. was recovered from all farms; *C. coli* (91.8%) was the dominant species, followed by *C. hyointestinalis* (7.0%). *Campylobacter* spp. are intrinsically resistant to lincosamides. Resistance among *C. coli* was high for macrolides (73.2–74.5%), ketolides (67.5%) and tetracyclines (53.5%). There was no resistance to ciprofloxacin or florfenicol, and only one isolate (0.6%) was gentamicin-resistant.

Table 6 Antimicrobial resistance pattern for *Salmonella* species (n=84), proportion susceptible, intermediate and resistant.



* Rank of antimicrobial agents based on World Health Organization's categorisations of critical importance in human medicine: (Collignon et al., 2016) Rank I, Critically important; Rank II, Highly important.

NEW ZEALAND

Industry background

New Zealand pigs are raised indoors or outdoors, although outdoor farming is limited to areas with suitable climate (mainly Canterbury).²⁸ Most pork produced in New Zealand is sold on New Zealand's domestic market as unprocessed cuts or after further processing. For

²⁸ [New Zealand Pork](#) (accessed 21 June 2018).

the year ending September 2017, 0.7 million pigs were processed at export plants and abattoirs, and approximately 47,000 tonnes of pork was produced (Beef + Lamb NZ, 2018).

Metaphylactic administration of antibiotics occurs in the pig industry when required, with the antibiotics being administered via food or water (Hillerton & Allison, 2015). Macrolides, predominantly tylosin, are used in the pig industry to treat ileitis (MPI, 2016). Tetracyclines are used to control respiratory disease in pigs (Heffernan *et al.*, 2011).

AMR in pig animal pathogens

No data were identified.

AMR in pig commensals

E. coli

E. coli (n=303) were isolated from swabs of dressed pig carcasses during 2009/10 (Heffernan *et al.*, 2011). Of these *E. coli* isolates, 35% were susceptible to all 17 antimicrobials tested against this bacterial species. Almost half (49%) were resistant to tetracycline and approximately one-third were resistant to streptomycin (32%) or sulfamethoxazole (33%). There were lower levels of resistance to spectinomycin (24%), chloramphenicol (10%), ampicillin (9%) and trimethoprim (8%). None of the *E. coli* isolates produced ESBL or AmpC β -lactamase. There was no resistance to 3rd generation cephalosporins, fluoroquinolones or gentamicin.

In another study, faecal samples were collected from healthy finisher pigs on three conventional farms with some exposure to antibiotics, and one organic farm where no antimicrobial agents were used (Nulsen *et al.*, 2008). In total, 375 presumptive *E. coli* isolates were obtained from 390 faecal samples from individual pigs. It should be noted that of these isolates, 30 underwent further speciation and one was identified as *Yersinia enterocolitica*. It is possible that some of the other isolates were not *E. coli*.

Of the 375 presumptive *E. coli* isolates, 197 (53%) were resistant to one or more antimicrobials. Resistances against tetracycline (48%), and to a lesser extent streptomycin (20%), were the highest measured. No resistance to ciprofloxacin was observed and the authors reported that this reflects low usage of fluoroquinolones in the pig industry after these were banned for use as growth promoters in 2000.

There were differences between the conventional farms and the one organic farm, e.g. *E. coli* resistance to tetracycline was 60% among the 296 *E. coli* isolates from the conventional farms compared with 5% (n=79) at the organic farm. The authors further noted that there were some differences in resistance patterns between farms, e.g. almost all *E. coli* isolates from one conventional farm were resistant to tetracycline compared with less than half from the other two farms. The farm, from which isolates demonstrated highest tetracycline resistance prevalence, used in-feed chlortetracycline followed by oxytetracycline, for over a year prior to the study.

Enterococcus spp.

E. faecalis (n=228) and *E. faecium* (n=57) were isolated from swabs of dressed pig carcasses during 2009/10 (Heffernan *et al.*, 2011). There was no resistance to ampicillin or

vancomycin in either species. Just over half (53%) of the *E. faecalis* isolates were susceptible to all of the nine antimicrobials tested. Resistances to tetracycline (43%), erythromycin (28%) and a high concentration of streptomycin (21%) were notable for *E. faecalis*. Of the *E. faecium* isolates, 31.6% were fully susceptible and no resistance to ampicillin, a high concentration of gentamicin, or vancomycin was observed. Around one third or more of *E. faecium* isolates were resistant to ciprofloxacin (33%), quinupristin/dalfopristin (30%) or tetracycline (39%), and 25% were resistant to erythromycin.

At farm level, faecal samples were collected from healthy pigs on three conventional farms with some exposure to antibiotics, and one organic farm where no antimicrobial agents were used (Nulsen *et al.*, 2008). All four farms were farrow-finish operations (i.e. breeding though to slaughter). From 390 faecal samples, 353 *Enterococcus* spp. were isolated.

Of the total number of *Enterococcus* isolates, 68% (241/353) were resistant to one or more antimicrobials (22% of isolates were resistant to four). Of the 273 *Enterococcus* isolates from conventional pig farms, over half were resistant to erythromycin, streptomycin or tetracycline, and almost half (49%) were resistant to virginiamycin. There were differences between the conventional farms and the one organic farm (n=80), e.g. *Enterococcus* spp. resistance to erythromycin was 69% across the conventional farms and 1% on the organic farm, and no resistance to streptomycin or virginiamycin was observed among *Enterococcus* spp. from the organic farm.

The authors reported increased resistance to erythromycin, virginiamycin and high-level streptomycin on one conventional farm over time, which corresponded with the introduction of lincomycin and spectinomycin, plus one month of tiamulin (with chlortetracycline), to the weaner and grower feeds. Use of lincomycin and tiamulin, plus tylosin, on conventional farms was the proposed cause of *Enterococcus* spp. resistance to erythromycin and virginiamycin.

As part of an investigation of how widespread a vancomycin-resistant *E. faecalis* (VRE) clone is in New Zealand (Manson *et al.*, 2003), seven faecal samples from pigs VRE were tested but VRE were not detected (Cook, 2003).

AMR in pig zoonotic pathogens

Campylobacter spp.

Data available for AMR among *Campylobacter* isolates from pigs are very limited:

- Fourteen thermotolerant *Campylobacter* isolates (7 *C. jejuni* and 7 *C. coli*) from pig offal samples (liver, kidney and heart) were all susceptible to ciprofloxacin, nalidixic acid and tetracycline (Harrow *et al.*, 2004). Five isolates (one *C. jejuni*, four *C. coli*) were resistant to erythromycin. These erythromycin-resistant isolates were further tested against chloramphenicol (all were susceptible) and clindamycin (all were resistant). These resistant isolates were not clonal and molecular analyses showed that nucleotide variations in the 23S rDNA contributed to erythromycin resistance.
- The number of *Campylobacter* isolates (n=11) from rinsates of pig carcass swabs were too small to draw conclusions over AMR trends in a 2009/10 study (Heffernan *et al.*, 2011).

Salmonella spp.

AMR among *Salmonella* spp. isolated from pigs or pig products are lacking. In a 2009/10 study only six isolates were obtained for AMR testing, which are too few to draw conclusions (Heffernan *et al.*, 2011). ESR continues to periodically test a sample of non-typhoidal *Salmonella* isolates from animals, foods and the environment for AMR.²⁰ Detailed data to support this current review are not readily available.

ANTIMICROBIAL RESISTANCE IN POULTRY MEAT AT PRODUCTION, PROCESSING AND RETAIL

All AMR data identified for inclusion in this review relates to chickens.

AUSTRALIA

Industry background

The Australian chicken meat industry had a gross production value of \$2.7 billion and retail value of approximately \$6.6 billion in 2015/16²⁹. The Australian Bureau of Agricultural and Resource Economics and Sciences forecasts the 2018/19 slaughter of 676 million chickens, producing 1,250 kt of meat (of which 41 kt is exported), with a gross value of \$2.9 billion³⁰.

Chicken meat production in Australia is dominated by a small number of large, vertically integrated, privately owned businesses that typically contract out the growing of their chickens to independent chicken growers. Approximately 700 contract growers rear about 80% of Australia's meat chickens. The industry has a strong presence in rural and regional communities, directly employing about 40,000 people with a further 100,000 jobs dependent on chicken meat production. The chicken meat industry purchases around five per cent of all grain grown in Australia.

Biosecurity on chicken farms is an essential element of good animal husbandry practice. The National Farm Biosecurity Manual for Chicken Growers (2010)³¹ applies to commercial meat chicken farms from the time of delivery of birds, until pick-up, slaughter or disposal of live birds. While the manual stipulates minimum requirements for any meat chicken farm, biosecurity measures in place on breeder farms would generally be more stringent, reflecting the economic importance and the extended life cycle of breeder flocks. The objectives of biosecurity are threefold, (i) to prevent the introduction of infectious disease agents to chickens (ii) to prevent the spread of disease agents from an infected area to an uninfected area, and (iii) to minimise the incidence and spread of microorganisms of public health significance. Implementation of biosecurity measures combined with good vaccination practices underpins the high health status of meat chicken production in Australia and ensures that antibacterial drug use can be minimised.

²⁹ [Agrifutures Australia Chicken Meat](#)

³⁰ [Agricultural Commodities Report](#)

³¹ National Farm Biosecurity Manual for Chicken Growers (2010) (http://www.chicken.org.au/files/_system/Document/Biosecurity/National%20Farm%20Biosecurity%20Manual%20for%20Chicken%20Growers%20-%20Feb%202010%20-%20web.pdf)

Fourteen antimicrobials classes, including 21 antimicrobial agents with antibacterial activity are approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use in poultry meat production. Only one of these antimicrobial agents (the streptogramin virginiamycin) is included in the ASTAG 'high' category of importance to human health. All other agents are considered of lesser importance or are not rated as they are not used for humans.

AMR in chicken meat pathogens

Relatively few studies have identified AMR in meat bird pathogens. In the study of Abraham *et al.* (2014b), 165 clinical *Salmonella* isolates from Australian food-producing animals in NSW (obtained from NSW Department of Primary Industries diagnostic specimens) were examined for antimicrobial resistance, but only four isolates were obtained from poultry, all belonging to serotype Typhimurium. Three isolates contained no resistant phenotypes and one isolate was resistant to ampicillin, sulphamethoxazole/trimethoprim and tetracycline. In an Australia-wide survey of antimicrobial resistance in 324 clinical *E. coli* isolates from food-producing animals, 32 isolates were obtained from poultry, though it is important to note that some of these isolates may have been derived from backyard poultry and zoological collections (Abraham *et al.*, 2015). Among the poultry isolates, moderate to high rates of resistance were observed to tetracycline (75%), streptomycin (21.9%) and sulphamethoxazole/trimethoprim (37.5%). Rates of resistance to other antimicrobial agents were consistently below 10% and included ampicillin (9.4%), ceftiofur, but not other 3rd generation cephalosporins (6.3%), and the aminoglycosides apramycin, neomycin and gentamicin (3.13%).

AMR in chicken meat commensals

E. coli

In 2007, Australia's peak advisory committee on food safety, The Food Regulation Standing Committee, commissioned Food Science Australia (now CSIRO) to conduct a pilot survey of foods to assess the presence of bacteria with antimicrobial resistance (AMR) (Barlow & Gobius, 2008). In this survey, samples of raw whole poultry (as well as beef mince, pork chops and iceberg lettuce) were collected each month between February 2007 and January 2008 from shops in Melbourne, Sydney, Brisbane and Perth. The food items selected were considered to be representative of an average consumer's shopping basket and all foods tested were of Australian origin. Bacteria were isolated from food samples and then tested to determine resistance to a range of different antibiotics.

High frequencies of *E. coli* AMR were observed to tetracycline (47%), ampicillin (38%), streptomycin (19%) and the combination of trimethoprim / sulfamethoxazole (22%). Multidrug resistance was observed in 20% of isolates and no resistance was observed to agents categorised by ASTAG as of high importance to human health, notably no resistance to 3rd generation cephalosporins, carbapenems or fluoroquinolones.

In 2016, the Department of Agriculture and Water Resources commissioned and funded the Australian Chicken Meat Federation to undertake a national AMR surveillance proof-of-concept study of bacteria in the gut of healthy meat chickens at slaughter. A systematic-random method of sampling was used to obtain 207 caecal samples from meat chickens

representing >90% national production. The objectives were to estimate the prevalence of resistance against specified antimicrobials amongst *E. coli*, *Salmonella* spp., *Enterococcus* spp., and *Campylobacter* spp. The MICs were interpreted according to CLSI veterinary and/or human clinical breakpoints or the EUCAST ECOFFs.

Of the 206 *E. coli* isolates, 63.1% were susceptible to all of the antibiotics tested and 5.8% were MDR. All isolates were susceptible to amoxicillin, ceftiofur, chloramphenicol, florfenicol, colistin or gentamicin. Non-susceptibility to tetracycline (19.4%), ampicillin (14.1%), and trimethoprim/sulfamethoxazole (8.7%) were substantially reduced compared to the samples collected in 2004 (DAFF, 2007). Two isolates demonstrated microbiological resistance to the fluoroquinolone class but the known resistance genes were not detected.

Enterococcus spp.

Barton & Wilkins (2001) reported the results of a study designed to assess the extent of AMR in enteric bacteria present on chicken carcasses. From 1286 carcass rinse samples, a total of 270 *Enterococcus* spp. were isolated, including 119 *E. faecium* and 48 *E. faecalis*. The authors concluded that 109 of the 270 isolates were vancomycin-resistant as they were isolated in the presence of 6 mg/L vancomycin. However, the breakpoint for vancomycin resistance is now >32 mg/L (Obeng *et al.*, 2013), necessitating a reinterpretation of the authors' conclusions. Thus 92 isolates were resistant when tested at 32 mg/L, with 86 shown by PCR to carry the *vanA* resistance gene. For the antibiotics of greatest human significance, current breakpoints (Obeng *et al.*, 2013) are ampicillin ≥ 16 , bacitracin >32, erythromycin ≥ 8 , flavomycin >8, gentamicin ≥ 16 , lincomycin ≥ 32 , tetracycline ≥ 16 , tylosin ≥ 16 , vancomycin >32, virginiamycin >32, indicating that with contemporary reinterpretation of the results, resistance is only now valid for ampicillin, erythromycin, gentamicin, tetracycline, tylosin and vancomycin. A moderate to high frequency of resistance was observed with bacitracin, erythromycin, gentamicin, tetracycline and vancomycin; antimicrobial agents ranked as important by ASTAG.

Following the recommendations of the JETACAR Report, the Australian Government DAFF conducted a pilot surveillance study to assess the prevalence of resistance to important antimicrobials amongst key indicator organisms found in the gut (caecum) of food-producing animals (DAFF, 2007). Sample collection for the pilot program commenced in November 2003 and was completed in July 2004. Samples of gut contents were obtained from healthy birds at 13 processing establishments in Queensland, NSW, Victoria and South Australia. Chickens yielded 238 *Enterococcus* isolates for susceptibility testing, assigned by biochemical testing to *E. faecium* (61 isolates), *E. faecalis* (123 isolates) and *E. hirae* / *E. casseliflavus* (33 isolates). The MICs of six antimicrobials (ampicillin, erythromycin, gentamicin, teicoplanin, vancomycin and virginiamycin) were assayed by broth or agar dilution according to CLSI methods. Application of contemporary breakpoints used by Obeng *et al.* (2013), are more relevant and have been used to reinterpret the results of the DAFF study. Amongst the 61 *E. faecium* isolated from chickens, there was no resistance observed to ampicillin, gentamicin, teicoplanin, or vancomycin, a low frequency of resistance to virginiamycin (1.6%), and moderate frequency of resistance to erythromycin (43%). No isolates displayed multidrug resistance. Amongst the 123 *E. faecalis* isolated from chickens there was no resistance observed to ampicillin, gentamicin, teicoplanin,

vancomycin or virginiamycin, but a high frequency of resistance to erythromycin (76%). No isolates displayed multidrug resistance.

Amongst the 33 *E. hirae* / *E. casseliflavus* isolated from chickens there was no resistance observed to ampicillin, gentamicin, teicoplanin, vancomycin or virginiamycin, but a high frequency of resistance to erythromycin (76%). No isolates displayed multidrug resistance.

Moderate to high frequency resistance was only observed to erythromycin. Importantly, no multidrug resistance was encountered in any isolates from any of the *Enterococcus* species and antimicrobial agents considered important to human health by ASTAG remained active, with all isolates susceptible to glycopeptides (teicoplanin and vancomycin), gentamicin and ampicillin and predominantly susceptible virginiamycin.

Retail chicken meat samples collected in the 2007/08 pilot survey of AMR in foods (Barlow & Gobius, 2008) were tested for *Enterococcus*. A total of 199 *Enterococcus* isolates were obtained during the 12 month sampling period, 92% of which were identified by PCR to be *E. faecalis*. No *E. faecium* isolates were identified. One hundred *E. faecalis* isolates were randomly selected for testing of susceptibility to 14 antimicrobial agents using Sensititre plates and CLSI breakpoints. Susceptibility to all antimicrobial agents was observed for 19% of the isolates. Significant frequencies of resistance were observed for erythromycin (48%), kanamycin (9%), streptomycin (5%), tetracycline (76%), and tigecycline (6%). A number of resistance phenotype patterns were observed with 52% of the isolates tested resistant to two or more antimicrobials. The most commonly observed patterns were tetracycline alone (24%) and erythromycin/tetracycline (36%). Only 11% of isolates were resistant to three or more antimicrobial classes and meeting the definition of MDR. No resistance was observed to agents categorised by ASTAG as of high importance to human health, notably no resistance to the glycopeptides (vancomycin and teicoplanin), the penicillins (ampicillin and penicillin) and the aminoglycoside gentamicin.

Obeng *et al.* (2013) completed a study to compare the antimicrobial resistance patterns in enterococci from intensively raised meat chickens, free range meat chickens and free range egg layers in Australia. Faecal samples were sourced from selected farms (155 from free range meat chickens, 69 from free range egg layer chickens and 87 from indoor meat chickens) and abattoirs (three) in Adelaide, South Australia between December 2008 and June 2009. *Enterococcus* isolates were tested for susceptibility to 11 antibiotics (ampicillin, bacitracin, ceftiofur, flavomycin, gentamicin, erythromycin, lincomycin, tylosin, tetracycline, vancomycin and virginiamycin). MIC values were determined on Mueller-Hinton agar plates containing two-fold serial dilutions ranging from 0.063 to 128 mg/ml. Antibiotics were prepared as described by the CLSI guidelines. In addition, isolates were evaluated by PCR for bacitracin (*bcrR*), tylosin (*ermB*), tetracycline (*tet(L)*, *tet(M)*, *tet(O)*, *tet(S)*, and *tet(K)*), gentamicin (*aac6-aph2*), vancomycin (*vanC* and *vanC2*), ampicillin (*pbp5*) and integrase (*int*) genes.

The predominant *Enterococcus* species identified were *E. faecalis* (198 isolates), *E. faecium* (47 isolates) and *E. gallinarum* (13 isolates), with small numbers of *durans*, *hirae*, *casseliflavus*, *avium* and *raffinosis*. As with their other study (Obeng *et al.*, 2014) and with the same limitations and caveats, the authors combined the results of all species before analysing the data and making comparisons between the different groups of chickens.

Resistance to bacitracin, ceftiofur, erythromycin, gentamicin, lincomycin, tylosin and tetracycline was found in all groups of chickens. In the free range meat chickens, 131 (91.6%), 123 (86%), 120 (83.9%) and 109 (76.2%) out of the 143 isolates were found to be resistant to bacitracin, lincomycin, tetracycline and tylosin, respectively. While from indoor meat chickens 71 (97.3%), 69 (94.5%), 63 (86.3%) and 54 (74%) out of the 73 isolates were found to be resistant to bacitracin, lincomycin, tetracycline and tylosin, respectively. Less resistance was found in the 58 isolates from free range egg layers to lincomycin (43.1%), tetracycline (32.8%), bacitracin (6.9%), erythromycin (5.2%) and tylosin (5.2%). A significant difference in resistance to lincomycin, tylosin and tetracycline was found between isolates from free range meat chickens and free range egg layers ($P < 0.05$). No significant difference was found between resistant isolates from indoor and free range meat chickens ($P < 0.05$). In total ($n=274$), widespread resistance to lincomycin, bacitracin, tetracycline and tylosin was found in 217 (79.2%), 206 (75.2%), 202 (73.7%) and 166 (60.6%) isolates, respectively. While moderate resistance to erythromycin, gentamicin, and ceftiofur was found to 155 (56.6%), 152 (55.5%), and 126 (46%) isolates, little resistance was found to flavomycin (7.3%) and ampicillin (3.6%). Notably, no resistance was found to vancomycin and virginiamycin in any of the different groups of chickens. A few of the isolates expressing resistance to ampicillin were found to express concomitant resistance to ceftiofur, the significance of which is unknown, especially as cephalosporins are not generally used in the treatment of human *Enterococcus* infections due to widespread intrinsic resistance.

Resistance to bacitracin, erythromycin and tetracycline was found to be correlated with the presence of *bcrR*, *ermB*, and *tet* genes in most of the isolates collected from meat chickens. Most bacteria encoding the *ermB* gene were found to express cross-resistance to erythromycin, tylosin and lincomycin.

While resistance to undifferentiated *Enterococcus* species was readily identified in free range and intensive production systems, there was less resistance in free range laying chickens than in meat chickens. However, resistances of greatest importance to public health were either present at low frequency (ampicillin resistance) or resistance was not detected, most notably all isolates were susceptible to the glycopeptide vancomycin and the streptogramin virginiamycin.

Obeng *et al.* (2014) studied the prevalence of AMR in enterococci and *E. coli* in meat chicken flocks during a production cycle and egg layer pullets during rearing. Environmental samples from feed, water and litter were collected and faecal samples were obtained from birds aged 3 to 39 days and 5 to 115 days from free-range meat chickens and intensively raised egg layer pullets from two different farms. *Enterococcus* spp. (252 isolates) were recovered for evaluation of phenotypic antimicrobial resistance and the presence of resistance genes. MICs of nine antibiotics for *Enterococcus* isolates – ampicillin, bacitracin, erythromycin, gentamicin, lincomycin, tetracycline, tylosin, vancomycin and virginiamycin were determined for each isolate using an agar dilution method based on CLSI guidelines.

The dominant *Enterococcus* species identified from the environment and from faecal samples included *E. faecium* and *E. faecalis* with no *E. gallinarum* or *E. casseliflavus* identified. Unfortunately, despite the different intrinsic antimicrobial resistance profiles, virulence factors and epidemiological characteristics, the authors grouped all *Enterococcus* species together for assessment and analysis of antimicrobial resistance.

However, resistance and resistance genes to tetracycline, bacitracin and the macrolides were observed in both free range meat chicken flocks and indoor egg layer flocks from all farms from the first week of age. Indeed, there appeared to be no significant difference in resistance phenotypes between the different farming operations. The authors conjecture that the early appearance of antimicrobial resistance may result from transmission from breeder flocks where antibiotic use may be more common than in on-farm meat chickens and layer pullets and hens. While this may be the case, the possibility of first exposure to a contaminated transport or on-farm environment was not assessed and is also plausible, though the authors note the differences in environments of free range and indoor operations. In either case there is great value in investigating the source of resistance in order to guide and monitor appropriate risk management interventions. The authors also hypothesise (but do not investigate) the possibility of cross contamination of feed with antibiotic agents leading to ongoing selection of AMR. In this study (Obeng *et al.*, 2014), unspciated *Enterococcus* isolates resistance to bacitracin, erythromycin, lincomycin, tylosin and tetracycline was common from the first week of age in both free range meat chicken farms and indoor layer pullet operations. Importantly, from a public health perspective, no resistance was observed to the highly important glycopeptide (vancomycin) and streptogramin (virginiamycin) classes of antibiotic.

The 2016 survey managed by the Australian Chicken Meat Federation (and funded by the Australian Government Department of Agriculture and Water Resources) found >50% of all *Enterococcus* isolates were susceptible to the antibiotics tested, with 17.5% classified as MDR. All *Enterococcus* isolates were clinically susceptible to vancomycin. Non-susceptibility to ampicillin in *E. faecium* was 55.8%, however this was not supported by the presence of known resistance genes but may be due to other resistance mechanisms. Resistance to tetracycline was common (40-46%), reflecting historical use in the industry, nevertheless there was a reduction in non-susceptibility (26-39%) from the survey in 2004 (46-77%; DAFF 2007). Unexpected levels of resistance to quinupristin-dalfopristin is suspected to be due to inappropriate breakpoints used to determine resistance.

AMR in chicken meat zoonotic pathogens

Campylobacter

In reviewing the epidemiology of AMR among *Campylobacter* spp. in Australia, Moore *et al.* (2006) noted that while human campylobacteriosis is very common in Australia, there is no evidence of fluoroquinolone resistance being acquired by the *Campylobacter* strains infecting Australians – all cases of infection with fluoroquinolone-resistant strains have been associated with foreign travel. The authors concluded that the lack of use of fluoroquinolone in food-producing animals in Australia likely accounts for the low frequency of fluoroquinolone resistance among *Campylobacter* spp. in Australia.

Barton & Wilkins (2001) reported the results of a study designed to assess the extent of AMR in enteric bacteria present on chicken carcasses. *Campylobacter* spp. were isolated from frozen chicken carcass rinses. Only 54 isolates could be recovered from 1347 rinse samples and for the purpose of the study were supplemented with a further 162 isolates obtained from 399 chicken intestinal samples, yielding a total of 142 *C. jejuni* and 74 *C. coli*. Isolates were subjected to susceptibility testing to 10 antibiotics (ampicillin, erythromycin,

gentamicin, lincomycin, neomycin, tetracycline, tylosin, ceftazidime, ciprofloxacin and clindamycin) using an unconventional tablet diffusion test based on the Kirby Bauer disc diffusion method. While resistance to ampicillin was present in more than 50% of isolates, and resistance to tetracycline and lincomycin was common, resistance to the macrolides (erythromycin and tylosin) was present in less than less than 5% (11/216) of isolates and only one isolate (1/216) displayed phenotypic ciprofloxacin resistance. The study of Barton and Wilkins (Barton & Wilkins, 2001) observed high proportions resistant to antibiotics seldom used in the management of human *Campylobacter* infections (β -lactams and lincosamides) and low proportions resistant to antibiotic classes of public health importance (macrolides and fluoroquinolones).

The prevalence of AMR among *Campylobacter* spp. was also investigated in the DAFF pilot surveillance study for food-producing animals (DAFF, 2007). Samples of gut contents were obtained from healthy birds at 13 processing establishments in Queensland, NSW, Victoria and South Australia. From 303 chicken samples, 131 *Campylobacter* (not speciated) isolates were available for susceptibility testing. The MICs of five antimicrobials (ciprofloxacin, erythromycin, gentamicin, nalidixic acid and tetracycline) were assayed by broth or agar dilution according to CLSI methods.

Tetracycline and erythromycin resistance (21% and 11% respectively) were detected in *Campylobacter* spp. (n=131). There was no multiple-resistance found. None of the *Campylobacter* spp. exhibited resistance to gentamicin, ciprofloxacin or nalidixic acid and 69% of isolated displayed susceptibility to all five agents tested. No resistance to the fluoroquinolones was observed and resistance to the macrolides was present in just 15 of 131 (11%) isolates tested.

Subsequent to the pilot AMR surveillance study for food-producing animals (DAFF, 2007), AMR among *Campylobacter* spp. was investigated in the corresponding Australian pilot survey of foods. A total of 175 *Campylobacter* isolates were collected during the 12-month sampling period, of which 100 were randomly selected for speciation (identifying 60 *C. jejuni* and 40 *C. coli*). AMR testing was performed against eight antibacterial agents, ciprofloxacin clindamycin erythromycin, florfenicol, gentamicin nalidixic acid, telithromycin (a macrolide in the ketolides subclass) and tetracycline. The overall level of AMR was very low. AMR was observed in two isolates (5%) of *C. coli* and three isolates (5%) of *C. jejuni*. Resistance to clindamycin (*C. coli*, 5%; *C. jejuni*, 1.7%), erythromycin (*C. coli*, 5%; *C. jejuni*, 1.7%) telithromycin (*C. coli*, 2.5%; *C. jejuni*, 3.3%) and tetracycline (*C. jejuni*, 1.7%) was recorded. No resistance to ciprofloxacin, florfenicol, gentamicin or nalidixic acid was observed. There was low frequency of resistance in both species of *Campylobacter* (*C. jejuni* and *C. coli*) especially to antibacterial agents of greatest public health interest, the macrolides (erythromycin – resistance noted with 1/60 *C. jejuni* and 2/40 *C. coli* isolates) and fluoroquinolones (ciprofloxacin – no resistance observed).

The status of tetracycline resistance in Australian *C. jejuni* and *C. coli* isolates has been investigated (Pratt & Korolik, 2005). Human (36 *C. jejuni* and four *C. coli*) and chicken (eight *C. jejuni* and one *C. coli*) isolates from the culture collection of the Department of Applied Biology, Royal Melbourne Institute of Technology (RMIT) were subjected to tetracycline MIC determination using the CLSI agar dilution method. In addition, the distribution and localization of the tetracycline resistance gene, *tet(O)*, on plasmid and chromosomal DNA

was determined by Southern-blot experiments. The ability to transfer resistance to recipient strains was examined through conjugation studies and the identity of transconjugants was confirmed by PCR and *flaA*-restriction fragment length polymorphism analysis. High-level tetracycline resistance was observed, ranging from 32 to >256 mg/L. Plasmids were detected in 74% of isolates with plasmids between 30 and 40 kb in size most frequently isolated. *tet(O)* was present in all tetracycline-resistant isolates and in the majority of strains under study the *tet(O)* gene was chromosomally encoded. The authors concluded that the *tet(O)* gene that has been widely reported in *Campylobacter* strains throughout the world, was present in these Australian *Campylobacter* isolates. The results were consistent with other Australian surveys of AMR among *Campylobacter* spp. that have identified a high frequency of phenotypic tetracycline resistance.

In 2007 a study of antibiotic resistance among 125 *C. jejuni* and 27 *C. coli* isolated from poultry from 39 meat chicken farms in the South-East Queensland region was published (Miflin *et al.*, 2007). Isolate susceptibility to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, nalidixic acid and tetracycline was undertaken by both disc diffusion and by MIC testing using a standardised agar dilution method. Resistances observed in *C. jejuni* included ampicillin (17.6% of isolates), nalidixic acid (2.4%) and tetracycline (18.4%). No resistance was observed for chloramphenicol, ciprofloxacin, or erythromycin. For *C. coli*, resistance was observed to ampicillin (14.8%), erythromycin (11.1%) and tetracycline (14.8%). No resistance was observed in *C. coli* to chloramphenicol, ciprofloxacin or nalidixic acid. The overall level of resistance by both methods was not significantly different. The authors concluded that their study provided solid evidence that the majority of Queensland poultry isolates of *Campylobacter* spp. show little resistance to antibiotics that are either used in the poultry industry or are of public health significance. The prevalence of resistance observed was at the low end of what is reported in other studies in Australia (for example, (Barton & Wilkins, 2001)) with no macrolide or fluoroquinolone resistance in *C. jejuni*, and in *C. coli*, only 11.1% isolates were resistant to macrolides and no isolates were resistant to fluoroquinolones.

Phenotypic and genotypic antimicrobial susceptibilities and resistance genes among *Campylobacter* spp. isolated from poultry were investigated by Obeng *et al.* (2012). A total of 311 chicken faecal samples were collected from December 2008 to June 2009 from three poultry farms and three abattoirs located in South Australia. The farms included a free range and an intensive indoor commercial meat chicken operation, as well as a free range egg layer farm. All isolates were typed to genus and species level using appropriate primers and biochemical tests. Antimicrobial susceptibility to ampicillin, clindamycin, ciprofloxacin, erythromycin, gentamicin, lincomycin, tylosin and tetracycline were determined using an agar dilution method conducted according to CLSI guidelines. All isolates were also tested by multiplex PCR for the presence of *tet(O)* (tetracycline), *aph-3-1* (erythromycin), *cmeB* (multi-drug efflux pump) and *bla_{OXA-61}* (ampicillin) resistance genes. From the 311 faecal samples, 83, 54 and 27 *C. jejuni* were isolated from free range meat chickens, indoor meat chickens and free range laying chickens. In addition 26, 12 and 35 *C. coli* were isolated from free range meat chickens, indoor meat chickens and free range laying chickens. All *Campylobacter* isolates were sensitive to ciprofloxacin, gentamicin, erythromycin and tylosin. In *C. jejuni*, lincomycin resistance was 78.3%, 96.3% and 51.9%, tetracycline resistance was 0%, 40.7% and 5.6%, and ampicillin resistance was 60.2%, 3.7% and 33.3%,

on free range meat, free range layers and indoor meat chickens, respectively. Resistance to lincomycin, tetracycline and ampicillin was closely associated with the presence of *cmeB*, *tet(O)* and *bla_{OXA-61}* genes respectively. The absence of observed resistance to macrolides and fluoroquinolones provided evidence that *Campylobacter* isolates from South Australia possessed little resistance to antibiotics of public health significance.

AMR among 15 *C. jejuni* and five *C. coli* towards nine antimicrobials (gentamicin, azithromycin, telithromycin, erythromycin, ciprofloxacin, nalidixic acid, tetracycline, florfenicol and clindamycin) was assessed using the Sensititre (R) AST *Campylobacter* plates (Wieczorek *et al.*, 2013). The isolates were obtained during 2010-12 from chicken carcasses sampled after immersion chilling at the end of chicken meat processing. Only two of the 20 (10%) Australian isolates were resistant, one to tetracycline and one to nalidixic acid. Although the number of isolates tested was low, the results affirm the low frequency of AMR among *Campylobacter* spp. isolated from Australian poultry, especially resistance to antibiotic classes of public health importance.

A comparison of epidemiologically linked *C. jejuni* isolates from human (22 clinical diarrhoeal samples) and poultry (26 raw chicken meat samples) in two regions of Australia was undertaken by the Queensland Public Health Microbiology Laboratory (Lajhar *et al.*, 2015). AMR to nine antimicrobials (gentamicin, azithromycin, telithromycin, erythromycin, ciprofloxacin, nalidixic acid, tetracycline, florfenicol and clindamycin) was assessed using the Sensititre (R) *Campylobacter* plate. Most of the *C. jejuni* isolates (43/48, 89.6%) were susceptible to all nine antibiotics tested. Two unrelated isolates of chicken origin were not susceptible to florfenicol, while two other chicken isolates and one human clinical isolate were not susceptible to tetracycline. All chicken isolates were susceptible to gentamicin, azithromycin, telithromycin, erythromycin, ciprofloxacin, nalidixic acid and clindamycin.

The 2016/17 survey managed by the Australian Chicken Meat Federation (and funded by the Australian government Department of Agriculture and Water Resources) found that of the 204 *Campylobacter* isolates, 63% of *C. jejuni* and 86.5% *C. coli* isolates were susceptible to all the antimicrobials tested and 3% were classified as multidrug-resistant. All *Campylobacter* isolates tested were microbiologically susceptible to florfenicol and gentamicin, however non-susceptibility to ciprofloxacin was detected in 14.8% of *C. jejuni* and 5.2% of *C. coli* isolates, results which were supported by the presence of known point mutations in quinolone resistance determining regions of the target chromosomal genes. However, the levels detected were similar to those detected in meat chickens in other countries that also don't use fluoroquinolones (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2015). The genetic analysis of these isolates from the Australian survey suggests that the presence of these isolates in the gut of health chickens was likely transferred from humans to the chickens.

Salmonella

AMR among *Salmonella* spp. was investigated in the 2007 Australian pilot survey of AMR in foods (Barlow & Gobius, 2007). A total of 174 *Salmonella* isolates were identified during the 12 month sampling period and 100 isolates were randomly selected for susceptibility to 16 antimicrobial agents using Sensititre plates and CLSI breakpoints. Susceptibility to all antimicrobial agents was observed for 77% of the isolates. The types and prevalence of

resistances observed were amoxicillin-clavulanic acid (1%), ampicillin (4%), cefoxitin (1%), florfenicol (1%), nalidixic acid (1%), streptomycin (5%), tetracycline (16%), and trimethoprim/sulfamethoxazole (3%). Thus no resistance was observed to eight agents, including 1st, 2nd and 3rd generation cephalosporins (cefazolin, cefotaxime, ceftiofur, ceftriaxone) and carbapenems (meropenem), fluoroquinolones (ciprofloxacin), chloramphenicol and gentamicin. The observed patterns of resistance included 18 isolates with a single resistance, two isolates with resistance to two agents, and three isolates with resistance to three agents in different classes – thus 3% of isolates were MDR. The proportions resistant to tetracycline, ampicillin, streptomycin and the combination of trimethoprim/sulfamethoxazole were low. No resistance was observed to agents categorised by ASTAG as of high importance to human health, notably no resistance to 3rd generation cephalosporins, carbapenems or fluoroquinolones.

The 2016/17 survey managed by the Australian Chicken Meat Federation (and funded by the Australian Government Department of Agriculture and Water Resources) found that, of the 53 *Salmonella* isolates, 92.5% were susceptible to all the antimicrobials tested and none were MDR. Six isolates were non-susceptible to cefoxitin but there were no known genetic resistance mechanisms found to support this, and cephalosporins are not approved for use in poultry in Australia. Two isolates were non-susceptible to ampicillin, and one isolate each was non-susceptible to streptomycin and trimethoprim/sulfamethoxazole.

NEW ZEALAND

Industry background

Like Australia, the poultry industry in New Zealand is dominated by a small number of fully integrated companies managing all aspects of poultry rearing and poultry meat and egg production, with strict biosecurity protocols. Poultry raised for meat are raised in barns or free-range³². Chicken consumption is high in New Zealand, with an estimated consumption of 20 chickens per person per year. In 2014 there were 103 million chickens bred for meat, including 15 million free range birds. Smaller numbers of turkeys and ducks are also farmed for meat. Most poultry in New Zealand is produced for domestic consumption.

Metaphylactic administration of antibiotics occurs in the poultry industry when required, with the antibiotics being administered via food or water (Hillerton & Allison, 2015). Avoparcin was used as a feed additive in New Zealand broiler production from 1977 until June 2000 to control necrotic enteritis caused by *Clostridium perfringens* (Manson *et al.*, 2003). Avoparcin is closely related to vancomycin so vancomycin-resistant enterococci (VRE) are of concern in poultry. Clostridial enteritis is now controlled largely by the use of zinc bacitracin (Heffernan *et al.*, 2011; Muellner *et al.*, 2016). Zinc bacitracin is not considered an important antibiotic for human health.

Resistance to the macrolide erythromycin is also of interest because tylosin was used extensively as a prophylactic for broilers; resistance to tylosin provides cross-resistance to erythromycin (Manson *et al.*, 2004). Macrolides are not routinely used, but may be used in

³² [Poultry Industry Association of New Zealand](#) (accessed 21 June 2018).

direct response to health issues in flocks, such as the 2011/12 outbreak of femoral head avascular necrosis in broiler chickens (Muellner *et al.*, 2016).

No fluoroquinolones are registered for use in poultry in New Zealand (Muellner *et al.*, 2016). Tetracyclines are mostly used in breeder chickens to treat respiratory or digestive conditions, not in broiler chicken production.

The Poultry Industry Association of New Zealand (PIANZ) commissioned two studies on AMR, one in 2006 and the other in 2014. Results from the 2006 survey were published in two papers by Pleydell *et al.* (2010a; 2010b). The similar, unpublished study conducted in 2014 examined levels of antimicrobial resistance in *E. coli* (n=400), *E. faecium* (n=214), *E. faecalis* (n=186) and *C. jejuni* (n=200) isolated from poultry carcass rinses in six plants in New Zealand. Information from these studies has been included in the following sections.

The following antibiotics are not currently used within the poultry industry: cephalosporins, gentamicin, kanamycin, quinupristin-dalfopristin, ciprofloxacin, enrofloxacin, nalidixic acid, furazolidone, erythromycin (this is important when interpreting resistance patterns reported below).

AMR in chicken meat pathogens

No data identified.

AMR in chicken meat commensals

E. coli

E. coli isolates were obtained from routine testing of poultry carcasses from primary processors during the PIANZ-funded study in 2006 (Pleydell *et al.*, 2010b). Of the 407 isolates, 291 (71.5%) were susceptible to all antimicrobials tested. Resistance to two or more antimicrobials was observed for 21 (5.2%) isolates. One isolate was resistant to five antimicrobials (three aminoglycosides plus sulfasoxazole and tetracycline). While the results show the presence of MDR, the highest proportion of isolates resistant to any of the antibiotics tested was 18%, against cephalothin (26% of isolates demonstrated intermediate cephalothin resistance). No resistance to ESCs were detected.

In the follow-up study conducted in 2014, the following observations were made:

- Most of the 400 *E. coli* isolates were sensitive to all antimicrobials tested (60%), and of the remainder, most were resistant to a single drug (68%). Ampicillin and tetracycline resistance remains relatively low compared to other countries.
- Four isolates showed evidence of multi-drug (>3) resistance in 2014, compared to one in 2006.
- In contrast to 2006, no isolates were resistant to gentamicin or kanamycin (1.5% and 0.2% in 2006, respectively)
- Compared to 2006, there was an 8% increase in resistance to cephalothin, a 1st generation cephalosporin (from 18% to 26%).

In a separate study, 306 *E. coli* were isolated from whole carcass rinsates during 2009/10 (Heffernan *et al.*, 2011). Over half (56%) were susceptible to all 17 antibiotics tested. The highest levels of resistance were reported for sulphonamide (sulfamethoxazole, 31%),

tetracycline (12%) and streptomycin (10%), which were higher than those reported in the above 2006 study. Ampicillin resistance (5%) was similar to the 2006 study (4%) and cephalothin resistance was lower (2%). None of the *E. coli* isolates produced ESBL or AmpC β -lactamase. No isolates were resistant to 3rd generation cephalosporins, fluoroquinolones or gentamicin.

Enterococcus spp.

During 2000 and 2001, 900 enterococci were isolated from broiler faecal samples and 82 (9%) were vancomycin-resistant (Manson *et al.*, 2003). VRE isolates were only obtained from broiler farms that were using, or had used, avoparcin as a dietary supplement.

Of these 82 VRE, 73 were *E. faecalis* and nine were *E. faecium*. All contained the *vanA* and *ermB* genes, which resided on the same plasmid, and all were resistant to vancomycin and erythromycin. All isolates also demonstrated resistance to teicoplanin and bacitracin. High resistances to tetracycline (87%) and avilamycin (63%) were also observed.

The *E. faecalis* strains were identical or closely related by PFGE, suggesting clonality. The vancomycin-resistant *E. faecalis* clone was also identified in 12/15 faecal samples from poultry and one chicken breast sampled at retail (Cook, 2003). These isolates also had high-level resistance to vancomycin and bacitracin.

During 2002/03, 382 enterococci were isolated from broiler faecal samples, of which 152 (40%) were *E. faecalis*, 181 (47%) were *E. faecium* (Manson *et al.*, 2004). Of the 382 enterococci, 104 were from broilers not receiving antimicrobials in their diet but AMR patterns were similar to isolates from broilers fed antimicrobials. Overall, vancomycin resistance was similar to the 2000/1 study (6%). Resistances to bacitracin (99% with MIC \geq 256 μ g/ml) and erythromycin (65%) were high. There was some resistance to avilamycin (15% with MIC \geq 8 μ g/ml).

A follow-up study applied whole genome sequencing to 156 VRE isolates isolated in the above studies (Manson *et al.*, 2003; 2004) and from a pool of 66 broilers (Rushton-Green, 2017). The *E. faecalis* isolates were highly clonal (91 were multilocus sequence type (ST) 108) but there was low clonality among *E. faecium*. All isolates, but one, identified as the *E. faecalis* ST108 clone were resistant to bacitracin. Resistance to vancomycin, erythromycin, tetracycline and clindamycin was also common among this clone.

Resistance to vancomycin appeared to be lower in the PIANZ-funded study in 2006, being measured as 0.3% among *E. faecium* isolates (n=318) and 3.6% among *E. faecalis* isolates (n=83) (Pleydell *et al.*, 2010a). These isolates were from poultry carcasses rather than faeces. Resistance to zinc bacitracin was high (97% for *E. faecium*, 88% for *E. faecalis*). Approximately one-third of *E. faecium* isolates were resistant to tetracycline and erythromycin; for *E. faecalis* these values were 48% and 42% for each respective antibiotic. Almost all (96%) *E. faecium* isolates were resistant to furazolidone. Of all 401 enterococci, only one (an *E. faecalis*) was fully susceptible to all nine antimicrobials. Two *E. faecium* isolates were resistant to six of the nine antimicrobials. *E. faecium* clustered into resistance phenotypes and these were associated with integrated poultry companies, suggesting that company-level factors influenced development of AMR profiles.

In the follow-up PIANZ-funded study in 2014, the following observations were made:

- In common with the 2006 only one isolate was fully sensitive to all antimicrobials tested.
- In common with the 2006 survey high levels of resistance were observed to bacitracin (>93% for both species).
- In common with the 2006 survey the level of furazolidone resistance also remained high (75% of isolates).
- In the 2014 survey, 9% *E. faecalis* were identified as vancomycin-resistant (VREs) however, none showed complete resistance - all had zone sizes close to the CLSI breakpoint zone size for resistance (14 mm).
- Similar to the 2006 survey, quinupristin-dalfopristin resistance was higher within the *E. faecalis* isolates (91%, 84% in 2006) compared to *E. faecium* (5%, 14% in 2006), owing to intrinsic resistance to this antibiotic among the majority of *E. faecalis*.

Enterococci were isolated from poultry carcass rinsates taken at primary processors during the period 2009/10 (Heffernan *et al.*, 2011). No resistance to ampicillin, vancomycin or high-level gentamicin was detected among either *E. faecalis* (n=140) or *E. faecium* (n=158) isolates. Of the *E. faecalis* isolates, 17.9% were susceptible to all nine antibiotics tested. Of the 158 *E. faecium* isolates, 20.3% were fully susceptible. Very high bacitracin MICs (≥ 512 $\mu\text{g/ml}$) were measured for 95% of *E. faecalis* and 99% of *E. faecium* isolates. Resistance to erythromycin was 34% among *E. faecalis* isolates and 25% among *E. faecium*. A high proportion of the *E. faecalis* isolates were resistant to tetracycline (78%). The *E. faecium* isolates demonstrated higher resistances to a range of antibiotics, e.g., ciprofloxacin (46%), tetracycline (35%), quinupristin/dalfopristin (32%) and nitrofurantoin (30%).

AMR in chicken meat zoonotic pathogens

Campylobacter

Resistance to ciprofloxacin, erythromycin, nalidixic acid or tetracycline was not detected among 20 *C. jejuni* and two *C. coli* isolates from raw retail chicken carcasses during 2001 (Harrow *et al.*, 2004). While this study was small, it was supported by the larger PIANZ-funded study during 2005/06, which tested 193 *C. jejuni* isolates from retail poultry carcasses (Pleydell *et al.*, 2010b). Of the 193 isolates, 192 (99.5%) were susceptible to all six antimicrobials tested. One isolate was resistant to erythromycin. No resistance to tetracycline, ciprofloxacin, chloramphenicol, enrofloxacin or nalidixic acid was observed.

In the follow-up study conducted in 2014 the following observations were made:

- In contrast to the 2006 survey, 19 *C. jejuni* isolates (9.5%) were found to be resistant to ciprofloxacin, enrofloxacin, nalidixic acid and/or tetracycline. This represented a significant increase compared to 2006, when no ciprofloxacin, enrofloxacin or tetracycline resistance was found.
- Resistance to ciprofloxacin, enrofloxacin, nalidixic acid and tetracycline was found in 15 isolates and all were the same multilocus sequence type: ST 6964 (see below).

Resistance was also uncommon among *C. jejuni* isolated from poultry carcasses at processing plants during the period 2009/10 (Heffernan *et al.*, 2011). Of 295 isolates tested for AMR, 95.9% were susceptible to all seven antimicrobials tested. Just 2.7% were

ciprofloxacin-resistant, 0.3% were tetracycline-resistant and no erythromycin resistance was detected.

C. jejuni ST 6964 was first reported in New Zealand in 2014, having been isolated from retail poultry, broiler chickens and human stools (Muellner *et al.*, 2016). These isolates were found to demonstrate AMR. During 2014, 199 *Campylobacter* isolates from 123 chicken carcasses/pieces were tested for AMR. Fifteen isolates from eight chickens were resistant to ciprofloxacin and tetracycline and all were *C. jejuni* ST 6964. Swabs from pooled caecal contents from chickens at slaughter were tested for ciprofloxacin and tetracycline-resistant *Campylobacter* spp. during 2015. Of 195 swabs, 72 (37%) yielded presumptive *Campylobacter* spp. resistant to both antibiotics. Between the period July 2015 and September 2015, an additional 8/15 caecal swabs (pooled samples from breeder flocks) also produced presumptive *Campylobacter* spp. isolates that were resistant to ciprofloxacin and tetracycline.

The whole genomes from 29 *C. jejuni* ST6964 poultry isolates identified during 2015 were evaluated for genomic markers for resistance to fluoroquinolones (point mutation in *gyrA*), macrolides (23s rRNA mutation), tetracycline (*tetO*) and β -lactams (*bla*_{OXA-61}) (Subharat, 2017). All 29 isolates had the *gyrA/tetO/bla*_{OXA-61} profile. The 23s rRNA mutation was not present. The molecular results were supported with phenotypic AMR testing by disk diffusion assay. All 29 isolates were resistant to the fluoroquinolone ciprofloxacin, tetracycline, and the β -lactams oxacillin and penicillin. All 29 isolates were susceptible to the macrolide erythromycin.

Those with the *bla*_{OXA-61} gene (all 29 poultry isolates) had smaller zones when tested against ampicillin (10 μ g) compared to those without (isolates from other sources), but the zone size difference was not significant. However, for other β -lactams (30 μ g of each of amoxycloxacilanic acid, cefaclor, cefepime or ceftriaxone), the zones were significantly smaller for isolates with *bla*_{OXA-61} (all 29 poultry isolates) compared to those without this gene. This suggested the gene conferred at least partial resistance to these antibiotics.

All 29 isolates were also resistant to the cephalosporin cephalixin (30 μ g), owing to the presence of *bla*_{OXA-61}. A trend was observed where larger zone diameters correlated with progressively newer generations of cephalosporins, e.g. cephalixin (1st generation) < cefaclor (2nd generation), ceftriaxone (3rd generation) < cefepime (4th generation).

Salmonella

AMR among 436 *Salmonella* isolates described as being from a “poultry” source are available (Broughton *et al.*, 2010). These isolates were selected from non-human *Salmonella* isolates received by ESR between 2002 and 2007. They were submitted by diagnostic veterinary laboratories, the national surveillance programme for processed meats and commercial laboratories that refer isolates from food and environmental sources. One-fifth of these isolates were not susceptible (i.e. demonstrated resistance or intermediate susceptibility) to streptomycin. The proportion of non-susceptible isolates was very low ($\leq 3\%$) or zero for the other antibiotics tested.

ESR continues to periodically test a sample of non-human *Salmonella* isolates for AMR but detailed data are not readily available. Other data on the AMR of *Salmonella* spp. isolated

from poultry are scarce. Only three isolates were obtained during routine testing of poultry carcasses from primary processors (Pleydell et al., 2010b). None of these demonstrated resistance to 12 antibiotics but the results are too limited to draw conclusions. Similarly, only three *Salmonella* isolates were obtained from whole carcass rinsates taken during 2009/10, and none were resistant to a panel of 18 antibiotics (Heffernan et al., 2011).

ANTIMICROBIAL RESISTANCE IN EGGS AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

Australian consumers eat an average of 231 eggs per person per year, with significant growth forecast³³, including 2018/19 forecast sales of 351 million dozen with a gross value of \$855 million. At June 2017, the Australian flock size totalled 27.5 million pullets (young hens not yet laying) and 19.3 million layers.

Poultry are commercially farmed for egg production in every state and territory in Australia except the Northern Territory. With a lack of grain production in the Northern Territory, the cost of transporting feed is prohibitive to commercial production. In 2012, there were 301 commercial egg farms in Australia. Eggs are produced and retailed as cage, barn or free-range which refers to the production system in which the hens are housed. There are both advantages and disadvantages to each of these styles of housing and the decrease in cage housing, and increase in barn and free-range, is driven by consumer demand. Egg production requires the same husbandry skills and animal care as for poultry meat production, however, there is the added requirement of processing and quality control of the eggs that is associated with food production standards and regulations, including Eggs Standards of Australia (ESA)³⁴ and FSANZ Primary Production and Processing (PPP) Standard for Eggs and Egg Products³⁵.

Bird health management is an important aspect of commercial egg production as it directly affects egg productivity and therefore commercial viability – healthy hens are productive hens. The key principles of poultry health management focus on preventing and minimising disease. In commercial poultry production, disease will spread through a flock very quickly and the primary goal is to prevent the onset of disease or parasites. Good hygiene practices and the use and application of a stringent farm quarantine program are the key elements for preventing disease. The National Farm Biosecurity Technical Manual for Egg Production (2015)³⁶ (currently being updated to include sections on antimicrobial resistance and zoonotic diseases) applies to commercial table egg production farms (layer farms) from the time of delivery of day old chicks until depopulation of the spent layer hens, including transportation and delivery of point of lay pullets. The biosecurity objectives for laying

³³ [2017 Annual Report of Australian Eggs](#)

³⁴ [Eggs Standards of Australia \(ESA\)](#)

³⁵ [FSANZ Primary Production and Processing \(PPP\) Standard for Eggs and Egg Products](#)

³⁶ [National Farm Biosecurity Technical Manual for Egg Production \(2015\)](#)

chickens are threefold, (i) to prevent the introduction of infectious disease agents to chickens' (ii) to prevent the spread of disease agents from an infected area to an uninfected area, and (iii) to minimise the incidence and spread of microorganisms of public health significance. Implementation of biosecurity measures combined with good vaccination practices underpins the high health status of laying chickens and egg production in Australia and ensures that antimicrobial agent use can be minimised.

AMR in layer pathogens

No data were identified.

AMR in layer commensals

No data were identified.

AMR in layer zoonotic pathogens

Salmonella

A published Australian study has described an investigation of AMR of non-typhoidal *Salmonella* spp. isolated from 33 commercial caged layer flocks in NSW and South Australia (Pande et al., 2015). A total of 145 *Salmonella* isolates from 7 serotypes (Agona, Anatum, Infantis, Mbandaka, Oranienburg, Typhimurium and Worthington) were obtained from dust samples, egg belts, faeces and shell washes. All isolates were subjected to AST against 12 antibacterial agents (amoxicillin, ampicillin, cefotaxime, ceftiofur, cephalothin, ciprofloxacin, chloramphenicol, gentamicin, neomycin, streptomycin, tetracycline and trimethoprim) using the broth microdilution method with results interpreted according to established CLSI guidelines or other widely accepted guidelines where CLSI breakpoints were not available. In addition, all 145 *Salmonella* isolates were screened for total of 20 ARGs and the presence of integrons by either multiplex or uniplex PCR. Of the 145 isolates, 133 (91.7%) were susceptible to all tested antibacterial agents. In addition, no MDR phenotypes were identified. Antimicrobial resistance was observed to amoxicillin and ampicillin (5.5%), tetracycline (4.1%), cephalothin (2.0%) and trimethoprim (0.7%), while no antimicrobial resistance was observed in any isolate to the 3rd generation cephalosporins cefotaxime and ceftiofur, the fluoroquinolone ciprofloxacin, chloramphenicol or the aminoglycosides gentamicin, neomycin and streptomycin. A low frequency of *Salmonella* isolates (4.8%) carried ARGs and a class 1 integron. The most commonly detected ARGs among the *Salmonella* isolates included blaTEM (2.07%), tet A (1.38%) and dhfrV (0.69%).

The authors concluded that the low prevalence of AMR among these *Salmonella* isolates represented a minimal public health risk associated with the emergence of MDR *Salmonella* spp. from the Australian layer industry. However, the authors also noted that regular surveillance over a larger geographical area and comprehensive nationwide sampling is needed to identify any changes in AMR patterns among *Salmonella* isolates in the egg industry.

The Department of Agriculture and Water Resources, with assistance from Australian Eggs, Scolexia Pty Ltd and the Microbiology Diagnostic Unit *Salmonella* reference laboratory, funded an AMR surveillance proof-of-concept study to identify the most appropriate mechanisms for obtaining and performing antimicrobial susceptibility testing on *Salmonella*

isolates obtained from Australian layer shed environments (Veltman et al., 2018). A total of 307 *Salmonella* isolates from the years 2015-2018 and proportionally representative of the number of layer flocks in each Australian state were obtained from reference, research and State Department of Health laboratories as well as directly from drag swab samples of Tasmanian shed environments. AST was performed by broth microdilution using Veterinary Reference Card panels for 16 antimicrobials. Three main serotypes comprised approximately one third of the collection – Typhimurium (61/307; 19.9%), Senftenburg (45/307; 14.7%) and Agona (37/307; 12.1%).

Non-susceptibility was observed to occur at a low level to streptomycin (8/307; 2.6%), sulfisoxazole (7/307; 2.3%), chloramphenicol (4/307; 1.3%) and tetracycline (3/307; 1%). Very low levels of non-susceptibility were observed to ampicillin (2/307; 0.7%), cefoxitin (2/307; 0.7%), azithromycin (1/307; 0.3%) and trimethoprim-sulfamethoxazole (1/307; 0.3%). All isolates were susceptible to amoxicillin-clavulanate, ceftiofur, ceftriaxone, ciprofloxacin, colistin, florfenicol, gentamicin and kanamycin. A very high proportion of *Salmonella* isolates (295/307; 96.1%) from Australian layer shed environments was susceptible to all 16 antimicrobial agents tested. Eight isolates (2.6%) were resistant to one antimicrobial class, and one isolate (0.3%), a *S. Typhimurium* strain, was resistant to two antimicrobial classes (represented by ampicillin and tetracycline). Three isolates (one of each of the serotypes Havana, Montevideo and Typhimurium) exhibited a MDR phenotype (1%).

Overall, the results (Table 7) confirm the low AMR status among *Salmonella* isolates from Australian caged and free range layer farm environments, which likely reflects the combination of restrictions on antimicrobial use, and in particular, on critically important antimicrobial agents including fluoroquinolones, 3rd generation cephalosporins and colistin, combined with effective non-antimicrobial disease control mechanisms.

NEW ZEALAND

Industry background

More than one billion eggs are produced each year by the commercial egg industry. In 2014 there were 3.4 million caged layers and 450,000 free ranged layers. The majority of eggs are produced by chickens held in conventional cages but these are being replaced with colony cages, free-range or barn systems in accordance with a Code of Welfare for layer hens, introduced in 2012 (National Animal Welfare Advisory Committee, 2012). While the majority of eggs are sold as whole table eggs, liquid egg (fresh or pasteurised, whole or separated, with or without other ingredients) and dried egg are also manufactured in New Zealand.

AMR in layer pathogens

No studies were identified for inclusion in this review.

AMR in layer commensals

No data were identified.

AMR in layer zoonotic pathogens

No data were identified.

Table 7. Minimum inhibitory concentration (MIC) distributions established for 16 antimicrobials against 307 *Salmonella* spp. isolates from commercial layer shed environments in Australia.

	Number and percentage of isolates with MICs (mg/L) at: ^a																					
Antimicrobial agent	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	%NWT ^b [95% CI]	%NS ^c			
Amoxicillin-clavulanate								290 94.5	15 4.9	0 —	2 0.7	0 —	0 — ^d					— ^e	0.0			
Ampicillin								276 89.9	26 8.5	3 1	0 —	0 —	0 —	2 0.7					0.7 [0.1–2.3]	0.7		
Azithromycin					0 —	0 —	0 —	1 0.3	39 12.7	237 77.2	28 9.1	1 0.3	1 0.3					—	0.3			
Cefoxitin						0 —	3 1	107 34.9	183 59.6	12 3.9	2 0.7	0 —					0.7 [0.1–2.3]	0.7				
Ceftiofur					0 —	1 0.3	26 8.5	270 87.9	10 3.3	0 —	0 —					0.0 [0.0–1.2]	—					
Ceftriaxone						307 100	0 —	0 —	0 —	0 —	0 —	0 —	0 —	0 —					—	0.0		
Chloramphenicol									5 1.6	122 37.9	176 57.3	4 1.3	0 —					0.0 [0.0–1.2]	1.3			
Ciprofloxacin	236 76.9	70 22.8	1 0.3	0 —	0 —	0 —	0 —	0 —	0 —										0.0 [0.0–1.2]	0.0		
Colistin					0 —	42 13.7	164 53.4	96 31.3	5 1.6	0 —										—	0.0	
Florfenicol								0 —	5 1.6	147 47.9	152 49.5	3 1	0 —	0 —	0 —					0.0 [0.0–1.2]	0.0	
Gentamicin						122 37.9	168 54.7	16 5.2	1 0.3	0 —	0 —	0 —					0.0 [0.0–1.2]	0.0				
Kanamycin									294 95.8	9 2.9	4 1.3	0 —	0 —	0 —	0 —	0 —					—	0.0
Streptomycin									5 1.6	54 17.6	196 63.8	44 14.3	5 1.6	1 0.3	2 0.7					2.6 [1.1–5.1]	2.6	
Sulfisoxazole												16 5.2	31 10.1	174 56.7	67 21.8	12 3.9	7 2.3	—	2.3			
Tetracycline										304 99	0 —	0 —	0 —	3 1					1 [0.2–2.8]	1		
Trimethoprim/sulfamethoxazole					304 99	2 0.7	0 —	0 —	0 —	0 —	1 0.3										0.3 [0.0–1.8]	0.3

- ^a Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGNF card. MICs > than highest concentration available are indicated in the shaded region
^b Vertical green lines indicate EUCAST ECOFF values; CLSI VET01S susceptible (blue) and resistant (red) breakpoints; NARMS breakpoint (red dashes)
^c Percentage non-wild-type (EUCAST)
^d Percentage non-susceptible, CLSI or NARMS (orange)
^e Not applicable
^f Not defined |

ANTIMICROBIAL RESISTANCE IN SEAFOOD AND SHELLFISH AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

Australian aquaculture production has continued to grow in volume and gross value over the past decade. The sector includes the propagation of over 40 species of aquatic animals including shellfish (e.g. oysters, mussels), a variety of fresh and saltwater fish (e.g. salmon, tuna, barramundi, perch, trout, kingfish, cobia and cod), prawns, abalone and saltwater crocodiles (Hayakijkosol et al., 2017).

Total aquaculture gross value of production in 2015/16 was \$1.3 billion, with salmonids the dominant sector contributing \$718 million of this value. In 2015/16, 56,300 tonnes of seafood was produced through aquaculture, which was twice as much as that produced during 2005/06 (Mobsby & Koduah, 2017). Aquaculture's share of total fishery and aquaculture production value increased from 34% in 2005/06 to 43% in 2015/16 (Australian Bureau of Agricultural and Resource Economics and Sciences, 2017). Further investment since 2015/16 is expected to see volumes and value of aquaculture production in Australia continue to grow.

Consumption of seafood among Australians has remained at approximately 15 kg/person/year for the decade leading up to 2015/16 (Australian Bureau of Agricultural and Resource Economics and Sciences, 2017) (see Figure 2 below). Whilst the bulk of this seafood is cooked prior to consumption, there are some increasing trends also for consumption of uncooked seafood such as sashimi in Japanese restaurants. This change in food preparation has the potential to alter risks for transfer of microbes and their AMR from seafood to humans.

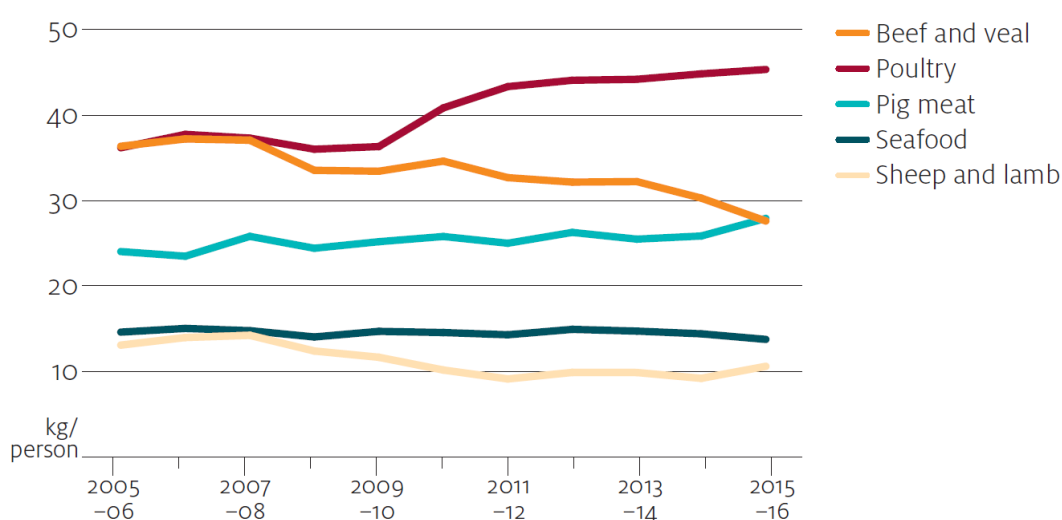


Figure 1: Australian per-person apparent consumption of meats and seafood, 2005/6 to 2015/16 (Australian Bureau of Agricultural and Resource Economics and Sciences, 2017).

Much of the microbial food safety risks associated with seafood consumption relate to seafood processing and handling, such as listeriosis, rather than the organisms associated with growing the seafood. Antibiotic use is generally very low relative to volumes of production. Where used, antibiotics are for control of clinical diseases. Antimicrobial use for growth promotion is not practised within aquaculture industries. Antibiotic stewardship within the aquaculture industry is generally good across larger suppliers who utilise industry veterinarians to ensure that usage conforms to appropriate use guidelines, as has been developed for other terrestrial food production animals. This includes ensuring appropriate investigations are made to determine if the cause of disease is bacterial, and that diagnostic laboratories are used to confirm pathogen identity and antimicrobial sensitivity testing. Some laboratories offer MIC testing, others offer disc diffusion test methods. Given there are no clinical breakpoints available for aquaculture pathogens, epidemiological cut-off values are used as an indication of isolate susceptibility and the development of resistance.

In addition, veterinary advice is provided on whether husbandry or infrastructure changes could assist in limiting or preventing future bacterial disease outbreaks and thereby avoid the use of antibiotics. Where serious bacterial pathogens emerge, finfish industries are encouraged to invest in vaccine development. The Fisheries Research and Development Corporation has supported several vaccination projects over the past decade as aquaculture production has expanded, and has recently coinvested with Tasmania's salmon industry in the establishment of the Centre for Aquatic Animal Health and Vaccines.

Antimicrobial use in aquaculture is undertaken with a prescription from a registered veterinarian. Prescriptions include advice on appropriate product withholding periods to ensure products with unacceptable adverse residues are not available for human consumption. FSANZ has a temporary MRL for oxytetracycline in fish of 0.2mg/kg. No other antimicrobials carry an MRL for any seafood commodity, so from a food safety perspective antibiotics are not permitted in seafood at levels above the limit of laboratory detection.

Presently, there are no fully registered antimicrobial products for use in any of the aquaculture sectors. The salmon industry has a Minor Use Permit for oxytetracycline to control some bacterial diseases. Historically other finfish enterprises have had Minor Use Permits also covering oxytetracycline. The National Aquaculture Council has assisted finfish industries (other than salmon) to get the Minor Use Permit re-issued. For other antibiotics, these are made available through off-label provisions through legislation of state jurisdictions regarding the use of veterinary medicines.

Other than oxytetracycline, antibiotics such as trimethoprim and potentiated sulphonamides are used at times with salmon. The development of locally produced efficacious vaccines for major endemic diseases has led to a marked reduction in antimicrobial usage by the Tasmanian salmon industry (Carson, 2017). For the period 2010-17 antibiotic use was <5g/t of salmon produced and the industry imposed a ban in 2003 on using oxolinic acid.

Some parts of the prawn farming and shellfish sectors occasionally utilise small volumes of erythromycin in their hatchery phase of production. No antimicrobials are used in either of these industries in the nursery or growout phases of their operations.

Antibiotic use is uncommon in the trout farming industry. Where required, it is based on laboratory diagnosis and under the guidance of veterinary prescription. The largest farms participate annually in the National Residue Survey. They have a record of freedom from antibiotic residues in their harvest product.

The Australian Barramundi Farmers' Association members participate in an accreditation and certification scheme that requires antibiotic use to be minimised and where use occurs, it is uniformly under the prescription and guidance of a registered veterinarian. No prophylactic use occurs. The industry participates in national testing schemes for antimicrobials and has demonstrated freedom from residues in its products annually (Anon, 2017).

AMR in aquaculture pathogens, commensal and zoonotic bacteria

Very few published studies have examined AMR among bacteria isolated from aquaculture species in Australia. Akinbowale *et al.* (2006) subjected 104 aquaculture bacterial isolates (100 Gram-negative and four Gram-positive) to AST for 19 antimicrobial agents using NCCLS (now CLSI) human breakpoints for Enterobacteriaceae. The isolates included saltwater and freshwater pathogens isolated from food-producing and aquarium industry fin fish species, and environmental as well as commensal isolates. The population was dominated by *Vibrio* spp. (approximately 60% of the 104 isolates) and *Aeromonas* spp. (21%). A high proportion of *Vibrio* isolates were resistant to aminopenicillins and 1st generation cephalosporins, but few were resistant to tetracycline and none to florfenicol or high ASTAG rating antimicrobials. A higher proportion of *Aeromonas* spp. were resistant to tetracycline. It is difficult to determine the significance of this study given the diversity of bacterial species and the use of non-standard techniques for aquaculture pathogen susceptibility testing, which brings into question the definition of resistance.

Akinbowale *et al.* (2007) screened 129 *Pseudomonas* spp. and 90 *Aeromonas* spp. isolates obtained from freshwater rainbow trout for resistance to 15 antimicrobials. Only intrinsic resistance to existing drugs was detected and all isolates were susceptible to gentamicin and ciprofloxacin. Akinbowale *et al.* also screened a collection of twenty tetracycline-resistant aquaculture isolates (mainly *Aeromonas* spp.) and identified a number of tetracycline resistance genes in fifteen of the isolates by PCR. *tetM* (50%) was the most common determinant, followed by *tetE* (45%), *tetA* (35%) and *tetD* (15%). Five of the genes were transferable by conjugation to *E. coli* indicating the potential for horizontal transmission (Akinbowale *et al.*, 2007).

Ndi & Barton (2011b) investigated the occurrence of class 1 integrons in *Aeromonas* spp. isolates from rainbow trout. Class 1 integrons were detected in 28/90 (31%) of isolates, and in addition to sulphonamide (*sul1*) and quaternary ammonia (*qac1*) resistance genes, some integrons contained the streptomycin resistance gene *aadA2* in their variable region. *tetC*

was also identified in some isolates but was not integron-associated. Ndi & Barton (2012) also investigated the occurrence of class 1 integrons in *Pseudomonas* spp. isolated from rainbow trout. Class 1 integrons were detected in 30/129 (23%) isolates and *aadA* streptomycin resistance genes were also detected in nearly half the isolates positive for integrase. The *mexA* multidrug efflux pump gene was detected in 85 isolates and 59/92 isolates tested also were positive for the cadmium resistance gene *cadA*. It is important to note that these are environmental organisms and the presence of resistance genes may not be directly related to antimicrobial use in the industry.

Watkinson *et al.* (2007) assessed the presence of antimicrobial resistance in *E. coli* (n = 50) isolated from native rock oysters in the Brisbane River exposed to waste water treatment plant discharges and found the prevalence of resistance to six tested antimicrobial agents was low ($\leq 4\%$).

Retrospective Tasmanian salmon aquaculture pathogen MIC data for oxytetracycline (170 isolates representing 16 species) and trimethoprim (178 isolates representing 15 species) obtained by the Tasmanian Department of Primary Industries, Parks, Water & Environment over a ten year period indicates the majority of isolates were not resistant to oxytetracycline with MIC₅₀ values of 1 µg/mL and MIC₉₀ values of 2 µg/mL (J Carson, personal communication).

NEW ZEALAND

Industry background

The New Zealand seafood industry harvests a diverse range of wild and aquaculture species but most are raised in natural conditions without any inputs of feed³⁷. King salmon are raised in pens but according to the industry, biosecurity procedures and the absence of any native salmon species mean that antibiotics are not administered³⁸. Approximately 600,000 tonnes of seafood (excluding aquaculture) is harvested from New Zealand waters each year contributing to a value of \$1.79 billion in exports in 2016.

AMR in seafood pathogens

No studies were identified for inclusion in this review.

AMR in seafood commensals

No data were identified.

AMR in seafood zoonotic pathogens

No data were identified.

³⁷ [Seafood New Zealand Website](#) (accessed 21 June 2018).

³⁸ [Aquaculture New Zealand website](#) (accessed 21 June 2018).

ANTIMICROBIAL RESISTANCE IN HORTICULTURE AT PRODUCTION, PROCESSING AND RETAIL

AUSTRALIA

Industry background

Australia's horticulture industry comprises fruit, vegetables, nuts, flowers, turf and nursery products³⁹. The industry is mainly made up of small-scale family farms, but these are now influenced by a growing trend to medium and larger scale operations. Australian farmers continue to adjust their operations and adopt new technologies to respond to the opportunities and challenges of agricultural production in Australia including, increased competition from imported fresh and processed produce, market price pressures, challenging or adverse seasonal conditions. For example, greenhouse vegetable production of premium quality fruiting vegetables (tomatoes, capsicums, and cucumbers) grown in soilless systems has been increasing.

In 2013/14 Australian horticulture (excluding wine grapes) had a gross value of \$8.7 billion, ranked third behind the meat and grain industries. The major product groups had the following gross value of production: Fruit and nuts: \$3.2 billion; vegetables: \$3.5 billion; nursery, cut flowers and cultivated turf: \$1.3 billion.

In 2014/15 the value of exported fresh and processed fruit, vegetables, nuts, and nursery products was \$2.1 billion. The export of fresh produce (particularly fruit) is limited by quarantine restrictions in a number of countries including Japan, USA, Vietnam, South Korea, Taiwan and mainland China.

There is no information to indicate the direct use of antimicrobials in Australian horticulture for control of microbial plant disease, however the use of animal manure fertiliser and irrigation water from sources shared with food animal industry production may be potential entry points for AMR bacteria and their propagation in horticulture supply chains.

Two studies have assessed the presence of AMR in Australian horticultural products; one original scientific article and one pilot survey report. Both studies report AMR in commensal or potential zoonotic pathogenic bacteria.

AMR in horticulture plant pathogens

No data were available for horticulture plant pathogenic bacteria in Australia.

AMR in horticulture commensals

Bacteria of the family Enterobacteriaceae isolated from post-harvest, retail-ready strawberries packaged in punnets were tested for AMR to 14 antimicrobials (Kurtböke et al.,

³⁹ Horticulture fact sheet. http://www.agriculture.gov.au/ag-farm-food/hort-policy/horticulture_fact_sheet#trade-statistics

2016). Testing of 113 isolates showed the highest percentage of resistance to ampicillin and cefoxitin, 100% respectively, in the genus *Pantoea* (n=23). Eighty percent of *Escherichia* isolates (n=15) were resistant to ampicillin and 96% of *Citrobacter* (n=24) were resistant to cefoxitin.

AMR in horticulture zoonotic pathogens

A pilot survey for AMR bacteria in Australian food was conducted in 2007/08 to estimate the prevalence of AMR bacteria in selected foods purchased at retail outlets (Barlow & Gobius, 2008). *E. coli* isolation from retail lettuce, followed by AMR assessment, was included as a food/bacterium survey target, however the number of *E. coli* isolates (n=7) fell well-short of the survey design requirement of 100 isolates. However, AMR testing of the available isolates was continued.

Resistance to ampicillin (57.1%) was most prevalent. Resistance to amoxicillin/clavulanic acid (28.6%), cefazolin (28.6%), streptomycin (14.3%), tetracycline (28.6%) and trimethoprim/sulfamethoxazole (14.3%) was also identified.

Resistance to one or more antimicrobials was observed in 5 of 7 isolates (71%). MDR was observed in two isolates including resistance to ampicillin-streptomycin-tetracycline-trimethoprim/sulfamethoxazole (4 antimicrobials; 1 isolate; 14.3%) and amoxicillin/clavulanic acid-ampicillin-cefazolin-tetracycline (4 antimicrobials; 1 isolate; 14.3%).

NEW ZEALAND

Industry background

The horticulture industry is valued at \$5.6 billion (NZD) and is supported by 5,500 growers (Horticulture NZ, 2017). Consolidated statistics are not available for the weight of vegetables and fruits produced in New Zealand. Major fruit crops are stonefruit, pipfruit and kiwifruit. Most crops are produced outdoors. Indoor crops include table tomatoes and salad greens.

Research particularly focusses on streptomycin resistance among bacteria isolated from horticultural production areas because this drug is used in New Zealand for controlling a number of crop diseases (Vanneste, 2011):

- Pipfruit: *Erwinia amylovora* (fire blight)
- Stone fruit: *Pseudomonas syringae* pv. *syringae* (bacterial blast), *Xanthomonas arboricola* pv. *pruni* (bacterial spot)
- Tomatoes: *Pseudomonas syringae* pv. *tomato* (tomato speck), *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot), *Clavibacter michiganensis* pv. *michiganensis* (bacterial canker and wilt)
- Kiwifruit: *Pseudomonas syringae* pv. *actinidiae* (Psa; bacterial canker, first detected in New Zealand in 2010)

Streptomycin and kasugamycin are registered for controlling Psa on kiwifruit. Their use is restricted to certain conditions (Anonymous, 2016, 2017; Young, 2012). Streptomycin use is decreasing among kiwifruit growers (Zespri/KVH, pers. comm.).

Bacteria present in fresh water may be transferred to fresh produce through irrigation. One study has detected AMR among *E. coli* from Canterbury river waters (including MDR *E. coli*) (Schousboe *et al.*, 2015). Another study detected the presence of resistance genes in DNA extracts from biofilms of rocks submerged at 20 Southland freshwater sites (Winkworth-lawrence & Lange, 2016).

AMR in horticulture plant pathogens

Erwinia amylovora

Streptomycin resistance among *E. amylovora* isolates was first reported in 1993 after analyses of samples from infected trees in orchards of the Hawke's Bay, Bay of Plenty, Waikato and Gisborne regions (Thomson *et al.*, 1993). Streptomycin-resistant strains were detected in 7/35 orchards in the Hawke's Bay, all within a 10 km radius, but not in any of the 12 orchards located in other regions. In three Hawke's Bay orchards, saprophytic bacteria (species not identified) were also tested for streptomycin resistance and all three were resistant.

The streptomycin-resistant isolates were resistant to this antibiotic at a concentration of 1000 µg/ml. In contrast, streptomycin sensitive isolates had zones of 14 mm surrounding disks dipped in 10 µg/ml streptomycin and larger zones against higher concentrations.

Additional work showed that streptomycin resistance was due to a mutation in a chromosomal gene, *rpsL* (Thomson *et al.*, 1993; Vanneste & Voyle, 2002; Vanneste *et al.*, 2008).

Pseudomonas spp. and Xanthomonas spp.

During 2004/5, samples from nectarine, apricot and peach trees were taken from commercial orchards in Central Otago and both epiphytic and potentially pathogenic bacteria were isolated and tested for resistance to streptomycin (and copper) (Vanneste *et al.*, 2005). In 33/47 blocks tested, >50% of the bacterial isolates were resistant to streptomycin (these included *Pseudomonas* spp.). *Xanthomonas* spp. were isolated from 14/47 blocks and all 306 strains were susceptible to streptomycin. An additional sample from a commercial Hawke's Bay orchard was also analysed. Of 120 bacteria selected, 97% were resistant to streptomycin.

Another 46 samples were received from the Central Otago orchards during 2005/6 and of the 1,061 *Pseudomonas* isolates examined, 38% were resistant to streptomycin (Vanneste *et al.*, 2008).

Zespri and Kiwifruit Vine Health (KVH) have been monitoring *Psa* resistance towards streptomycin (and copper) since 2011, and towards kasugamycin since 2015. Monitoring is set to continue until 2021. Originally the resistance monitoring programme was based in Te Puke/Bay of Plenty region but from 2016 was expanded to 100 orchards over the North Island. The same vines are tested in spring, summer and autumn. Streptomycin resistance was first detected in autumn 2015. Since then, the percentage of isolates each season with resistance has decreased (<5% in November 2017). To date, no kasugamycin resistance has been detected.

The genes for streptomycin resistance (*strA*, *strB*) in *Pseudomonas* spp. (and the epiphytic bacteria *Pantoea agglomerans*) were carried either on a transposon identified as Tn5393 or on a plasmid (Vanneste *et al.*, 2008). In some isolates, the genes coding for streptomycin resistance were found on the same plasmid as those encoding copper resistance, so the use of one control agent may increase the proportion of the bacterial population resistant to the other. However, not all *Pseudomonas* spp. or *P. agglomerans* isolates that demonstrated resistance to streptomycin carried these genes so it was proposed that other resistance mechanisms existed (Vanneste & Voyle, 2002). The genes encoding streptomycin resistance have been detected on plasmids from two New Zealand *Psa* isolates (Colombi *et al.*, 2017).

AMR in horticulture commensals

No data were identified.

AMR in horticulture zoonotic pathogens

Salmonella spp.

Data on AMR among *Salmonella* spp. isolated from fresh produce are scarce and currently unsuitable for indicating trends:

- During a 2008/9 microbiological survey of fresh produce, only two isolates were recovered and neither demonstrated any AMR against a suite of 18 antibiotics (Heffernan *et al.*, 2011).
- Vegetable samples analysed during autumn/winter 2014 yielded *Salmonella* spp. isolates but the actual number was not reported; the authors noted that only 9/79 vegetables were positive for this pathogen (Wadamori *et al.*, 2016). The authors report resistance towards vancomycin, ampicillin and penicillin.

AMR in horticulture other bacteria

S. aureus

Only one very limited study indicates the potential for resistance among *S. aureus* on fresh produce. *S. aureus* was detected in most of the 79 vegetable samples from Canterbury farms and the authors reported that resistance to penicillin was common (Wadamori *et al.*, 2016).⁴⁰

E. coli

AMR resistance among 90 *E. coli* isolates obtained from a microbiological survey of fresh produce during 2008/9 was low (Heffernan *et al.*, 2011). Ninety percent of the *E. coli* isolates were susceptible to all of the antimicrobials tested. The highest resistance observed was to tetracycline (7% resistant). None of the *E. coli* isolates produced ESBL or AmpC β -lactamase.

⁴⁰ The author of this publication was contacted with a request for additional information but no response was received.

IDENTIFICATION OF ANTIMICROBIAL RESISTANCE DATA GAPS

Summary tables have been prepared to assess and compare the status of available AMR knowledge in each food sector for both Australia and New Zealand. The comparison of different food sector and country data has also facilitated the ranking of available knowledge where the availability of AMR data is designated Substantial (+++); Moderate (++); Limited (+); or None (-). This ranking has aided the identification of priority areas for further development of food sector-specific AMR knowledge, including surveillance.

The variations of available AMR knowledge for different food sectors, as well as the different levels of available AMR knowledge, have supported the recommendations for further investigation of AMR in the following report section on Future Surveillance.

Table 8. Summary of Red Meat AMR knowledge status and knowledge gap identification for Australia and New Zealand.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+++	<ul style="list-style-type: none"> Comprehensive AMR data is available from relevant microorganisms covering the production, processing and retail levels. Multiple studies exist on AMR prevalence among pathogens isolated from diseased cattle and bovine and ovine clinical isolates (Abraham et al., 2014b; Abraham et al., 2015; Goldspink et al., 2015; Izzo et al., 2011; Omaleki et al., 2016; Sparham et al., 2017; Stephens et al., 1993). Substantial effort has been placed in the assessment of AMR in commensal and indicator bacteria from beef and dairy cattle, and faecal samples (Barlow et al., 2015, 2017; Barton et al., 2003; DAFF, 2007) AMG has also been undertaken at the production and processing levels (Barlow et al., 2008, 2009). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> Given the size of the sheepmeat industry, there is an absence of AMR information on ovine sentinel bacteria.
New Zealand	++	<ul style="list-style-type: none"> Only a single study, although relatively comprehensive, has investigated AMR profiles of commensal bacteria from young calves (Heffernan et al., 2011).

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
		<ul style="list-style-type: none"> • The same study (Heffernan et al., 2011), which had limited isolate numbers and a larger report from 2010 (Broughton et al., 2010), focused on AMR profiles of <i>Salmonella</i> spp. from bovine and ovine sources. • Two studies have investigated AMR among <i>Campylobacter</i> spp. from the faeces and offal of beef and sheep, and dressed carcasses of very young (Harrow et al., 2004). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> • Absence of data on AMR profiles of major foodborne pathogens and sentinel bacteria in retail products. • Data on foodborne pathogens and sentinel bacteria from production and processing levels is also very limited.

+++ : Substantial AMR data available.

++ : Moderate level of AMR data available.

+ : Limited amount of AMR data available

- : No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

Table 9. Summary of Dairy AMR knowledge status and knowledge gap identification for Australia and New Zealand.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	++	<ul style="list-style-type: none"> Notably limited AMR information on clinical isolates of dairy cattle pathogens compared to available New Zealand data. One MRSA isolate was identified (Abraham et al., 2017b) and a small number of Mannheimia haemolytica isolates were tested for AMR (Omaleki et al., 2016). While there is limited AMR information on enterococci from dairy cow faeces (Barlow et al., 2017), more data exists on the AMR profiles of E. coli from dairy cattle caecal specimens and faeces (Barlow et al., 2015; DAFF, 2007; Jordan et al., 2005). A number of studies provide information on AMR among <i>Salmonella</i> spp. derived from faeces and dairy cattle. <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> Considering dairy cattle can enter the beef cattle supply, there is a crucial lack of data available on other important zoonotic bacteria e.g. <i>Campylobacter</i> spp. and pathogenic <i>E. coli</i> strains from dairy cattle. Information on AMR among bacteria derived from dairy products is limited to relatively small studies that investigated AMR in <i>Listeria monocytogenes</i> (Wilson et al., 2018) and enterococci (McAuley, 2017; McAuley & Craven, 2005). Information on AMR of foodborne pathogens such as <i>Salmonella</i> and pathogenic <i>E. coli</i> strains, and commensal bacteria derived from dairy farms, food processing environments and retail products is limited.
New Zealand	++	<ul style="list-style-type: none"> A substantial amount of data was identified on clinical isolates of dairy cattle pathogens e.g. <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., <i>Enterococcus</i> spp. and <i>Trueperella pyogenes</i> (Carman & Gardner, 1997; de Boer et al., 2015; Grinberg et al., 2005; McDougall et al., 2014; Petrovski et al., 2015; Petrovski et al., 2011; Salmon et al., 1998; Situmbeko, 2004).

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
		<p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> • Information on AMR of dairy animal commensals is limited to one study that analysed AMR among 209 <i>E. coli</i> isolated from dairy cow uteri (de Boer et al., 2015). • Only one study was identified that tested AMR of important foodborne pathogens (small number of <i>Campylobacter</i> isolates) from cattle faeces (Harrow et al., 2004). • Assuming dairy cattle also enter the beef cattle supply in NZ, there is a lack of AMR data available on foodborne pathogens and sentinel bacteria from dairy cattle. • Information on AMR of foodborne pathogens and sentinel bacteria derived from dairy farms, food processing environments and retail products is not available.

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+ Limited amount of AMR data available

- No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

Table 10. Summary of Pork AMR knowledge status and knowledge gap identification for Australia and New Zealand

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+++	<ul style="list-style-type: none"> Comprehensive AMR data is available covering the production, processing and retail levels. Extensive work has been done to determine AMR prevalence among animal pathogens and relevant human isolates (Abraham et al., 2015; Abraham et al., 2014b; Bettelheim et al., 2003; Dayao et al., 2014; Dayao et al., 2016; Jordan et al., 2009; Reid et al., 2017; Smith et al., 2010; Stephens, 2003; Van Breda et al., 2017). Extensive data are also available on AMR in commensal bacteria isolated from animals, faeces, carcasses, retail products (Barlow & Gobius, 2008; Barton et al., 2003; DAFF, 2007; Fard et al., 2011; Hart et al., 2004; Kidsley et al., 2018; McLellan et al., 2018; Obeng et al., 2012; Smith et al., 2016). Data also exists on AMR profiles and AMG of isolates of the problematic foodborne pathogen <i>Salmonella</i> 1,4,[5],12:i:- (Weaver et al., 2017). The Department of Agriculture and Water Resources national pilot survey for pork AMR was recently completed and reported AMR status of <i>E. coli</i>, <i>Enterococcus</i> and <i>Salmonella</i> isolated from Australian caged and free range layer farm environments.
New Zealand	++	<ul style="list-style-type: none"> Two relatively comprehensive studies on AMR profiles of commensal bacteria <i>E. coli</i> and <i>Enterococcus</i> spp. isolated from carcass and faecal samples were identified (Heffernan et al., 2011; Nulsen et al., 2008). A single study provides very limited data on AMR among <i>Salmonella</i> spp. and <i>Campylobacter</i> spp. derived from pigs (Heffernan et al., 2011). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> Lack of available data on commensal bacteria from retail foods. Data is also lacking on important foodborne pathogens from production, processing and retail levels, although

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
		the prevalence of some species, such as <i>Campylobacter</i> and <i>Salmonella</i> , are low.

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+ Limited amount of AMR data available

- No AMR data available

AMR: Antimicrobial resistance; AMG: Antimicrobial resistance gene screening

Table 11. Summary of Poultry Meat AMR knowledge status and knowledge gap identification for Australia and New Zealand

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+++	<ul style="list-style-type: none"> Two studies were identified that investigated AMR of a relatively large number of clinical isolates (<i>E. coli</i> and <i>Salmonella</i>) (Abraham et al., 2014a; Abraham et al., 2015). Substantial work has been done to determine the extent of AMR among enteric bacteria of chickens present in the gut, and on carcasses and retail products (Barlow & Gobius, 2008; Barton & Wilkins, 2001; DAFF, 2007; Obeng et al., 2013; Obeng et al., 2014). Comprehensive data is also available on AMR among <i>Campylobacter</i> spp. from chickens, their faeces and carcasses, retail products as well as human isolates (Barton & Wilkins, 2001; DAFF, 2007; Lajhar et al., 2015; Miflin et al., 2007; Obeng et al., 2012; Pratt & Korolik, 2005; Wieczorek et al., 2013). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> Comprehensive AMR knowledge of <i>Salmonella</i> spp. from food is lacking. AMR investigations of <i>Salmonella</i> spp. are limited to a 2007-2008 pilot survey of AMR in foods, in which 100 isolates were randomly tested.
New Zealand	++	<ul style="list-style-type: none"> Two comprehensive surveys were commissioned by PIANZ in 2006 and 2014, which looked at AMR profiles in the organisms <i>E. coli</i>, <i>Enterococcus</i> spp. and <i>Campylobacter</i> spp. (Pleydell et al., 2010a; Pleydell et al., 2010b). Additional studies have focused on AMR profiles in commensal organisms <i>E. coli</i> and <i>Enterococcus</i> spp., as well as in <i>Campylobacter</i> spp. and <i>Salmonella</i> (Cook, 2003; Heffernan et al., 2011; Manson et al., 2003; Manson et al., 2004). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> Scarcity of information remains available on <i>Salmonella</i> spp.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
		<ul style="list-style-type: none"> No available AMR data on the animal and foodborne pathogen <i>Clostridium perfringens</i>.

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+ Limited amount of AMR data available

- No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

Table 12. Summary of Eggs AMR knowledge status and knowledge gap identification for Australia and New Zealand.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+	<ul style="list-style-type: none"> Only a single study was identified that investigated AMR profiles of 145 <i>Salmonella</i> (7 serotypes) recovered from product, faeces and environmental samples from commercial layer flocks in NSW and SA (Pande et al., 2015). This study included AMG screening. The Department of Agriculture and Water Resources national pilot survey for egg <i>Salmonella</i> AMR was recently completed and indicated low antimicrobial resistance status of <i>Salmonella</i> isolated from Australian caged and free range layer farm environments. <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> A limited number of studies have focussed on <i>Salmonella</i> AMR, however there is no data available for indicator commensal bacteria <i>E. coli</i> and <i>Enterococcus</i>.
New Zealand	-	<p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> No data was identified

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+ Limited amount of AMR data available

-: No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

Table 13. Summary of Seafood AMR knowledge status and knowledge gap identification for Australia and New Zealand.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+	<ul style="list-style-type: none">Two studies investigated AMR patterns in a relatively large number of aquaculture isolates and isolates from fresh water trout. These constituted pathogens, commensals and environmental isolates (Akinbowale et al., 2007; Akinbowale et al., 2006).A single study assessed AMR of <i>E. coli</i> isolates from native rock oysters (Watkinson et al., 2007).Two studies provide some information on the presence of AMR genes in <i>Pseudomonas</i> spp. and <i>Aeromonas</i> spp. (also environmental species) that were isolated from rainbow trout (Ndi & Barton, 2011b, 2012). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none">Information on AMR of sentinel organisms and foodborne pathogens ranges from very limited to absent from production, processing and retail levels. However, sampling at processing level is impractical.Current limitations also include the absence of clinical breakpoints for aquaculture testing.
New Zealand	-	<p>Knowledge gap/s identified:</p> <ul style="list-style-type: none">No data was identified

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+: Limited amount of AMR data available

-: No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

Table 14. Summary of Horticulture AMR knowledge status and knowledge gap identification for Australia and New Zealand.

Country	Rank of available knowledge	Food AMR knowledge status and gaps identified
Australia	+	<p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> • No data available on AMR among plant pathogens. • Only one study was identified that tested for AMR among horticulture commensals from the family Enterobacteriaceae (Kurtböke et al., 2016). These were isolated from retail-ready strawberries. • Only a single study investigated AMR of 7 <i>E. coli</i> isolates isolated from retail lettuce (Barlow & Gobius, 2008). • There is an absence of data on AMR profiles of commensals and major foodborne pathogens (<i>Salmonella</i> spp., <i>Listeria monocytogenes</i>, <i>E. coli</i> O157:H7, <i>Campylobacter</i> and <i>Bacillus cereus</i>) from production, processing and retail levels.
New Zealand	+	<ul style="list-style-type: none"> • Limited data was found on AMR among plant pathogens e.g. <i>Pseudomonas</i> spp., <i>Xanthomonas</i> spp. and <i>Erwinia amylovora</i> (Colombi et al., 2017; Thomson et al., 1993; Vanneste & Voyle, 2002; Vanneste et al., 2008; Vanneste et al., 2005). <p>Knowledge gap/s identified:</p> <ul style="list-style-type: none"> • A scarcity of data was identified on AMR among <i>Salmonella</i> spp. isolated from fresh produce (Heffernan et al., 2011). • There is an absence of data on AMR profiles of commensals and major foodborne pathogens (<i>Listeria monocytogenes</i>, <i>E. coli</i> O157:H7, <i>Campylobacter</i> spp. and <i>Bacillus cereus</i>) from production, processing and retail levels.

+++ Substantial AMR data available.

++ Moderate level of AMR data available.

+ Limited amount of AMR data available

- No AMR data available

AMR: Antimicrobial resistance

AMG: Antimicrobial resistance gene screening

FUTURE SURVEILLANCE

Recommendations for future globally harmonized surveillance of food AMR in Australia and New Zealand

In support of initiatives for more effective global management of AMR, the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) published the guidance manual *Integrated Surveillance of Antimicrobial Resistance* (AGISAR, 2013). The guidance manual specifically recognises that challenges exist in meeting the objective of having worldwide harmonised AMR surveillance systems, and provides step-by-step approaches for countries designing such programs, using standardised and validated antimicrobial susceptibility testing methods and harmonised interpretive criteria. Importantly, it is recognised that harmonisation does not mean that all country programs should conduct their surveillance activities in exactly the same way. For example, it is acknowledged that local epidemiology and treatment of foodborne diseases, public health resources, laboratory capacity, government policies, production practices, food animal processing, distribution of food products, and pre-existing public health infrastructure can all influence the design of national monitoring programs. The AGISAR Manual (AGISAR, 2013) further illustrates flexible approaches to harmonised AMR surveillance through references to numerous international programs that have been implemented to date. Included among the representative international programs is Australia's Pilot Surveillance Program for Antimicrobial Resistance in Bacteria of Animal Origin reported in 2007 (DAFF, 2007).

Recommendations for globally harmonised active surveillance in Australia and New Zealand

The recent Australian *National Antimicrobial Resistance Strategy 2015-2019*⁴¹ and New Zealand *Antimicrobial Resistance Action Plan* both identify objectives for integrated active surveillance for AMR as part of wider One Health priorities. In the case of Australia, the 2018 publication of recommendations from the WHO Joint External Evaluation of IHR Core Capacities of Australia has also noted the need for prioritisation of '*mechanisms to achieve routine communication, coordination, and collaboration for AMR-related assessment, planning, and response (including outbreaks) across all jurisdictions and sectors (at least animal, human, food, and environment)*' (World Health Organisation, 2018). Relevant to these national objectives, this literature review has identified many variations in the practice of AMR surveillance in different food production, processing and retail sectors within each country and between Australia and New Zealand. It is apparent that the evident knowledge gaps span a spectrum, from absence of knowledge (e.g. AMR in New Zealand

⁴¹ Commonwealth of Australia 2015 *Responding to the threat of Antimicrobial Resistance - Australia's First National Antimicrobial Resistance Strategy 2015-2019*. <https://www.amr.gov.au/resources/national-amr-strategy>

eggs and Australian horticulture) to a mature knowledge position (e.g. AMR in Australian beef production) reported in peer-reviewed international journal publications.

In order to optimally address the knowledge gaps that currently exist in both Australia and New Zealand, support national public health objectives and support Australia and New Zealand's food export industries, recommendations for future action on AMR in food systems have been prepared. The following recommendations are presented in order of the priority considered necessary to deliver the most substantial impact.

Recommendation 1: A senior governance body (e.g. ASTAG) should develop the findings of this report and ensure that food AMR surveillance is included fully within the design and implementation of both Australian and New Zealand national objectives for integrated active surveillance for AMR. Including,

Australian National Antimicrobial Resistance Strategy 2015-2019⁴²

Objective 3. Develop nationally coordinated One Health surveillance of antimicrobial resistance and antimicrobial usage.

Priority Areas for Action

3.1.3 Targeted active surveillance programmes to determine the prevalence of antimicrobial resistance in indicator organisms in animal health and zoonotic organisms in food and the digestive tract of food animals.

3.6 Improve animal health and agriculture surveillance

3.7 Investigate requirements for surveillance in food

New Zealand Antimicrobial Resistance Action Plan

Objective 2. Surveillance and research – Strengthen the knowledge and evidence base about antimicrobial resistance through surveillance and research.

Priority Area for Action

Establish a coordinated national surveillance programme of antimicrobial resistance and antimicrobial use in humans, animals and agriculture.

In the event that full implementation of Recommendation 1 is not practical, the supplementary Recommendations 2-4 below are intended to address the most substantial AMR knowledge gaps associated with specific food industry sectors as identified in this review.

⁴² Commonwealth of Australia 2015. *Responding to the threat of Antimicrobial Resistance* - Australia's First National Antimicrobial Resistance Strategy 2015-2019. <https://www.amr.gov.au/resources/national-amr-strategy>

Recommendations for the implementation of food sector-specific pilot studies appropriate for the provision of harmonised surveillance data

Recommendation 2: As necessary, design and implement targeted pilot surveys for AMR in the specific industry sectors for which very limited or no AMR data are currently available. These food industry sectors are horticulture, eggs and seafood in both Australia and New Zealand.

Available AMR literature and data for Australian red meat (particularly beef), pork and chicken meat are assessed as Substantial (+++). In these Australian food sectors, AMR prevalence data for animal pathogen, sentinel indicator and zoonotic foodborne pathogen bacteria are largely available. In contrast, similar AMR data for New Zealand dairy, red meat, pork and chicken meat sectors, and the Australian dairy sector are considered of moderate completeness in terms of their ability to assess risks to human health, which supports the following:

Recommendation 3: As necessary, design and implement targeted survey approaches to address particular AMR knowledge gaps identified in the Australian dairy sector and New Zealand dairy, red meat, pork and poultry meat sectors.

Recommendations 2 & 3 must involve careful survey design that clearly shows how the sampling strategy (i.e. food types, point of sampling in the food chain) and measured AMR (i.e. target microbial species and AMR phenotypes/genotypes) will support future decision-making. This requires setting clear objectives for each survey and considering how the results will inform risk and mitigation.

In view of the rapid increase in the power of genomic technologies, coupled with the decreased costs of these technologies, we recommend the application of genomic technologies to AMR food safety surveillance.

Recommendation 4: Focus on the development and application of genomic technologies for efficiency gains and precision in food systems AMR surveillance. Consideration is to be given to them having the capacity to be implemented affordably and on a scale that addresses the complexity of the distribution of AMR in the food supply.

FUTURE AMR RESEARCH ACTIVITIES AND GENERAL RECOMMENDATIONS

The following details include:

- i. known current or planned food AMR research activities relevant to any future AMR surveillance activities that may be implemented subsequent to the consideration of this report; and
- i. additional recommendations for AMR research and/or surveillance that may be undertaken as standalone studies or as integrated components of the more extensive recommendations for globally harmonised AMR surveillance provided above.

AUSTRALIA

Red meat

- The Australian beef industry, through Meat and Livestock Australia and Australian Meat Processor Corporation intend to support a repeat study of the 2013 AMR survey using similar methodology and design, with reporting expected in 2020.
- MLA are also currently funding a study into the AMR of *E. coli*, *Salmonella* and *Enterococcus* from healthy sheep at slaughter with reporting to occur in 2019.

Dairy

- The current review has noted the limited availability of AMR data and knowledge concerning animal pathogens, non-bovine dairy animal systems, the dairy farm production environment and dairy food products.
- Studies of pathogens are mostly limited to *Salmonella* and *S. aureus*. Although pasteurisation can eliminate many pathogens, post-pasteurisation contamination can and does occur (e.g. *Listeria* in dairy food products). Bacteria which may be relevant in this regard are include *Listeria*, *Campylobacter*, *Bacillus cereus*, and *Clostridium perfringens*.

Pork

- A new research project funded by Rural Research and Development for Profit (RRDP) (Australian Government Department of Agriculture and Water Resources) with APL and Agrifutures Australia proposes to fully automate the process of isolation, identification, antimicrobial susceptibility testing and genotyping of major AMR surveillance indicator bacteria in pigs and poultry and compare the results with conventional methods ([Bettles, 2017](#)). It is proposed that samples will be submitted directly from slaughter age animals on farm, as part of industry quality assurance and product integrity programmes to reduce overall antimicrobial use which will promote a growing export industry. This will provide ongoing AMR surveillance data in two key intensive livestock industries and may mitigate the need to extend AMR surveillance to retail foods.

- Genes encoding resistance to 3rd generation cephalosporins have recently been identified on Australian pig farms with a history of ceftiofur use. Evidence that isolates containing these genes are likely to be disseminated onto retail pork and pork products and potentially increased drug resistance in humans is lacking. Therefore, precautionary principles should prevail and guide all efforts to reduce their prevalence and numerical abundance on farm. Following the publication of these findings, more industry veterinarians are choosing to voluntarily withdraw the off-label use of ceftiofur from their recommended individualised treatment lists and the RRDP surveillance project should provide further information on public health risk without the need to further test retail products.
- Genotyping studies have now shown that Australian pig farms are not immune to incursions by multidrug-resistant bacteria that have originated offshore. Strict biosecurity and a ban on importation of live animals into Australia precludes these isolates gaining entry through colonised livestock. However, current industry biosecurity practices do not extend to migratory or scavenging wild birds and piggery workers and veterinarians that regularly travel overseas. Preliminary genotyping studies have uncovered evidence that livestock-associated MRSA, fluoroquinolone-resistant commensal *E. coli* and monophasic *Salmonella* 1,4,[5],12:i:- may have entered Australian piggeries via these proposed external sources and may then be amplified via co-selection arising from use of low and medium ASTAG importance antimicrobials and possibly, heavy metals such as zinc and copper ([Abraham et al., 2018](#)). AMR is now recognised by the industry as a biosecurity issue and preventing entry and amplification of MDR organisms in Australian piggeries provides further incentive to reduce selection pressure by finding credible alternatives to current antimicrobial agents recommended for the treatment and control of endemic diseases.
- The recent detection of monophasic *Salmonella* 1,4,[5],12:i:- in Australia corresponding to internationally distributed ST34 (which is strongly associated with pork consumption) is a case in question concerning potential biosecurity incursion into Australian pork production systems ([Arnott et al., 2018](#)). Australian isolates from cases of food poisoning suggest both local (endemic) and overseas (i.e. returned travellers) acquisition of infection, with the small number of isolates resistant to 3rd generation cephalosporins (SHV-12 and CMY-2), colistin (mcr-3) and/or containing plasmid-mediated quinolone resistance genes all sharing close genomic similarity to international clones. A cluster of closely related isolates from humans also contained two isolates from retail pork, and most of these human cases were not associated with overseas travel. It cannot be determined if the pork was contaminated pre- or post-abattoir processing without also comparing *Salmonella* 1,4,[5],12:i:- isolates obtained from piggeries. However, the issues and implications surrounding source attribution from the application of whole genome sequencing as a molecular epidemiology tool need to be thoroughly dialogued with industry. It is recommended that resources should be focused on ensuring co-ordinated national surveillance of AMR in *Salmonella* isolates obtained from food through the current reference laboratories and State Departments of Health.

Poultry meat

- The importance of ensuring reliable systematic and ongoing antimicrobial susceptibility testing on animal isolates needs to be both recognised and supported with sufficient resources. Regrettably, the Australian Salmonella Reference Laboratory in Adelaide has not been testing the antimicrobial susceptibility of *Salmonella* isolates from animal sources since 2014. It is therefore recommended that resources should be focused on ensuring co-ordinated national surveillance of AMR in *Salmonella* isolates obtained from food through the current reference laboratories and State Departments of Health. Because of the widespread nature of backyard chicken production the surveillance programme should consider inclusion of representative premises in order to be able to detect potential novel and emerging resistances, which may arise from contact with wildlife or because of poorly regulated use of antimicrobial agents.

Seafood

- Since its inception, the Australian aquaculture industry has made a concerted effort to reduce antimicrobial use and find alternatives to antimicrobial agents for endemic disease control, such as efficacious vaccines. Current antimicrobial use is restricted to low ASTAG rating drugs. Ongoing surveillance based on susceptibility testing of aquaculture pathogens cultured from diagnostic sample submissions is likely to give practical and expedient measurement of antimicrobial resistance in the industry. The Department of Agriculture and Water Resources is currently funding an AMR surveillance proof-of-concept project focused on Australian aquaculture pathogens due for completion in October 2018.

Horticulture

- There is no information to indicate the direct use of antimicrobials in Australian horticulture for control of microbial plant disease, however the use of animal manure fertiliser and irrigation water from sources shared with food animal industry production may be potential entry points for AMR bacteria and their propagation in horticulture supply chains. Increasing market access opportunities for exported Australian horticulture products have been recently challenged by food safety outbreaks associated with Australian produce. While there has been no reported implication of AMR associated with such foodborne illness, arguably, the availability of AMR knowledge relevant to Australian horticulture production and export may provide additional measures of food safety assurance for market access.

NEW ZEALAND

Under New Zealand's AMR action plan, Objective 2 supports finalising and maintaining a list of priority organisms for surveillance and reporting for human health (Ministry of Health and Ministry for Primary Industries, 2017a). With respect to human health and potential foodborne exposure, the most important AMR bacteria have been identified as (Pullon *et al.*, 2016; Williamson & Heffernan, 2014):

- MRSA;
- ESBL-producing Enterobacteriaceae (particularly ESBL *E. coli* and *Klebsiella pneumoniae*); and
- *Clostridium difficile*, which is intrinsically resistant to many common antimicrobials.

In addition to these, vancomycin-resistant enterococci continue to be isolated from New Zealanders and both *Campylobacter* spp. and *Salmonella* spp. are important foodborne pathogens that should be watched closely for increasing AMR. The emergence of *C. jejuni* ST 6964 demonstrates the potential for resistant strains to appear in the food supply.

A number of ongoing studies in cattle were identified by Burgess and French ([Burgess & French, 2017](#)), examining AMR in the following: *S. aureus* (conducted by Dr Pippa Scott, University of Otago), ESBLs (conducted by Sara Burgess, Massey University), antimicrobial replacements (Prof Greg Cook, University of Otago), and antimicrobials and mastitis (Jane Lacy-Hulbert, DairyNZ).

Activities towards standardising and enhancing AMR surveillance in human health are also being driven under New Zealand's Antimicrobial Resistance Action Plan, Objective 2, as are efforts towards standardising the methodology and reporting of AMR identified in human health laboratories (Ministry of Health and Ministry for Primary Industries, 2017b). In terms of AMR of pathogens isolated from animals and plants, it is intended that international laboratory testing standards are implemented and a national reporting system established.

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APPENDICES

Appendix 1. Additional details of literature search data sources and search strategy.

Key Australian and New Zealand research publications and reports included for Literature Search Phase 1- initial backwards and forwards citation searches.

AU Report:

Shaban RZ, Simon GI, Trott DJ, Turnidge J & Jordan D (2014). Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia. CC BY 3.0. Report to the Department of Agriculture, Griffith University and University of Adelaide, Australia.

AU Paper:

Smith, M., Jordan, D., Chapman, T., Chin, J.-C., Barton, M. D., Do, T., Fahy, V., Fairbrother, J. & Trott, D. J. (2010). Antimicrobial resistance and virulence gene profiles in multi-drug resistant enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhoea. *Veterinary microbiology*, 145, 299-307.

NZ Report:

Heffernan H, Wong TL, Lindsay J, Bowen B and Woodhouse R. (2011). A baseline survey of antimicrobial resistance in bacteria from selected New Zealand foods, 2009-2010. MAF Technical Paper No: 2011/53. Available from <https://www.mpi.govt.nz/processing/agricultural-compounds-and-vet-medicines/antimicrobial-resistance/>

NZ Paper:

Pleydell E, Rogers L, Kwan E and French N. (2010) Evidence for clustering of antibacterial resistance phenotypes of enterococci within integrated poultry companies. *Microbial Ecology* 59(4):678-88.

Literature and Grey Literature Search Strategy for the Identification of Data for AMR in Food in Australia and New Zealand.

Data selection

Consortium researchers, in partnership with CSIRO professional Information Management staff, defined and undertook the appropriate search strategy to identify both published and grey literature. The customised methodology, including all relevant science database search engines that were accessed, literature websites (e.g. national and international surveillance reports), and citation tracking are recorded below.

Grey literature that was not otherwise identified through digital searching was accessed through research and industry networks, including peak industry bodies and relevant working groups and committees concerned with AMR management and mitigation in Australia and New Zealand.

Published and grey literature resulting from research conducted in Australia and New Zealand from 1998 onwards (post JETACAR report) was surveyed and included:

- Published and unpublished reports, theses, surveillance reports, reviews, systematic reviews, proceedings, meta-analyses and risk analysis.
- Scientific expert opinion reports

A hierarchy of quality evidence was implemented with published literature having a greater weighting than unpublished literature. Research of poor quality and design was excluded.

Search strategy:

A search strategy utilizing relevant keywords and phrases was constructed to capture relevant literature on AMR in Australia and New Zealand from 1998 to the current date.

An initial keyword search for AMR in red meat was conducted using the selected databases to validate the search (Appendix 2). The results were analysed to determine the sufficient relevance to the required outcomes and the search will then be re-constructed with the advice of key researchers to achieve the most relevant results set.

Selected Databases:

- Web of Science database platform (with specific emphasis on the FSTA database – Food Science and Technology Abstracts and BIOSIS) The Web of Science is the platform for the Web of Science Core Collection. This Collection is a multidisciplinary index, with searchable author abstracts, covering the journal literature of the sciences (Science Citation Index Expanded), social sciences (Social Sciences Citation Index) and arts and humanities (Arts and Humanities Citation Index).
- Scopus – for its strength in grey literature. Scopus is the largest abstract and citation database of peer-reviewed literature: scientific journals, books and conference proceedings. With more than 57 million journal records and 90,000 books Scopus delivers a comprehensive overview of the world's research output in the fields of science, technology, medicine, social sciences, and arts and humanities. Scopus features smart tools to track, analyse and visualize research
- Livestock Library - is an online or virtual library that provides access to quality information to support Australia's livestock industries. Its target audiences are all sectors of livestock production industries and the general public. When searching the Livestock Library users are concurrently searching the 24,000 documents in the Livestock Library Research Database and an estimated 70,000 documents on selected industry web sites.
- PubMed - is a service of the US National Library of Medicine® that: Provides free access to MEDLINE®, the NLM® database of indexed citations and abstracts to medical, nursing, dental, veterinary, health care, and preclinical sciences journal articles
- ProQuest – ProQuest is a single gateway for access to millions of documents from thousands of sources, covering research and subject areas like these: Health & Medicine, and Science & Technology

- Cochrane Library - The Cochrane Library is a collection of evidence-based medicine databases
- BioOne - BioOne.1 and BioOne.2 provides full-text access to over 160 bioscience research journals published by small societies and non-commercial publishers
- SourceOECD - Provides access to the full text of all monographs, reports, studies, periodicals and statistics produced by the Organisation for Economic Co-operation and Development (OECD).

Limiters applied to the search were:

Limiter: Australia* or "New Zealand*"

Date Limiter: 1998 – 2018

De-duplication: after a search of selected databases and grey literature sources was conducted and results collated, duplicates were assessed utilizing the Find duplicates function of EndNote reference management software.

Grey Literature Databases and Data Sources:

Grey literature was a key resource for the search outcomes. Cited reference searches were conducted on key papers to identify original sources, reports and literature not indexed in mainstream databases. Bibliographies of key reports were also searched to identify further relevant sources not indexed elsewhere.

Relevant websites of organizations were interrogated for relevant reports and other grey literature.

Grey literature and web sources included Australian, New Zealand and International sources. Relevant grey literature sources were identified with the assistance of experts in the field of AMR. International grey literature sources were included to identify where Australian and New Zealand are referred to or compared to International research. Sources of grey literature and web sources were interrogated to identify relevant theses, surveillance reports, reviews, systematic reviews, proceedings, meta-analysis and risk analysis reports. Sources searched included, but were not be restricted to:

- New Zealand Ministry of Health, Royal Society of New Zealand, Australian Department of Agriculture and Water Resources, Australian Department of Health, New Zealand Ministry of Primary Industries, New Zealand College of Public Health Medicine, CIJIG, AIHW, WHO, FAO, OIE, USDA, NTIS
- OpenGrey - A multidisciplinary database with content from a range of European sites with research reports, conference papers, dissertations and other types of grey literature covering science, biomedical science, social science and humanities.
- Global Health Observatory (GHO) data- GHO is a gateway to comprehensive health-related data and statistics from all around the world.
- CORE (COncecting REpositories) CORE provides searchable access to millions of research papers from repositories and Open Access journals.

Results Output and Organisation:

- Relevant reports and papers were identified and saved into EndNote reference management software.
- A password-secured access internet file repository 'ANZ-Food-AMR' was established on the CloudStor facility provided by Australia's Academic and Research Network (AARNet) and accessible by all Australian and New Zealand project personnel.
- All literature records identified through the search strategy were saved in relevant EndNote library files (including pdf publication files where possible) and made available to all project personnel.

SYSTEMATIC LITERATURE REVIEW WORKSHEET FOR BEEF AMR								
Record Identification and Screening	Database searched	Date of Search	Search Terms	Notes	Filters / Limiters applied ²	# of Records retrieved	# of Records excluded after screening ³	# of Records included
	BIBLIOGRAPHIC DATABASES:							
	Web of Science	29/01/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR "Australia")		1998-2018	81		
	Scopus	29/01/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR "Australia")		1998-2018	62		
	PubMed	30/01/2018	((("antimicrobial resistance"[Title/Abstract] OR "antibiotic resistance"[Title/Abstract] OR "antibacterial drug resistance"[Title/Abstract] OR "antibacterial resistance"[Title/Abstract]) AND ("New Zealand" OR "Australia"))		1998-2018	28		
	ProQuest	30/01/2018	((("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND (cattle OR cow* OR beef OR bovine* OR steer* OR calf OR bull* OR veal* OR "bos Taurus")) AND su(("New Zealand" OR Australia*))		1998-2018/excluded blogs and wire feeds/Anywhere except fulltext	91		
	Livestock Library	2/02/2018	Advanced Search - Full text search - ("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR Australia*) and (cattle or cow* or beef or bovine* or steer* or calf or bull* or veal* or "bos Taurus")	Poor Advanced search structure, had to search by keyword phrases and no direct export function to EndNote	1998-2018	51		
	Cochrane Library	2/02/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR Australia*) and (cattle or cow* or beef or bovine* or steer* or calf or bull* or veal* or "bos Taurus")	Most indexed material regards human research	1998-2018	21		
	BioOne	5/02/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR Australia*) and (cattle or cow* or beef or bovine* or steer* or calf or bull* or veal* or "bos Taurus")	Most indexed material regards human research				
	SourceOECD	5/02/2018	All Fields containing 'antimicrobial resistance' Published Between 1900 and 2018 AND Full Text containing 'cow or cattle or veal or beef'	Can only narrow to pre-2003 and 2018, so had to search by whole range and manually filter		112		
Eligibility	GREY LITERATURE DATABASES:							
	Open Grey	5/02/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance")	- search structure does not support refining easily, had to manually filter the result. Mostly European content	1998-2018	2		
	GHO	5/02/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR Australia*) and (cattle or cow* or beef or bovine* or steer* or calf or bull* or veal* or "bos Taurus")			0		
	CORE	5/02/2018	("antimicrobial resistance" OR "antibiotic resistance" OR "antibacterial drug resistance" OR "antibacterial resistance") AND ("New Zealand" OR Australia*)	Poor search structure, no Advanced search, ran separate searches		675	2	
	OTHER SOURCES (Eg. handsearching, personal unpublished sources etc):							
	Websites searched: • New Zealand Ministry of Health, Royal Society of New Zealand, Australian Department of Agriculture and Water Resources, Australian Department of Health, New Zealand Ministry of Primary Industries, New Zealand College of Public Health Medicine, CIJIG, AIHW, WHO, FAO, OIE, USDA, NTIS							
	RECORDS AFTER DUPLICATES REMOVED: ⁴				134 duplicate records removed		217	

Appendix 2. Example output worksheet for initial AMR in beef in Australia and New Zealand search record results.

Appendix 3. Food AMR stakeholder organisations in Australia and New Zealand engaged for AMR grey literature discovery.

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The authors express their gratitude to the many people involved in the Australian and New Zealand food industries who contributed information and advice to this review.

Industry consultation

The following organisations were consulted about this review and asked to provide relevant information or data where available. Consultation was over the phone and/or by e-mail, using the standard introductory letter to provide details.

Australia

Sector	Organisation
Government and Regulatory Authority	Food Standards Australia and New Zealand
	Australian Department of Health
	OzFoodNet
	Australian Department of Agriculture and Water Resources
	Tasmanian Department of Primary Industries, Parks, Water and Environment
Red meat	Meat & Livestock Australia
Dairy	Dairy Australia
Pork	Australian Pork Limited
Poultry meat	AgriFutures Australia
Eggs	Australian Eggs
Horticulture	Horticulture Innovation Australia
	Fresh Produce Safety Centre
Seafood	Fisheries Research and Development Corporation
	Tasmanian Salmonid Growers' Association Ltd (TSGA)
	Future Fisheries Veterinary Service Pty Ltd

New Zealand

Sector	Organisation
Government and Regulatory Authority	New Zealand Ministry for Primary Industries
	New Zealand Ministry of Health
Red meat	Meat Industry Association of New Zealand
	Beef + Lamb New Zealand Inc.
	Beef + Lamb New Zealand Ltd.
	New Zealand Pork
	Deer Industry Association of New Zealand
Dairy	Dairy Companies Association of New Zealand (DCANZ)

Sector	Organisation
	DCANZ member: Fonterra New Zealand
	DCANZ member: Dairy Goat Co-operative
	DCANZ member: Danone
	DCANZ member: Goodman Fielder
	DCANZ member: Miraka
	DCANZ member: Oceana Dairy
	DCANZ member: Open Country Dairy
	DCANZ member: Synlait Milk
	DCANZ member: Tatua Co-operative Dairy Company
	DCANZ member: Westland Milk Products
	DCANZ member: Yashili New Zealand Dairy Company
	DairyNZ
Poultry meat	Poultry Industry Association of New Zealand
Eggs	Egg Producers Federation of New Zealand
Seafood	Seafood New Zealand*
	Safe New Zealand Seafood Programme
Horticulture	Horticulture New Zealand (HortNZ)
	HortNZ product group: New Zealand Apples & Pears
	HortNZ product group: New Zealand Asparagus Council
	HortNZ product group: Blackcurrants New Zealand
	HortNZ product group: Boysenberries New Zealand
	HortNZ product group: New Zealand Buttercup Squash Council
	HortNZ product group: Tomatoes New Zealand
	HortNZ product group: Summerfruit New Zealand
	HortNZ product group: New Zealand Kiwifruit Growers
	HortNZ product group: Onions New Zealand
	HortNZ product group: New Zealand Persimmon Industry Council
	HortNZ product group: Potatoes New Zealand
	HortNZ product group: Strawberry Growers New Zealand
	HortNZ product group: New Zealand Tamarillo Growers Association
	HortNZ product group: New Zealand Avocado
	HortNZ product group: Blueberries New Zealand
	HortNZ product group: New Zealand Citrus Growers
	HortNZ product group: New Zealand Feijoa Growers Association
	HortNZ product group: New Zealand Kiwiberry Growers
	HortNZ product group: New Zealand Passionfruit Growers Association
	HortNZ product group: Processed Vegetables New Zealand
	Zespri

Sector	Organisation
	United Fresh
Other	Massey University
	University of Otago
	Institute of Environmental Science & Research

* Seafood New Zealand consulted with their industry group members on our behalf.