Antimicrobial resistant bacteria in dairy cattle: A review.

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Summary

The development and transmission of bacterial antimicrobial resistance (AMR) is a complex and multifaceted process. One of the main drivers identified for the development and spread of AMR is the use of antimicrobials in human and veterinary medicine as well as for agricultural use. Although agricultural use from high antimicrobial use sectors such as pigs and poultry contribute to the emergence of resistant strains (and transmission via food does occur), current evidence indicates that human use is believed to be the main driver for emergence and persistence of AMR in humans. Despite New Zealand having a low use of antimicrobials in food producing animals compared with other developed countries there is room for improvement. In the dairy sector blanket dry cow therapy (DCT) is being used on some farms; where targeted treatment would suffice. In addition, judicious use of antimicrobials, (particularly those identified as being red tier by the NZVA) is required.

In New Zealand there is no evidence to date that the use of antimicrobials in dairy cattle has resulted in the emergence of pathogens that are multidrug resistant. At present the risk of AMR developing in bacteria carried by dairy cattle and potential transmission to humans is not able to be assessed because of the lack of New Zealand data. However, research carried out overseas suggests that there is the potential for antimicrobial resistance to increase due to the use of antimicrobials in the dairy industry, particularly through the use of third and fourth generation cephalosporins. Although there is limited evidence for the transmission of antimicrobial resistant bacteria and their genes between dairy cattle and humans, it is clear that antimicrobial use can lead to bacteria in the gut of dairy cattle developing AMR.

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Risk areas

Potential risks have been identified as outlined below with and without changes being made to current antimicrobial practices. In order to give a risk rating to these risks more data is required as will be discussed within this review.

Potential risks associated with:

- **No changes to current antimicrobials usage practices**

    1. Damage to national and international reputation
    2. Reduced market access
    3. Development and transmission of antimicrobial resistance

Risks one and two could be mitigated through the targeted use of antimicrobials and by minimisation of the use of antimicrobials identified as 'red tier' by the New Zealand Veterinary Association (NZVA). In order to assess the risk and develop suitable mitigation strategies for the development and transmission of AMR on dairy farms more information is needed as will be described in more detail within the review.

- **Targeted use of antimicrobials**

    1. Reduction in animal health
    2. Reduction in milk production

Risk one could potentially be mitigated through more education as described below.

Recommendations

- **Education**

  To ensure more prudent use of antimicrobials and better prevention and control of disease, more education to farmers and veterinarians is required around:

  - Importance of monitoring herd status and infection control
  - Reduced use of third and fourth generation cephalosporins
  - Correct usage of teat sprays
  - Internal teat sealant administration

- **Surveillance and research**

  As outlined in Section 7 there are a number of areas where more research is required to assess the risk for the development and transmission of antimicrobial resistant bacteria in New Zealand. Priorities include:

  - Prevalence data are required particularly around the incidence of methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae carried by dairy cattle and in-contact humans
  - Assessment of risk factors and transmission pathways
  - Assessment of the role of biocides and disinfectants used on dairy farms, in the selection AMR bacteria.

- **Risk assessment of different AMR scenarios and their impact on the dairy industry**

  Assess the impact on the dairy industry (e.g. on milk production and animal health) as a result of different AMR scenarios.

- **Collaboration**

  There are currently a number of small research projects being carried out by different groups in the area of antimicrobial use and resistance in New Zealand dairy cattle. Greater collaboration is required, along with a more coordinated approach to the funding of research projects and the dissemination of research outcomes to end-users.

  Stakeholders of relevance to antimicrobial use in the dairy industry are listed in Appendix 1.

- **Assessment of economic benefits**

  One of the main barriers towards reducing antimicrobials is the perceived cost to the farmer of implementing other strategies to prevent disease. Cost benefit analyses need to be undertaken to show whether there are economic benefits to the more prudent use of antimicrobials.

- **Improved systems for data capture of antimicrobial use**

  To date the main way in which antibiotic consumption data is captured is through sales records. For those antimicrobial products that do not have a defined use it is difficult to determine how the antimicrobial is being used. It is a requirement for farmers to keep animal treatment records, but these are currently not publicly accessible and are difficult to analyse. It is recommended that this data is captured in a defined and standardised electronic format.

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Table of Contents

List of Figures

List of Tables

Abbreviations

AMR	Antimicrobial resistance
CC	Clonal complex
DCT	Dry cow therapy
ESBL	Extended-spectrum beta lactamase
ITS	Internal teat sealant
MLST	Multi locus sequence typing
MRSA	Methicillin resistant *Staphylococcus aureus*
CA-MRSA	Community associated MRSA
HA-MRSA	Hospital associated MRSA
LA-MRSA	Livestock associated MRSA
NZVA	New Zealand Veterinary Association
TMR	Total mixed ration

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Definitions

Antibiotics	Substances produced by a microorganism (microbial origin) that is generally only active against bacteria.
Antimicrobials	Substances (synthetic or of microbial origin) that destroys or inhibits the growth of microorganisms in general (i.e. bacteria and fungi).
Cephalosporins	A class of β-lactam antibiotics. The first generation of cephalsporins developed, were mainly active against Gram-positive bacteria. Subsequent generations have increased activity against Gram-negative bacteria. Third and Fourth generation cephalosporins are regarded as critically important for human health by the WHO.
Clonal complex	A group of bacterial strains, derived from a common ancestor, which share a similar (but not neccersarily the same) core genotype profile. A clonal complex may contain multiple sequence types.
Horizontal gene transfer	The movement of genetic material from one cell to another (except via cell division from a parent cell to its offspring) via transduction, transformation or conjugation (refer also to Figure 1).
Microbiome	A community of microorganisms found in a particular environment e.g. the human gut.
Mobile genetic element	Pieces of DNA (e.g. transposons or plasmids) that can move within a genome or between bacterial cells
Multi locus sequence typing (MLST)	The DNA sequence comparison of multiple house-keeping genes.
Multidrug resistant	Resistance to three or more classes of antimicrobials.

Mutation	An alteration in the DNA sequence of a gene generally by the insertion, deletion or change in a single base unit.
Sequence type	During the process of MLST each unique sequence is allocated an allele number, the combined allelic profile (from multiple house-keeping genes) is then allocated a sequence type number.
spa type	The *spa* gene, which encodes a specific staphylococcal protein A, contains a variable repeat region, which is used for *spa* typing. Each unique repeat is allocated a *spa* type number.
Zoonotic disease	A disease which can be transmitted from animals to humans.

1 Introduction

Antimicrobial resistance (AMR) is now a global issue that has been described as one of the most important issues for both human and animal health[1]. Infections caused by AMR bacteria are more difficult to treat, often resulting in increased severity of infection and mortality rates.

Multidrug resistant pathogens have long been associated with hospital acquired infections; however, more recently the importance of AMR in community related infections, as a result of clonal spread and horizontal gene transfer, has emerged as a major concern [2]. This is particularly important because community related AMR can easily be transmitted to a wider population. The carriage of multidrug resistant bacteria in livestock could have important implications for the transmission of bacterial AMR to humans in the community. Livestock, including dairy cattle have been identified as a potential reservoir of AMR bacteria [3]. The transmission of AMR bacteria and their genes from livestock to humans could occur directly through occupational/lifestyle exposure or indirectly via other pathways such as the food chain, contaminated waterways or wild animals. In the case of dairy cattle there is still a lack of information on the risk of potential transmission of AMR bacteria and their genes from dairy cattle to humans. The main potential issue of concern is the development of resistance in the population of gut microbes (the 'microbiome') of livestock and then the potential for the transmission of AMR genes from commensal organisms in the gut to pathogens that usually subsist in animals, but that can infect humans, i.e. zoonotic pathogens [4].

The use of antimicrobials in both human and animals has been identified as a main driver of antimicrobial resistance AMR [5]. This can be seen over time; resistance has generally developed not long after the introduction of each new antimicrobial, [6]. Antimicrobials were first used for the treatment of human infection in the 1930s and at the end of World War II were introduced for the treatment and prevention of disease in livestock. In dairy cattle, this resulted in the successful treatment of bacterial infections causing mastitis such as *Streptococcus agalactiae*. This was followed with the use of antimicrobials for disease prevention in animals. For example, antibiotic dry cow therapy (DCT) was developed in the UK in the 1950s and became routinely used during the 1960s as part of the 'five point plan' for

mastitis management [7, 8]. the prophylactic use of antimicrobials was introduced, for the prevention of mastitis, in the 1960s [8]. In New Zealand, the use of prophylactic antimicrobial DCT was not routinely used until the introduction of SCC penalties in 1993/1994 as it was not considered economical [9, 10].

In New Zealand, antimicrobial use in dairy cattle is predominantly used for the treatment and prevention of mastitis; therefore, the main focus of this review is on the mastitis causing pathogens that are of relevance to human health and tools to manage antimicrobial use better for mastitis treatment and prevention.

2 Bacterial pathogens of concern

2.1 Mastitis associated pathogens

Mastitis causing pathogens can be broadly divided into two groups: (1) contagious pathogens, which are transmitted from cow to cow and (2) environmental pathogens, which are isolated from the surrounding environment. The major contagious pathogens in New Zealand are *Streptococcus agalactiae* and *Staphylcoccus aureus*. *Str. agalactiae* is now largely under control and has virtually disappeared in New Zealand [11, 12]. This is a result of good hygienic practice and the ability to treat *Str. agalactiae* more easily compared with *S. aureus*. *Streptococcus dysgalactiae* is unusual in that it is considered both a contagious and environmental pathogen. Other environmental bacterial pathogens include *Str. uberis*, members of the Enterobacteriaceae family (e.g. *Escherichia coli* and *Klebsiella* species, also referred to as coliform mastitis), *Pseudomonas aeruginosa, Bacillus cereus, Arcanobacterium pyogenes, Serratia species, Enterococcus* species and *Nocardia* species. Of these *Str. uberis* is the most common cause of environmental mastitis in New Zealand [13]. The most common cause of coliform mastitis is *E. coli* [14]. Coliform mastitis is more commonly associated with the intensive farming practices used in many parts of the Northern Hemisphere; where the use of feed pads, indoor housing and supplementary feeds is more common practice compared with practices associated with pasture based farming. In recent years there has been an increase in the number of feed pads and animal shelters in New Zealand, which has been identified as a potential risk for the increase of environmental mastitis in New Zealand, particularly coliform mastitis [14]. However, there is limited data on the incidence of coliform mastitis and whether it is associated with more intensified farming practices in New Zealand.

2.2 Clinical relevance to humans

2.2.1 Staphylococcus aureus

S. aureus is commonly found on the skin and in the nose. Some strains of *S. aureus* are pathogenic and can cause skin and soft tissue disease, which in New Zealand is generally caused by methicillin susceptible *S. aureus*. Some strains of *S. aureus* are methicillin resistant (MRSA). In New Zealand MRSA prevalence is estimated to be 8 – 10 % in humans [15]. Methicillin resistance is determined by the presence of a mobile genetic element, found on the chromosome, containing either the *mecA* or *mecC* gene [16, 17].

S. aureus strains are commonly typed based on their multi-locus sequence type (hereafter referred to as sequence type) or their *spa* type (refer to Definitions). Certain sequence types are more commonly associated with livestock and others with humans. However, this barrier appears to be coming increasingly indistinct, showing that those strains isolated from livestock have the potential to be transmitted humans [18].

2.2.2 Enterobacteriaceae

Enterobacteriacae are a group of gram-negative bacteria that form part of the natural flora in the mammalian gut. Some members of the Enterobacteriaceae family, such as *E. coli and Klebsiella pneumoniae* are opportunistic pathogens and can cause both hospital and community related infections [19-21]. It is the development of multidrug resistance in pathogenic strains of Enterobacteriacae that is of particular concern to the health sector as infections caused by these strains are often harder to treat, resulting in increased severity and duration of infection. The development of multidrug resistant community related infection by members of the Enterobacteriaceae family is a relatively new phenomenon and in New Zealand is a common cause of urinary tract infections. These organisms can also be a cause of septicaemia and pneumonia. One mechanism of AMR in members of the Enterobacteriaceae family is due to the production of extended-spectrum β-lactamases (ESBL) [22]. In addition, ESBL producing strains often contain other mechanisms of resistance, causing them to be multidrug resistant, where they are resistant to three or more classes of antimicrobials.

3 Antimicrobial use in dairy cattle

Despite AMR being raised as a global issue it is estimated that by 2030 antimicrobial use will increase by 67% [23]. Globally cattle has the lowest average annual consumption compared with use in the other food animals such as chicken and pigs [23]. New Zealand has a low use of antimicrobials in food animals generally and in the study by Hillerton, et al. [24] was the third lowest of the 30 countries investigated.

In dairy cattle, antimicrobials are used for a variety of reasons including DCT, mastitis, endometritis, metritis and neonatal diarrhoea. A study carried out in the Waikato region indicated that 86% of antimicrobial use for dairy cattle in New Zealand is for DCT and the treatment of mastitis [25] (Table 1); compared with other countries antimicrobial use in New Zealand dairy cows appears to be low [26-31]. However, it is difficult to make comparisons between studies as various methods are used for assessing antimicrobial use.

Table 1. Estimation of average daily usage rates of antimicrobials for selected New Zealand dairy farms

Reason for treatment	Average antibiotic drug use rate (daily doses/cow/year)
Dry cow therapy	0.68[a]
Mastitis	0.67[a]
General	0.32[a]
Endometritis	0.06[a]
Metritis	0.03[a]
Neonatal diarrhoea	< 0.01[b]

[a] Data from [30]
[b] Data from [29]

A variety of antimicrobials are used for the treatment and prevention of disease in dairy cattle as listed in Tables 2, 3 and 4. Many of these antimicrobials have been classed as being “critically important” by the World Health Organisation (WHO) and the OIE (Organisation Mondiale de la Santé Animale) [32], as indicated in Tables 2 and 4 by the acronyms CIA and VCIA respectively. In New Zealand the NZVA has published guidelines for the judicious use of antimicrobials in dairy and has classified antimicrobials as green, yellow or red based on the WHO classes [33]. It is the use of the third and fourth generation cephalosporins (highlighted in grey in Table 2 and 4) that are of particular concern, because third and fourth generation cephalosporins have been attributed to the development of AMR in certain bacteria as will be discussed in Section 4.3 [34]. The use of fluoroquinolones and macrolides have also

been classified as critically important by the WHO and red tier by the NZVA and are also used within the dairy sector. It is largely unknown how fluoroquinolones are used as they do not have a defined use. Tylosin is the major macrolide used; in the dairy sector and is likely to be predominantly used for mastitis prevention

Every 2 – 4 years MPI carries out a sales analysis for antimicrobials that are important to human health and are sold for horticulture or veterinary purposes. In the most recent sales analysis it was found that there was a 55% rise in sales of third and fourth generation cephalosporins, compared with the previous sales analysis report [35]. The reason for this increase is unknown, but was predominantly attributed to ceftiofur use in dairy cattle [35]. The third generation cephalosporin ceftiofur is in a variety of products and is generally used for treating infections other than mastitis, such as metritis. Anecdotal evidence suggests that in some cases third generation cephalosporins are being used, for example for foot-rot, when other antimicrobials such as penicillin would suffice. The fourth generation cephalosporin cefquinome is in the product Cobactan® LC, which has been marketed for the treatment of respiratory disease, foot-rot, acute *E. coli* mastitis mastitis.. Sales of macrolides increased by 25% for veterinary use between the previous two MPI antimicrobial sales analyses, although macrolides are predominantly used in the pig and poultry industries [35]. In 2013/2014 3.5% of the total macrolide sales were attributed to a new tylosin based product, which was registered for cattle use only.

How “critically important” antimicrobials are being used in the New Zealand dairy industry is largely unknown. In the 2009 – 2014 sales analysis 74 – 81% of all DCT sales were penicillin products. In a study by Bryan and Hea [36], which assessed antimicrobial use across four New Zealand regions (Southand/Otago, North Canterbury, Manawatu and Taranaki), it was found that penicillins were the most commonly sold antimicrobial followed by macrolides and cephalsporins. Some penicillins (e.g. ampicillin) are regarded as “critically important” whereas others are “highly important” (e.g. cloxacillin). In a study carried out by McDougall, et al. [37] which analysed antimicrobial use across 80 dairy herds it was found that those antimicrobials classed as “critically important” had an average daily usage rate of 0.05 compared with for 1.06 for those classed as “highly important”. In addition, 20% of the dairy farms analysed were not using antimicrobials classed as “critically important”. However, these data were obtained through two veterinary practices and

are therefore not representative of what is occurring across New Zealand. Differences in antimicrobial use have also been noted between regions as well as herd sizes [36]. However, the reasons for these differences have not been assessed.

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Table 2. Intramammary antimicrobials used in New Zealand cattle

Active antimicrobial combination	Label use	Restrictions	Product(s)	WHO/OIE classification[a]	NZVA classification[b]
Amoxycillin and Clavulanic acid	**Lactating cow:** treatment of mastitis (broad spectrum)		Clavulox L.C.	CIA, VCIA	
Cefquinone	**Lactating cow:** treatment of mastitis caused by *S. aureus, Str. uberis, Str. dysgalacia, E. coli* and Enterobacteriaceae	Recommended for treatment only after culture and susceptibility indicates use	Cobactan LC	CIA, VCIA	
Cefuroxime	**Lactating cow:** treatment of mastitis caused by susceptible organisms		Maxalac L.C.; Spectrazole Milking Cow	HIA, VHIA	
Cephalexin	**Lactating cow:** treatment of clinical mastitis treatment by susceptible organisms		Rilexine LC	HIA, VHIA	
Cephalonium	**Dry cow:** treatment of subclinical mastitis and prevention of new infections during the dry period	Dry cow formulations for use in cows with >30 days until calving (range 30-49 days)	Cefamaster; Cepravin Dry Cow	HIA, VHIA	
Cefapirin	**Dry cow:** treatment of subclinical mastitis and prevention of new infections during the dry period	Dry cow formulations for use in cows with >35 days until calving	Cefa-Safe	HIA, VHIA	
Cloxacillin	**Dry cow:** treatment of most mastitis and decreases new infections during the dry period **Lactating cow:** treatment of mastitis caused by Gram-positive organisms *(Staph & Strep*)	Dry cow formulations for use in cows with >30 days until calving (range 30-35 days)s	Juraclox LA 600 Dry;Orbenin DC; Orbenin Endure; Orbenin LA; Nitroclox LA	HIA, VCIA	
Cloxacillin and Ampicillin	**Dry cow:** treatment and prevention of mastitis **Lactating cow:** treatment of mastitis caused by susceptible organisms	Dry cow formulations for use in cows with >30 days until calving (range 30-49 days)	Bovaclox Dry Cow; Cloxamp DC500; Cloxamp DC600; Dryclox DC; Dryclox Xtra; Lactaclox	CIA (ampillin), VCIA (cloxacillin and ampicillin)	
Lincomycin and Neomycin	**Lactating cow:** treatment of mastitis caused by Gram positive and Gram negative organisms (including *E. coli*)		Lincocin Forte S	CIA, VCIA (neomycin only)	

Oxytetracycline, Oleandomycin and Neomycin[c]	**Lactating cow:** treatment of mastitis caused by susceptible organisms		Mastalone	CIA (oleandomycin and neomycin), VIA (all)	
Penicillin G	**Lactating cow:** treatment of mastitis caused by *Str. Uberis*	Not to be used for treatment of non-susceptible organisms	Intracillin 1000 Milking Cow; Lactapen G	CIA, VCIA	
Penicillin G and Cloxacillin	**Lactating cow:** treatment of mastitis caused by susceptible Gram-positive organisms		Penclox 1200	CIA (penicillin), VCIA (penicillin and cloxacillin)	

[a] CIA: Critically Important Antimicrobial, HIA: Highly Important Antimicrobial, VCIA: Veterinary Critically Important Antimicrobial, VHIA: Veterinary Highly Important Antimicrobial

[b] Refer to the NZVA antibiotic judicious use guidelines [33]

[c] Oxytetracycline is classed as green tier, oleandomycin (which belongs to the macrolide class) as red tier and neomycin (which belongs to the aminoglycoside class) as yellow tier by the NZVA

Table 3. Intrauterine antimicrobials used in New Zealand cattle

Active antimicrobial combination	Label use	Restrictions	Product(s)	WHO/OIE classification	NZVA classification[b]
Cephapirin	**Intrauterine:** treatment of subacute and chronic endometritis		Bomacure; Metri-Clean, Metricure	HIA, VHIA	
Oxytetracycline	**Intrauterine**: treatment or prevention of uterine infections		Oxyfoam Forte	HIA, VCIA	

[a] CIA: Critically Important Antimicrobial, HIA: Highly Important Antimicrobial, VCIA: Veterinary Critically Important Antimicrobial, VHIA: Veterinary Highly Important Antimicrobia

[b] Refer to the NZVA antibiotic judicious use guidelines [33]

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Table 4. Systemic antimicrobials used in New Zealand cattle

Active antimicrobial combination	Label use	Restrictions	Product(s)	WHO/OIE classification	NZVA classification
Amoxycillin	**Injectable:** treatment of Gram positive and some Gram-negative infections sensitive to Amoxycillin, including secondary bacterial infections in viral disease		Betamox LA; Moxylan; Vetrimoxin	CIA, VCIA	
Amoxycillin and Clavulanic acid	**Injectable:** broad-spectrum treatment of infections		Clavulox; Noroclav,	CIA, VCIA	
Ceftiofur	**Injectable (short-acting):** treatment of foot-rot (specified organisms), respiratory disease (specific organisms), metritis, Gram-negative and Gram-positive bacterial disease **Injectable (long-acting):** treatment of foot-rot (specified organisms), respiratory disease (specific organisms), metritis and endometritis in at-risk cows	Not for use in bobby calves; some products have a prudent use recommendation for use only under certain circumstances	Calefur; Excenel RTU; Eficur, Cefaguard; Cefanil; Excede LA; Kelacef	CIA, VCIA	
Cefquinome	**Injectable:** treatment of respiratory disease caused by *Pasturella multocida* and *Manheimia haemolytica*, foot-rot, acute *E. coli* mastitis	Carries a special precaution warning recommending prudent use	Cobactan 2.5%	CIA, VCIA	
Cephalexin	**Injectable:** broad-spectrum treatment of infections		Cephalexin	HIA, VHIA	
Enrofloxacin[c]	**Injectable:** treatment of *Pseudomonas* mastitis, or for the treatment of osteomyelitis	Prudent use recommendation for confirmed indicated disease	Baytril 10%	CIA, VCIA	
Marbofloxacin[c]	**Injectable:** treatment of respiratory and Gram-negative infections	Prudent use warning for only severe and/or resistant infections	Marbocyl 10%	CIA, VCIA	
Oxytetracyline	**Injectable:** broad-spectrum treatment of local and systemic infections susceptible to oxytetracycline		Alamycin 10, Alamycin LA; Alamycin 300; Bivatop 200; Engemycin; Oxytertra LA; Oxytetra MA 10%; Oxytetrin LA; Tetraguard LA	HIA, VCIA	

Penethamate	**Injectable:** treatment of mastitis caused by Gram-positive organisms, respiratory infections, metritis and foot-rot		Mamyzin; Penethaject	CIA, VCIA	
Penicillin (procaine penicillin G, benzathine penicillin, Procaine benzyl penicillin)	**Injectable:** treatment of infections susceptible to penicillin		Bovipen; Depocillin; Duplocillin LA; Intracillin 300; Intracillin L.A.; Masticillin RTU; Norocillin; Norocillin LA; Ovipen; Phoenix pharmacillin 300; Vetguard peng-300	CIA, VCIA	
Tilmicosin[c]	**Injectable:** treatment of respiratory disease cuased by *Pasturella multocida* and *Manheimia haemolytica*		Micotil 300; Tilmovet 300	CIA, VCIA	
Trimethoprim and Sulfonamide (various)	**Oral bolus and injectable formulation:** treatment of various systemic and localised bacterial disease including secondary bacterial infections	Oral bolus not to be used in bobby calves	Amphoprim bolus; Amphoprim; Norodine 24, Tribrissen 48%	HIA, VCIA	
Tulathromycin[c]	**Injectable:** treatment and control of respiratory disease caused by *Pasturella multocida Haemophilus somnus,* and *Manheimia haemolytica*	Prudent use recommendation to monitor response to treatment, and perform culture and susceptibility tests	Draxxin	CIA, VCIA	
Tylosin[c]	**Injectable:** treatment of tylosin susceptible mastitis, infections including mastitis, respiratory infection, metritis, foot-rot		Tylan 200; Tylo 200; Tylofen (with NSAID); Tyloguard; Tylovet	CIA, VCIA	

[a] CIA: Critically Important Antimicrobial, HIA: Highly Important Antimicrobial, VCIA: Veterinary Critically Important Antimicrobial, VHIA: Veterinary Highly Important Antimicrobial

[b] Refer to the NZVA antibiotic judicious use guidelines [33]

[c] Enrofloxacin and marbofloxacin belong to the fluoroquinolone class; tilmicosin, tulathromycin and tylosin belong to the macrolide class of antimicrobials

4 Development and transfer of AMR

4.1 Mechanisms of AMR development

The basis for the development and spread of AMR bacteria in human and animal populations is complex and multifaceted [5]. The use of antimicrobials in human and animal health is a main driver for the development of AMR. However, there are also other factors that can also come into play such as the use of other compounds, for example heavy metals, biocides and disinfectants may select for AMR [38]. In *S. aureus* the plasmid borne *qacA* gene, which can confer resistance to quaternary ammonium compounds and the disinfectant chlorhexidine, may also be found alongside other genes that confer resistance to some antimicrobials [39-41].

Acquired AMR occurs through random mutations in a gene, or by gaining gene(s) through horizontal transfer of DNA (Figure 1). Many of the genes that are associated with AMR are located on plasmids or other genetic mobile elements, making them easily transferable between bacteria. Addition of an antimicrobial to an environment selects for the resistant population. Recurring exposure to an antimicrobial can lead to the clonal spread of the resistant population. In some cases development of AMR comes at a fitness cost to the bacterial cell and if the selection pressure (e.g. the antimicrobial) is removed the bacterial population will revert to a predominantly susceptible population. For example, the discontinued use of cephalosporins as a growth promoter in Danish pigs resulted in a significant decrease in ESBL producing *E. coli* in pigs on-farm [42]. AMR genes are inherently present in the natural environment and have been described as a potential source of novel antimicrobial resistant mechanisms [43-45].

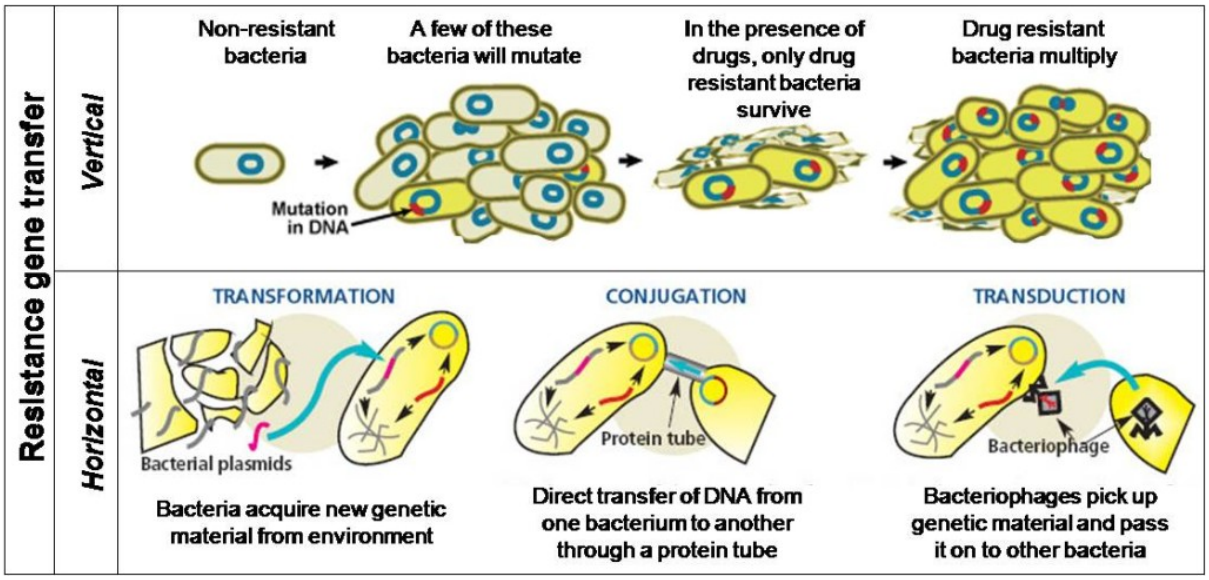

Figure 1. Mechanisms for the acquisition of antimicrobial resistance. The development of AMR can occur via mutations in the chromosomal DNA and subsequent clonal spread (**a**); or through horizontal gene transfer via transduction or through the transfer of plasmids and mobile genetic elements via transformation and conjugation (**b**) (image taken from: https://www.assignmentexpert.com/blog/why-bacteria-are-resistant-to-penicillin/).

The spread of AMR bacteria and their genes to humans and animals is a complex process and could potentially occur via multiple pathways as illustrated in Figure 2. In the case of dairy cattle there is still a large knowledge gap around the potential transmission routes between dairy cattle, humans and the environment. The direct transfer of AMR bacteria or their genes from dairy cattle could potentially occur through direct contact with animals; for example, by farmers or veterinarians, or else through the consumption of dairy products. The transmission of AMR bacteria and their genes between dairy cattle and humans appear to be rare, but there is evidence that it may occur. The remainder of this section will focus on what is known around the development of resistance and transmission of three main groups of AMR bacteria: MRSA, ESBL producing Enterobacteriaceae and Lactic acid bacteria.

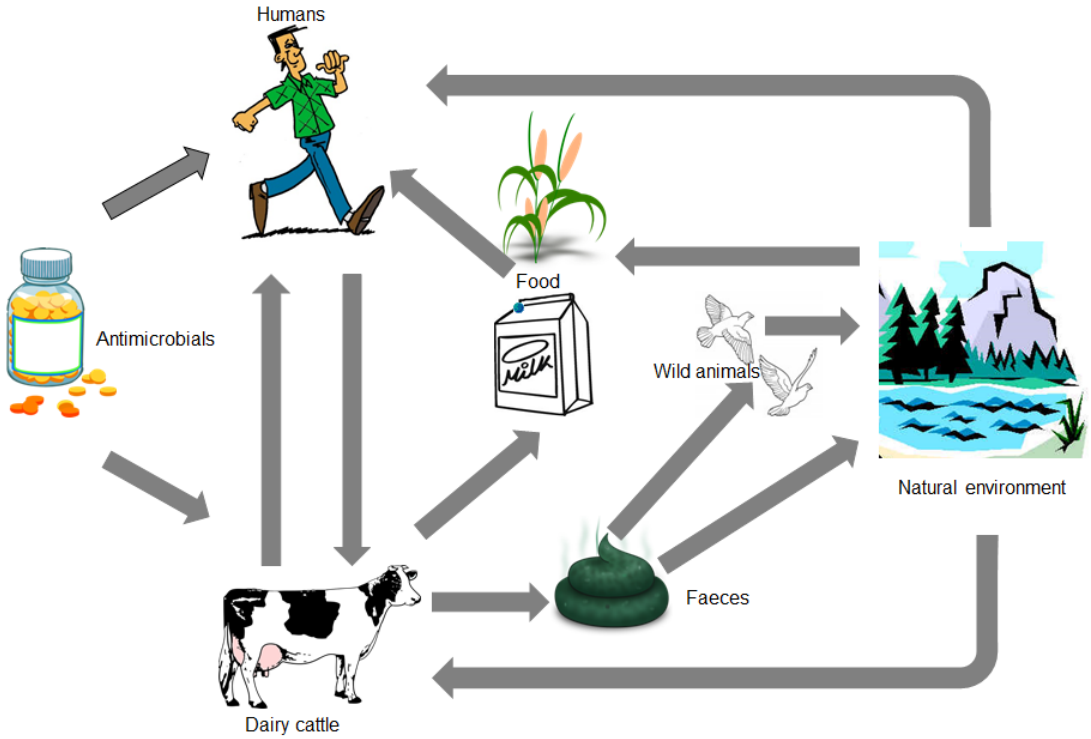

Figure 2. Potential transmission pathways for the spread of AMR (Adapted from Woolhouse and Ward [46]).

4.2 Methicillin resistant *Staphylococcus aureus* (MRSA)

MRSA from hospitals, the community and livestock are generally genetically distinct, and hence the terms hospital associated MRSA (HA-MRSA), community associated (CA-MRSA) and livestock associated (LA-MRSA) [47]. However, recently the line between these different distinctions has become less clear suggesting that transmission has occurred between humans and animals [18]. In Europe LA-MRSA is mainly due to sequence type ST398 and related strains belonging to clonal complex (CC) 398 as well as ST97, whereas ST9 is more commonly found in Asia [48]. The two main LA-MRSA sequence types in Europe: ST398 and ST97 have both been isolated from dairy cattle or bulk milk [49, 50]. Recently, nine human clinical isolates were identified as belonging to CC 398 in New Zealand [51]. To our knowledge LA-MRSA has not been associated with dairy cattle in New Zealand.

The transmission dynamics of MRSA between humans and animals is complex and needs further research. Where transmission events do appear to be occurring overseas, they are predominantly associated with pig farms [52]. In the study by Dahms, et al. [53], 78 farm workers from pig, poultry and dairy farms were tested for

MRSA. Of these MRSA was only found in pig workers. In this study no samples originated from the dairy cattle so it is unknown whether MRSA was actually present on the dairy farms sampled. There is evidence of transmission of MRSA between humans and dairy cattle, but these appear to be rare. In a study by Harrison, et al. [54], whole genome sequencing was used to show that a clinical human case of MRSA (ST130) was linked to an isolate from a cow, where all isolates originated from the same farm. Low resolution genotyping techniques, such as pulse field gel electrophoresis (PFGE), have been used to determine the genetic relatedness of isolates from both humans and dairy cattle isolates off MRSA positive farms. Some of these isolates have the same genotype suggesting transmission may have occurred from animals to humans or vice versa [55, 56].

Despite the high incidence of MRSA carriage in livestock there appear to few cases that lead to clinical MRSA infection [57]. Data analysed from eight European countries attributed 1.7 % of human clinical MRSA isolates to the main LA-MRSA sequence type, ST398 [58]. The carriage of LA-MRSA in humans seldom results in a MRSA infection. Few studies have evaluated the impact of human carriage of LA-MRSA on infection and other aspects of health. A recent study suggested that persistent carriage of MRSA in pig farmers, over a one year period, did not impact health [59]. However, the authors acknowledge that the study is limited, particularly with respect to the lack of a suitable control group, and that more work is required to confirm the findings.

4.3 ESBL producing Enterobacteriaceae

AMR in Enterobacteriaceae can be caused by a number of different mechanisms. This review will focus on one type of resistance mechanism: extended-spectrum β-lactamase (ESBL) production, which can be associated with both human and livestock isolates [22]. It is also recognised by the WHO as being a resistance mechanism of global concern [1].

In the dairy cattle context, studies suggest that there is an association between the use of third and fourth generation cephalosporins (such as ceftifur and cefquinome as highlighted in Tables 2 and 4) and the likelihood of a dairy farm being ESBL positive, but there appears to be no association between overall antimicrobial use and an increased likelihood of being ESBL positive [60-63].

Few studies have assessed the potential for transmission of ESBL producing Enterobacteriaceae and their genes between dairy cattle and humans. In a study by Dahms, et al. [64] samples from livestock, including dairy cattle, as well as humans in contact with livestock, were screened for ESBL producing Enterobacteriaceae. Of the 24 human samples from cattle farms, three tested positive for ESBL producing Enterbacteriaceae, with only one of these isolates showing the same MLST sequence type as an isolate originating from a dairy cattle faecal sample. The authors suggest this may indicate zoonotic transmission.

There are multiple factors that may play a role in transmission of ESBL producing Enterobaciaceae and their genes in the dairy farm environment as shown in Figure 3. Horigan, et al. [65] have suggested that further data are needed in order to assess the risk of transmission, between and within humans, dairy cattle and their environment in the UK, particularly concerning the prevalence of ESBL producing Enterobacteriaceae in dairy cattle, bulk milk, dairy products, and the dairy farm environment. This lack of data is even more apparent in New Zealand, where there is no information on the prevalence of ESBL producing Enterobacteriace in dairy cattle, bulk milk, dairy products, or the dairy farm environment.

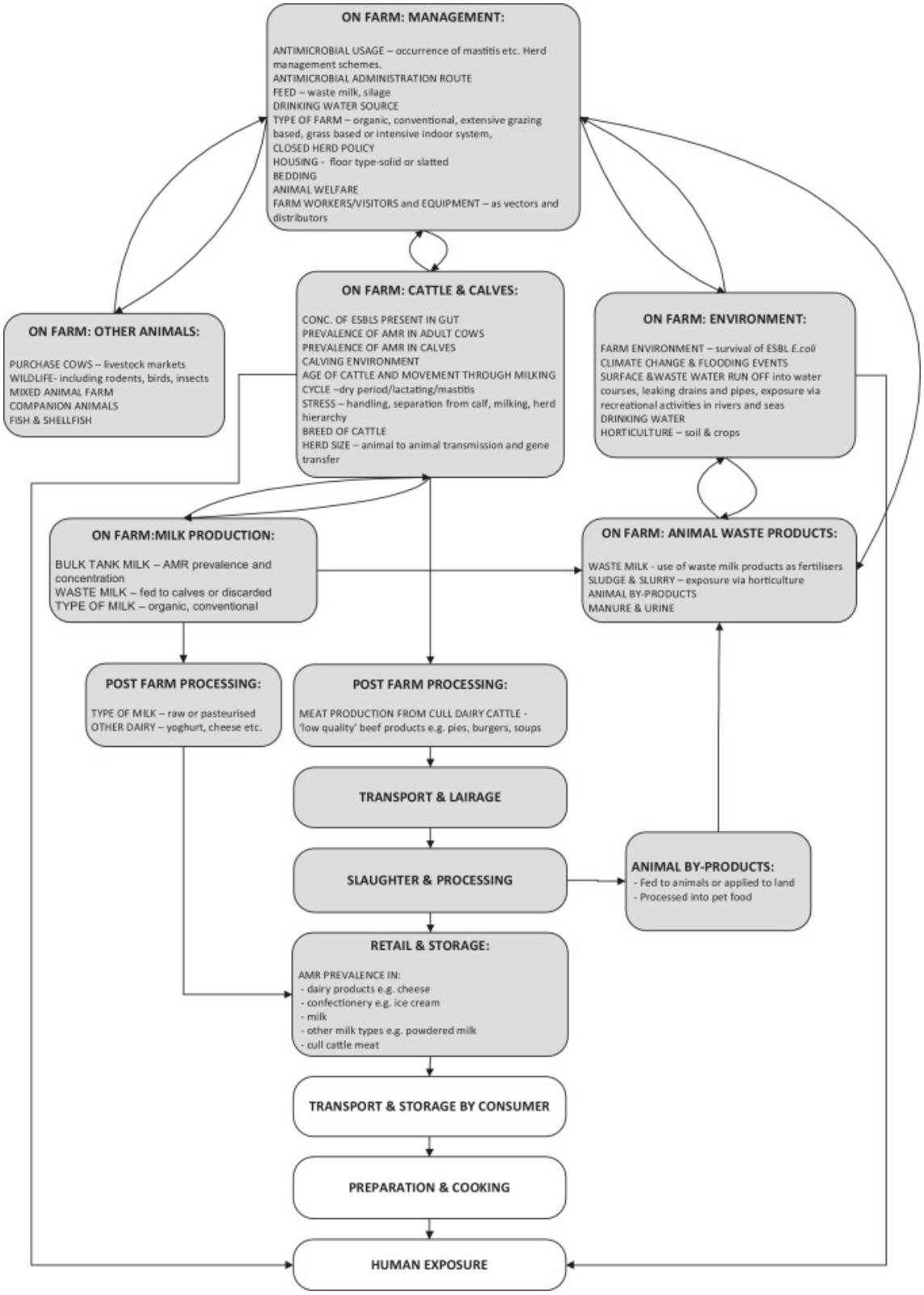

Figure 3. Factors that may be involved with the development and transmission of antimicrobial resistance on a dairy farm (taken from Horigan, et al. [65]).

4.4 Development of AMR in Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) are fundamental to making many dairy products. Of recent interest is whether LAB may harbour AMR genes that can be passed on to opportunistic pathogens in the gut. Some LAB appear to naturally have a higher antimicrobial resistance profile towards many antimicrobials, for example, vancomycin [66]. However, more recent reports suggest that some strains have acquired additional antimicrobial resistance mechanisms [67, 68]. The presence of acquired resistance genes has been identified as a potential health risk and it is recommended that all dairy starter strains be screened for antimicrobial resistance [69].

5 Prevalence of AMR in dairy cattle and milk

Studies of the occurrence of AMR mastitis causing pathogens in New Zealand bovine milk have focussed on the major mastitis causing pathogens *S. aureus*, *Str. uberis*, and *Str. dysgalactiae*, with strains of these species generally being susceptible *in-vitro* to antimicrobials used for the treatment of mastitis [70]. *In-vivo*, *S. aureus* is more difficult to treat compared with *Str. uberis* and *Str dysgalactiae* [71]. However, there are probably other factors that come into play such as cow health, ability to form biofilms, internalization into host cells and host-pathogen interactions [72]. To our knowledge there appears to be no evidence for an increase in AMR of mastitis associated isolates of *Streptococcus* spp. and *S. aureus* [72, 73]. However there is a limited amount of information on whether the use of antimicrobials in dairy cattle has selected for the carriage of AMR bacteria, for example in the nose for MRSA or the gut for ESBL producing Enterobacteriaceae. Given the lack of data in New Zealand on the prevalence of MRSA and ESBL producing Enterobacteriaceae the remainder of this section has mainly used data from overseas.

5.1 Methicillin resistant *Staphylococcus aureus* (MRSA)

MRSA in New Zealand cow's milk, appears to be very rare or non-existent [74, 75]. To our knowledge only one isolate originating from cow's milk has been classified as being MRSA; however, the clinical importance of this isolate was uncertain as it was derived from a mixed susceptible/resistant culture [74]. Overseas, MRSA has been isolated from dairy cattle (milk as well as from nasal and skin swabs); however, the prevalence of MRSA in dairy cattle is low compared with other livestock such as veal calves and pigs (Table 5). In many cases where MRSA has been isolated from dairy cattle the animals are located on mixed livestock farms, including pigs.

Table 5. Prevalence of MRSA in dairy cattle, cow milk, in contact humans and other livestock.

Sample type	Location	Prevalence of MRSA	Reference
Healthy cows – nasal swab	Belgium	9.9% (14/141 farms[c])	Nemeghaire, et al. [76]
Healthy cows – skin swab after bleeding	Netherlands	3.9 % (16/411 healthy dairy cattle)	van Duijkeren, et al. [77]
Cows[a,b] – nasal swab from MRSA positive farms	Netherlands	12.5 % (4/32 cows)	Feßler, et al. [55]
Cows – milk	Sweden	0.55 % (4/730 *S. aureus* isolates)	Unnerstad, et al. [78]
Cows[a,b] – milk from MRSA positive farms	Netherlands	3.4 % (62/1839 cows)	Feßler, et al. [55]
Cows[a, b] – milk from MRSA positive farms	Germany	12 % (19/160 cows)	Spohr, et al. [79]
Cows – milk from bovine mastitis cases	Belgium	4.4 % (19/430 *S. aureus* isolates)	Bardiau, et al. [80]
Bulk milk	Germany	4.1 % (14/388 milk samples)	Tenhagen, et al. [81]
Bulk milk	Minesota, USA	1.3 % (2/150 milk samples)	Haran, et al. [82]
Bulk milk	Korea	10.6 % (5/47 farms)	Song, et al. [83]
Milk derived from subclinical or clinical mastitis cases	Belgian	9.3 % (11/118 *S. aureus* isolates, originating from farms with subclinical or clinical mastitis cases)	Vanderhaeghen, et al. [84]
Raw milk	Shaanxi, China	1.4 %	Wang, et al. [85]
Raw milk from retail stores	Iran	2.0 % (21/1035 raw milk samples)	Jamali, et al. [86]
Farmers in contact with cows[a]	Netherlands	50 % (6/12 farms)	Feßler, et al. [55]
Dairy veterinarians[a]	British Cattle Veterinarian Association Congress	2.6 % (8/307 veterinarians)	Paterson, et al. [87]
Veal calves[a]	Belgium	46.1 % (48/104 farms[c])	Nemeghaire, et al. [76]
Veal calves	Germany	39.9 % (267/670 calves)	Tenhagen, et al. [81]
Veal calves	Netherlands	28 % (602/2151 calves)	Graveland, et al. [88]
Pigs	Canada	24.9 % (71/285 pigs)	Khanna, et al. [89]
Pig farms	Canada	45 % (9/20 farms)	Khanna, et al. [89]
Pig farms	Netherlands	56 % (28/50 farms[d])	Van Den Broek, et al. [57]

Pig farmers	Germany	55 % (20/36 farmers)	Dahms, et al. [53]
Pig farmers	Netherlands	30 % (15/50 farmers)	Van Den Broek, et al. [57]
Pig farmers	Canada	20 % (5/25 farmers)	Khanna, et al. [89]

[a] farms had previously been identified as MRSA positive
[b] Some of the farms analysed also had pigs
[b] Surface swabs were collected after bleeding
[c] Based on nasal swabs from 20 calves per farm were pooled together and tested for MRSA.
[d] Based on nasal swabs from pigs and dust samples.

5.2 ESBL producing Enterobacteriaeceae

The prevalence of ESBL producing Enterobacteriaceae in New Zealand dairy cattle is unknown. To our knowledge only two studies has been carried out on the prevalence of AMR *E. coli* in New Zealand livestock. Heffernan, et al. [90] evaluated whether *E. coli* isolates obtained from calves, pigs and poultry were AMR; none of the isolates were found to be ESBL producers. In the study by Pleydell, et al. [91] the level of AMR Gram negative bacteria on chicken carcasses were assessed and it was found that none of the *E. coli* isolates tested were ESBL producers. Overseas both the herd prevalence and incidence of ESBL producing Enterobacteriaceae in dairy cattle varies greatly between studies (Table 6). However, it is difficult to compare studies as the selection criteria of farms and samples as well as the methods used for determining the presence of ESBL producing Enterobacteriaceae differs between studies. The prevalence of ESBL producing Enterobacteriaceae from farms with lactating cattle is significantly higher than those with other adult cattle [92, 93].

Table 6. Prevalence of ESBL producing Enterobacteriaceae in dairy cattle and milk.

Sample type	Country	Prevalence	Reference
Cow slurry samples	Netherlands	41.0 % (41/100 herds positive for ESBL producing *E. coli*)	Gonggrijp, et al. [60]
Ground fecal samples from	United Kingdom	35.4 % (17/48 farms positive[a] for ESBL producing *E. coli*)	Snow, et al. [62]
Fecal, dust and boot swab samples	Germany	93.3 % (28/30 farms positive[a] for ESBL producing *E. coli*), 41.1 % (37/90) cow fecal samples positive for ESBL producing *E. coli*	Schmid, et al. [93]
Fecal samples from cattle[b]	Switzerland	1.6 % (1/61 cattle fecal samples positive for ESBL producing Enterobacteriaceae)	Geser, et al. [94]
Fecal samples from dairy cattle	Japan	5.2 % (20/381 dairy farms positive for ESBL producing Enterobacteriaceae)	Ohnishi, et al. [95]
Fecal samples[c] from milk producing cows	United Kingdom	30.0 % (200/660 fecal samples positive for ESBL producing Enterobacteriaceae).	Watson, et al. [92]
Fecal samples from calves	Switzerland	13.7 % (17/124 calf fecal samples positive for ESBL producing Enterobacteriaceae)	Geser, et al. [94]
Bulk tank milk samples	Switzerland	0 % (0/100 bulk milk samples ESBL producing Enterobacteriaceae)	Geser, et al. [94]
E. coli isolates from mastitis milk	Switzerland	1.5 % (1/67 of *E. coli* isolates were ESBL producers)	Geser, et al. [94]
E. coli isolates from mastitis milk	France	0.4 % (6/1427 of *E. coli* and *K. pneumoniae* isolates were ESBL producers)	Dahmen, et al. [96]
Fecal samples from milk producing cows	United Kingdom	45.3 % (72/159 fecal samples CTX-M positive *E. coli*)	Randall, et al. [97]
Waste milk	United Kingdom	66 % (6/9 waste milk samples from three farms previously found to be positive for ESBL producing *E. coli*).	Randall, et al. [97]

[a] based on one sample from a farm testing positive
[b] included dairy and beef cattle
[c] samples were taken from one farm that had previously been found to be an ESBL positive farm

6 Mastitis management

The most effective way of preventing mastitis is through effective farm management as outlined in SmartSAMM (Smart Approach to Minimising Mastitis). SmartSAMM were originally based on the five point plan outlined by the National Institute for Research into Dairying (NIRD): (1) Treat and document clinical cases; (2) Disinfection of teats after milking; (3) Dry cow antimicrobial therapy (4) Cull established infective cases (5) Maintenance of milking equipment [10]. In 2011 the SAMM plan was extended, because of an increase in somatic cell counts (SCC), and renamed SmartSAMM [10]. This extended SAMM plan is more flexible; it recognises that each farm is different and therefore may require tailored strategies to minimise mastitis. In New Zealand there appears to be a general consensus that the most appropriate way to ensure more prudent use of antimicrobials and prevention of disease is through good management practice as opposed to some of the alternatives that are available, as will be described in Section 6.2.

Table 7 summarises some of the good management practices recommended by DairyNZ for the prevention of new mastitis infections. A key good management practice is the correct use of teat spray. Correct teat spray usage requires the teat spray to be evenly sprayed around the entire teat and for it to be made up to the required concentration. It has been shown that repeated exposure to suboptimal concentrations of chlorhexidine (which is used in teat sprays) can result in an increased resistance to chlorhexidine and may also contribute to the development of resistance to some antimicrobials [39, 98, 99].

The key to ensuring farmers follow good practice is through education. This is particularly important for more intensive farming where there is a higher risk of teats becoming infected with environmental microorganisms such as coliforms. In the case of more intensive farming units, washing, drying as well as the use of teat spray is recommended for preventing coliform mastitis [14]. Another important risk factor for coliform mastitis is feed. In a study by Lacy-Hulbert, et al. [100], cows were feed total mixed ration (TMR) or pasture over three lactation seasons and monitored for the incidence of mastitis; it was found by

season two that those cows feed TMR had a significantly higher incidence of coliform mastitis.

Table 7. Farm management practices important for the control of mastitis (adapted from DairyNZ guidelines http://www.dairynz.co.nz/animal/mastitis/)

Season	Component	Importance	Previous work
Calving	Environment	The environment is an important reservoir of environmental mastitis causing pathogens that can be transferred to the cow during calving.	Gomes and Henriques [101], Lopez-Benavides, et al. [102]
	Heifer welfare	Heifers generally have a higher incidence of mastitis compared with older cattle; therefore steps should be taken to ensure their welfare	De Vliegher, et al. [103], McDougall, et al. [13]
Lactation	Maintenance of milking equipment	Milking machines that do not function correctly can cause: damage to teats, spread of bacteria, and ineffective milking of the cow	Dufour, et al. [104]
	Teat disinfection	Teat spray reduces the incidence of new mastitis infections	Williamson and Lacy-Hulbert [105]
	Milking hygiene	Poor hygiene can result in spread of bacteria	Hovinen and Pyörälä [106], Dufour, et al. [104]
	Udder health	Damaged teats are more difficult to clean and prone to infection	Hovinen and Pyörälä [106]
	Environment	Pathogens can be picked up from muddy tracks and racers leading to the milking shed.	Lopez-Benavides, et al. [102]
	Treating and documenting clinical cases	Treating and documentation enables more rapid detection of cases	
	Monitoring SCC	Monitoring bulk SCC is used as an indirect indicator of subclinical mastitis. It is recommended by SmartSAMM that individual cow SCC are carried out twice a month over the lactation season in order to monitor herd health.	Harmon [107], Reneau [108]
Drying off	Appropriate strategy	DairyNZ SmartSAMM Technote 14 provides information on selecting the appropriate strategy for drying off (antimicrobial treatment versus ITS versus no treatment).	McDougall and Compton [109]
	Cull	Cows with reoccurring infections and/or consistently high SCC should be culled	
Drying period	Regular checks	It is recommended by SmartSAMM that cows are checked weekly. To do this some farmers may need additional facilities in the area, in order to bring the cows in for checking.	

6.1 Internal teat sealants

The most recognised alternative to antimicrobial DCT is the use of internal teat sealants (ITS). These have been shown to be effective at preventing new mastitis infections from environmental pathogens over the dry period [8, 110, 111]. Teat sealants are currently available with and without antimicrobials. However, those without antimicrobials have been shown to be as effective at preventing new mastitis infections from environmental pathogens over the dry period for those farms with low SCC. ITS is made from bismuth sub-nitrate, which prevents bacteria from moving up the teat canal into the udder. In the past, bismuth sub-nitrate was a cause of black spot in cheese [112]. This problem has been resolved by the installation of clarifiers into milk processing plants. Research is ongoing into alternative substances to bismuth sub-nitrate, but at present no other options are available on the market.

Several challenges prevent ITS from being more widely used. The insertion of ITS and regular monitoring of cows afterwards increases the workload. There is also a risk of infection if ITS is not administered correctly, under strict hygienic conditions. This risk is low if the procedure is carried out correctly [71]. At present it is generally veterinarians who are administering ITS to heifers. DairyNZ is currently looking at the practicalities of being able to use more ITS across New Zealand dairy farms as at present there are not enough trained administers of ITS to increase the use of teat sealant across New Zealand. As part of the DairyNZ project[1]: “prudent use of antimicrobials for mastitis – pilot study dry cow mastitis” further studies will be carried out to compare the efficacy of ITS with and without antimicrobials. It has been estimated that the use of ITS could decrease antimicrobial use by 50 % in the dairy industry [71].

[1] Refer to https://www.dairynz.co.nz/about-us/investment/summaries-and-reports/prudent-use-of-antimicrobials-for-mastitis-pilot-study-dry-cow-mastitis-rd1442/.

6.2 Antimicrobial alternatives

6.2.1 Vaccines

Vaccines are commonly used in countries such as the UK and USA where more intensive farming is used and mastitis rates are higher than those in New Zealand. In New Zealand the vaccine Startvac[2] is available; this is a combination vaccine for *E. coli*, *S. aureus* and coagulase negative staphylococci. However, given approximately 75 % of mastitis cases in New Zealand are caused by *S. uberis*, these vaccines have a limited effect on mastitis. The *E. coli* component of this vaccine is a J5 product, which has been shown to reduce the severity of *E. coli* mastitis but not the incidence. Companies in the UK and USA have been trying to develop a vaccine for *S. uberis* since the early 1990s without success. This is in part probably because *S. uberis* represented by multiple strains making it difficult to find a target.

6.2.2 Phage therapy

Bacteriophages have been successfully used to lyse and control the growth of mastitis causing isolates of *S. aureus and E. coli* [113-116]. To our knowledge there is only one published study that has analysed the effectiveness of bacteriophage for the treatment of *S. aureus* bovine mastitis, with mixed results [117]. Several reasons have been suggested for the inability to develop an effective bacteriophage treatment including inactivation of the bacteriophage by milk components or the immune success as well difficulties in the bacteriophage accessing the bacterial cells within biofilms and host cells [101]. To date phage therapy has not been successful for use for mastitis causing *Streptococcus uberis* (personal communication Craig Billington, ESR, 2016). This appears to be in part because *S. uberis* cells clump together rendering cells inaccessible to the phage. To our knowledge there are no commercially available phage therapy cocktails for the treatment of mastitis.

6.2.3 Research into additional alternatives

Other alternatives such as nanoparticles, probiotics, cytokines and plant derived natural compounds have been tested all with little success [101, 118-124]. For information on these alternatives the reader is referred to the review by Gomes and Henriques [101].

[2] Refer to http://www.agilis.nz/products/startvac/.

6.3 Targeted use of antimicrobials

There are mixed reports on the benefits of reducing prophylactic antimicrobial DCT. There is general agreement that all dairy cattle have the potential to develop new infections over the dry period. Many studies have shown (both overseas and in New Zealand) that prophylactic antimicrobial treatment of dairy cattle over the dry period reduces the incidence of new infections (particularly those caused by *Streptococcus* spp.) in the subsequent lactation season [125-129]. However, with the introduction of ITS in the early 2000s and a reduction in the prevalence of transmissible mastitis causing pathogens such as *Str. agalactiae* there is a reduced need for prophylactic antimicrobial treatment of dairy cattle [130]. There is now beginning to be a shift in thinking as to how antimicrobials are being used on dairy farms and therefore a move towards targeted use of antimicrobials. For example, in the UK the British Cattle Veterinary Association is recommending that ITS be used for DCT where antimicrobials are not needed and that prophylactic antimicrobial treatment is not used [131]. In New Zealand, Purata dairy farms have led the way with the development of an antimicrobial stewardship plan in 2014 with the result that the number of antimicrobial doses have halved [132, 133]. This aligns with the NZVA objective: “By 2030 New Zealand will not need antibiotics for the maintenance of animal health and wellness”.

For targeted use of antimicrobials, the question still arises which cattle should be treated with antimicrobials? At present subclinical mastitis cannot be determined based on the visual appearance of the teats. Biggs, et al. [130] provide some suggestions on how to identify Individual cows that may need antimicrobial DCT, including using a combination of individual cow SCC, herd history and bacterial detection methods.

Bacterial test kits are available for on-farm use but these are expensive and time-consuming. The Rapid Mastitis test (also known as the California Mastitis Test) gives an indication of somatic cell count and therefore the presence of subclinical mastitis. It uses a four-well (one for each quarter) plastic paddle for mixing milk with the Rapid Mastitis Test reagent, where somatic cells are lysed and react with the RMT reagent to form a gel. The Check-Up kit is used to give an indication of the type of pathogen present, and therefore enables decisions around choice of antimicrobial to administer. It requires the purchase of an incubator, and includes a manual and

poster to determine the most common pathogens. The milk sample is streaked onto the Check-Up test agar plate, incubated for 24 hours and then compared with the reference manual.

7 Research needs

- **Simplification of on-farm diagnostic tools**

Simplified on-farm diagnostic tools for identifying subclinical mastitis would enable more informed decision making around farm management practices. However, there is no simple answer to the development of rapid simple diagnostic tools. Research into this area for a variety of applications has been ongoing for many years and as yet there has been no 'silver bullet' answer to developing microbiological diagnostic tools that are rapid, inexpensive, easy to use and provide results similar to traditional culture methods.

- **Surveillance and antimicrobial usage data**

At present there is very little data on the incidence of AMR pathogens causing disease in New Zealand cattle; perhaps more importantly, there is also insufficient data on the carriage of commensal AMR bacteria. These data are not only important for dairy cattle, but also for other reservoirs that may come into contact with dairy cattle such as humans, wild animals, and the natural environment. On-farm use of antimicrobials is also insufficiently monitored in this country, and without surveillance or antimicrobial usage data it is difficult to assess whether the development and transmission of AMR is an issue in New Zealand. At present MPI reports on total veterinary and agricultural antimicrobial sales, which are of importance to human medicine [35]. However these reports do not provide information on how antimicrobials are used unless they have a specific use. Mark Bryan (VetSouth Ltd, Winton) and Scott McDougall (Cognosco, AnexFVC, Morrinsville) have been carrying out surveillance work on antimicrobial use in dairy cattle [30, 36, 134]. However, these studies are also based on antimicrobial sales in certain New Zealand regions and may not be representative of actual usage across New Zealand.

- **Assessment of risk factors and transmission pathways**

Risk factor analysis around transmission pathways is a key step to improving understanding AMR bacteria on New Zealand farms. To assess potential transmission pathways high resolution genotypic data (preferably from whole genome sequencing) of AMR isolates from various sources would be required.

- **Are biocides and disinfectants drivers of AMR?**

There is the potential for chemicals other than antimicrobials to drive AMR. At present there is work being carried out looking at Chlorhexidine as a driver of AMR. There may be other chemicals that need to be assessed, such as biocides being used on-farm and in dairy manufacturing that could potentially drive AMR.

- **Fundamental research**

The development of AMR is a complex process and there are still many questions around how AMR develops and remains in an environment. For example, in the dairy context there is a lack of information on the role of LAB in spreading AMR and whether genes from AMR bacteria naturally present in milk could be a reservoir of AMR genes.

8 Current research projects

Table 8. Current AMR projects associated with dairy cattle in New Zealand

Project	Lead investigator	Institution
Transmission of *S. aureus* in the rural community	Dr Pippa Scott	Department of Pathology, University of Otago, Christchurch
The carriage of ESBL producing Enterobacteriacae in ruminant livestock	Dr Sara Burgess	^{m}EpiLab, Institute of Veterinary and Biomedical Sciences, Massey University
Development of new disinfectants for agriculture (including replacements for chlorhexidine)	Prof Greg Cook	Department of Microbiology & Immunology, University of Otago
Prudent use of antimicrobials for mastitis	Dr Jane Lacy-Hulbert	DairyNZ, Waikato

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Appendix 1: List of stakeholders

The following lists stakeholders relevant to antimicrobial use in the New Zealand dairy industry:

- Government Ministries: Primary Industries; Environment, Foreign Affairs and Trade; Business Innovation and Employment.
- Dairy NZ
- Dairy Companies Association of New Zealand (DCANZ)
- Other individual dairy companies
- Meat Industry Association (MIA)
- Beef+Lamb New Zealand
- Federated Farmers of NZ
- NZ Veterinarian Association
- Agcarm

Refer also to the NZVA AMR framework [135].