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Reflection paper on the use of macrolides, lincosamides and streptogramins (MLS) in animals in the European Union: development of resistance and impact on public and animal health

Draft

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Executive summary

In 2011, the European Medicines Agency (EMA) has published a reflection paper on the use of macrolides, lincosamides and streptogramins (MLS) in food-producing animals in the European Union (EU). Macrolides and lincosamides are widely authorised and used in food-producing and companion animals, as well as in human medicine, where they play a critical role in the treatment of bacterial infections, particularly in respiratory and soft tissue indications. Streptogramins are not currently authorised for veterinary use in the EU. However, owing to their shared sites of action and resistance mechanisms, these classes were reviewed alongside. More than a decade has now elapsed, and the CVMP considers it relevant to review the status of these classes, and to update the information to the state of the art.

In 2021, a concept paper was published proposing this review, focusing on the different classification status of macrolides by the Antimicrobial Advice ad hoc Expert Group (AMEG), which has categorised them as belonging to AMEG Category C, and the classification assigned by the World Health Organization (WHO) as High Priority Critically Important Antimicrobial (HP-CIA) (EMA/CVMP/AWP, 2021). Although this discrepancy has been mitigated in 2024, with the publication of the WHO list of medically important antimicrobials for human medicine, where macrolides were re-classified as Critically Important Antimicrobials (CIA) and considering the evidence provided by recent consumption data, which places macrolides within the top three of most-used classes both in human (in the community) and veterinary medicine (in food-producing animals), this revision was nevertheless considered pertinent and needed. Therefore, considering the need of interconnecting the different sectors linked with the risk of development of antimicrobial resistance (AMR), the CVMP's Antimicrobial Working Party (AWP), developed this reflection paper in close collaboration with the Committee for Medicinal Products for Human Use (CHMP)'s Infectious Diseases Working Party (IDWP), and the CVMP's Environmental Risk Assessment Working Party (ERAWP) and Safety Working Party (SWP-V), in a 'One Health' approach. Where needed, other relevant stakeholders were also contacted to provide input on the data included in the paper.

This reflection paper provides a comprehensive review of the use of MLS within the EU, examining their pharmacological properties, patterns of use, the development of resistance, and implications for both public and animal health. This reflection paper also provides an environmental perspective on the role of MLS residues and resistant determinants emitted into the environment by treated animals.

The document highlights the critical role of MLS antibiotics in treating respiratory, gastrointestinal, and musculoskeletal infections in livestock, particularly swine, cattle, sheep, and poultry species. However, their extensive use has contributed to the emergence of AMR, particularly among pathogens such as *Campylobacter coli*, *Brachyspira* spp., and *Mycoplasma* spp. Resistance mechanisms include target site modification, efflux pumps, and enzymatic inactivation, with cross-resistance observed across the MLS group. Environmental assessments reveal that MLS residues and resistance genes persist in manure and soil, posing risks of transmission to human and wildlife populations.

While sales data from 29 EU/EEA countries indicate a downward trend in the use of lincosamides in food-producing animals between 2017 and 2024, for macrolides sales have fluctuated during this period, with the lowest value observed in 2022. Sales of group treatment forms remain prevalent for both macrolides and lincosamides. For companion animals, sales of macrolides have declined, while sales of lincosamides have increased.

The paper underscores the need for prudent use, improved dosing strategies, and enhanced surveillance to mitigate the risk of AMR. It also calls for further research into pharmacokinetics/pharmacodynamics (PK/PD) relationships and the development of veterinary-specific

clinical breakpoints. It also recommends a One Health approach, linking regulation, stewardship, surveillance, and prevention, to safeguard the antimicrobial effectiveness of MLS and limit resistance in animals and humans.

CVMP Recommendations for action

Based on the results of this comprehensive review and the reflection on the possible actions to address identified needs, recommendations for actions that could be taken to mitigate the development of resistance and impact on public and animal health due to the use of MLS in animals in the EU are presented:

- The CVMP's pilot Dosage review and adjustment of selected veterinary antibiotics (ADRA) project aims to review current dosing regimens of specific antimicrobials to ensure achievement of sufficient pharmacokinetic/pharmacodynamic (PK/PD) targets and subsequently to minimise the risk of resistance selection. A priority list of substances has been established (including e.g. tylosin) and the work will be supported by the Dosage Review and Adjustment of Established Veterinary Antibiotics temporary Working Party (ADRA tWP). Authorised dosing regimens of veterinary medicines containing macrolides and lincosamides may not be suitable, particularly for established products with sparse data (e.g., tylosin). Any review should consider the appropriate PK/PD index and related target values (selected based on the desired clinical outcome), as well as the treatment duration (including the minimum duration), if feasible.

- The European Committee on Antimicrobial susceptibility Testing (EUCAST) and regulatory bodies are encouraged to promote the standardisation of susceptibility testing and establishment of veterinary clinical breakpoints specific to animal species and infections for macrolides and lincosamides to enable proper interpretation of results. Human-derived breakpoints are unsuitable, as they rely on human pharmacological data and cannot be reliably extrapolated to veterinary contexts. Microbiological resistance can be determined using epidemiological cut-off values (ECOFFs); however, they are not designed for clinical use but can help clinicians identify pathogens with reduced susceptibility. Advancing knowledge of PK/PD relationships and microbiological criteria is essential to establishing veterinary-specific breakpoints and ensuring prudent antimicrobial use.

- National Competent Authorities are encouraged to support the education of veterinarians, farmers and manure operators on the risk of antimicrobial residues, including MLS residues and resistance genes found in manure and soil as a potential threat to human health after exposure via the environment. Prudent use of antimicrobials in accordance with good veterinary and husbandry practices (e.g. manure management), as well as measures to mitigate their emission, ought to be considered to reduce the risk of human exposure.

- The possibility of including other macrolides and lincosamides in the panel of antimicrobial substances to be included in AMR monitoring in food-producing animals and foodstuffs could be explored by the European Food Safety Authority (EFSA). This monitoring would facilitate early detection of emerging trends and spread of macrolide- and lincosamide-resistant bacteria and antimicrobial resistance genes in animal commensals and zoonotic pathogens (e.g., *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp. and *Enterococcus* spp.).

- The EMA, the EFSA and the European Centre for Disease Prevention and Control (ECDC) could further investigate potential associations between usage and resistance, based on the consumption data by animal species collected from annual surveillance European Sales and Use of Antimicrobials for Veterinary Medicine (ESUAvet) reports, and resistance data from other relevant European reports. This investigation could be delivered through interagency collaboration. For example,

exploring the relationship between usage and resistance in different animal species and target pathogens for lincosamides and for third-generation macrolides (e.g. tulathromycin).

- Marketing authorisation holders of VMPs containing third-generation macrolides (e.g. tulathromycin and gamithromycin), should transition to including the recommended standard sentences for antimicrobials available in the latest 'Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP/383441/2005-Rev.1 Corr.1) in the product information of such veterinary medicinal products (VMPs), since these antimicrobials should be reserved for cases where first-line antimicrobials have failed or where susceptibility testing confirms a need.
- Regulatory bodies, governmental agencies and academia are encouraged to support and initiate further research in:
  - Pharmacokinetics and pharmacology to optimise dosing regimens for existing antibiotics. It is recommended to use *in vitro* media that more closely represent the biophase in which the target bacteria are located, to enable a robust PK/PD analysis. These efforts would enhance the responsible use of these classes, reduce the risk of selection for resistance, potentially replace conventional dose-determination studies, and complement clinical trials.
  - The field of environmental science, fundamental research on the spread and/or persistence of resistance via the environment and the associated quantitative risk of back-transmission to humans and animals and explore potential risk mitigation measures, in particular for mobile and linked ARGs conferring resistance to MLS.
  - Clinical data in various animal species and contexts, to allow the establishment of specific veterinary clinical breakpoints for specific target pathogens to be treated with macrolides and lincosamides.

Notwithstanding the list of recommendations above, the CVMP holds the view that AMR should not be considered in isolation, but a comprehensive response to the problem is needed. Implementation of prudent use principles remains a cornerstone for containing resistance, together with biosecurity and other measures that promote animal health and thereby reduce the need for treatment, in a 'One Health' approach.

1. Background

Macrolides are an important class of antibiotics in human medicine, widely used to treat conditions such as upper and lower respiratory tract infections, sexually transmitted infections, and soft tissue infections. In its most recent update, the WHO (WHO, 2024) classified macrolides as CIA, primarily due to their role in treating campylobacter infections, particularly in children. In previous revisions, macrolides were categorised as HPCIA. In veterinary medicine, macrolides are extensively used to treat diseases common in food-producing animals. The World Organisation for Animal Health (WOAH, 2025b) lists them as Critically Important for Veterinary Antimicrobials (VCIA). Within the EU, they are placed in Category C of the AMEG classification (EMA/CVMP/CHMP, 2019). In the EU, macrolides are among the few options for treating haemorrhagic digestive tract disease in pigs (*Lawsonia intracellularis*) and footrot in sheep and goats. They are also crucial for treating mycoplasma infections in pigs and poultry species. Newer macrolides are among the limited options available for treating respiratory tract infections caused by bacteria resistant to other antimicrobials in AMEG Category D.

WOAH classifies lincosamides as Veterinary Highly Important Antimicrobials (VHIA), while AMEG places them in Category C. In human medicine, lincosamides, primarily clindamycin, are used to treat

infections caused by *Streptococcus pyogenes* (β-haemolytic group A), including invasive disease, staphylococcal infections, such as complicated skin and soft tissue infections (including those due to community-acquired methicillin-resistant *Staphylococcus aureus*, or MRSA), anaerobic infections, tonsillitis, and dental infections. They are categorised as Highly Important Antimicrobials (HIA) by WHO (WHO, 2024). Lincosamides are also widely used in veterinary medicine, in both companion and food-producing animals within the EU and globally.

By contrast, streptogramins and ketolides are not authorised for veterinary use in the EU, although streptogramins are classified by WOAH as Veterinary Important Antimicrobials (VIA) and are used in animals in certain third countries. No ketolides are currently authorised for human use in the EU. Both ketolides and streptogramins are included in AMEG Category A. Streptogramins, namely pristinamycin, are only authorised in France for human use in the EU. WHO classifies streptogramins as HIA, while ketolides are designated as 'authorised for use in humans only' (WHO, 2024).

Although MLS are structurally distinct, they are often considered together because they share properties, including overlapping binding sites on the 50S ribosomal subunit. Consequently, several bacterial species possess genes that confer resistance to more than one drug within this group (Prescott et al., 2000; Roberts, 2011). Modification of the bacterial target site often results in cross-resistance between macrolides, lincosamides, and streptogramin B, known as the MLS_B resistance phenotype.

This reflection paper reviews the use and indications of the MLS group in veterinary and human medicine across the European Union. It provides updated information on MLS chemical structures, mechanisms of action, pharmacokinetics/pharmacodynamics, and AMR mechanisms. The paper also examines how veterinary use could contribute to the emergence of AMR, how resistance determinants may spread among animals, humans and the environment, and the resulting consequences for public and animal health. Finally, the paper identifies the need for animal and environmental risk management measures, highlights knowledge gaps, and suggests areas for further research.

2. General drug characteristics

2.1. Macrolides and derivatives (azalides, ketolides, macrocycles)

2.1.1. Structure and mechanism of action

Macrolides constitute a large family of protein synthesis inhibitors composed of a macrocyclic lactone ring of varying sizes, to which one or more deoxy- or amino-sugar residues are attached (Dinos, 2017). Macrolide antibiotics and their derived classes (azalides, ketolides, and macrocycles) are classified according to the size of the macrocyclic lactone ring. Macrolides with a 12-member ring structure are no longer in use.

The first macrolide, erythromycin, was discovered in the early 1950s and is based on a 14-membered lactone ring. It is an organic compound produced by the actinomycete *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) (Zhanel et al., 2001). The first macrolide intended for veterinary use was the 16-membered spiramycin, introduced in the early 1960s, followed by erythromycin and tylosin (also with a 16-membered ring) (Prescott, 2008). Chemically modified variants of tylosin authorised in the EU, also containing a 16-membered lactone ring, include tylvalosin (acetylisovaleryltylosin), approved in 2004 for pigs, and tildipirosin, approved in 2011 for pigs and cattle.

In the early 1990s, a new generation of semi-synthetic macrolides, known as azalides, was introduced in human medicine, with azithromycin as the most prominent member. Azalides have one or more nitrogen atoms inserted into their 15-membered lactone ring (Ballow & Amsden, 1992; Bryskier & Butzler, 2003). The first azalide approved for veterinary use in the EU was gamithromycin in 2008. Subsequent development led to tulathromycin, authorised for use in cattle and swine. Although classified among the azalides, tulathromycin is a semi-synthetic mixture of 13- and 15-membered rings, with three amine groups added. Azalides with this structure are termed triamilides.

At the beginning of the 2000s, another macrolide-related class, the **ketolides** (e.g. telithromycin, cethromycin), was developed (Bryskier, 2000; Hamilton-Miller & Shah, 2002). Ketolides are 14-membered antibacterials in which the L-cladinose moiety at position 3 is replaced with a keto group (Bryskier & Butzler, 2003; Xiong & Le, 2001). Lastly, fidaxomicin is classified as a macrocyclic antibiotic, and it is structurally and mechanistically distinct from macrolides (Kuijper, 2017). For the purpose of this reflection paper, fidaxomicin is considered out of the scope[1].

Macrolides act by binding to the nascent peptide exit tunnel near the peptidyl transferase centre of the 50S ribosomal subunit. With all macrolides, the macro lactone ring is similarly oriented in the ribosomal tunnel, and a key hydrogen bond is formed with A2058 (adenosine at position 2058) of the 23SrRNA (part of the 50S subunit, as well as other nucleotides). Macrolides were initially thought to block the ribosomal exit tunnel, causing the dissociation of the peptidyl-tRNA from the ribosome and thereby acting as general translational inhibitors. Recent evidence indicates that the blockage is not static and sequence-dependent, allowing a subset of peptides to be synthesized even in the presence of the macrolide. In these cases, macrolide interact with specific amino acid sequences of the growing peptide chain (nascent peptide) as it enters the exit tunnel in a way that partially bypasses the blockage, enabling longer peptides to form or delaying the halt of protein synthesis (Dinos, 2017; Graziani, 2021; Vázquez-Laslop & Mankin, 2018).

2.1.2. Spectrum of activity

Macrolides and their derivatives (azalides and ketolides) possess a moderately broad spectrum of activity (Table 1). They are active against most Gram-positive cocci, notably staphylococci, haemolytic streptococci, pneumococci, as well as Gram-negative cocci and several intracellular pathogens, such as *Mycobacterium* spp., *Chlamydia* spp., *Mycoplasma pneumoniae*, *Rhodococcus equi*, and *Legionella* spp. (Gordon, 2018; Van Bambeke, 2018a, 2018b; van Ingen, 2018; Wenzler & Rodvold, 2018). Gram-negative bacilli generally show low intrinsic susceptibility, except for some clinically essential genera, such as *Bordetella* spp., *Campylobacter* spp., *Chlamydia*, *Legionella* spp. and *Salmonella* spp.

Apart from *Shigella* spp. and *Salmonella* spp., members of the Enterobacterales are generally resistant to macrolides, although susceptibility varies among species (Bryskier & Butzler, 2003; D. Hardy et al., 1988). Unlike erythromycin and other 14-membered macrolides, **azalides,** such as azithromycin, can act against some Enterobacterales, owing to their ability to penetrate the outer membrane (Jelić & Antolović, 2016; Jones et al., 1988; Rise & Bonomo, 2007; Vaara, 1993). The triamilide tulathromycin, a semi-synthetic macrolide/azalide with three amine groups in its structure, has been introduced in veterinary medicine for the treatment of infections caused by *Actinobacillus pleuropneumoniae, Mannheimia haemolytica, Pasteurella multocida, Mycoplasma hyopneumoniae, Glaeserella parasuis, and Bordetella bronchiseptica*. Although not authorised in the EU for this indication, bacteriostatic activity against *Streptococcus suis* has also been reported (Y.-F. Zhou et al., 2017a).

[1] The classification of fidaxomicin in the literature varies, as some authors considered macrocycles as sub-class of macrolides, while others consider macrocycles as a separate antimicrobial class. For the purpose of this reflection paper, macrocycles (fidaxomicin) are considered a separate antimicrobial class.

Ketolides (e.g. telithromycin, solithromycin) have a broader spectrum than the 14- and 15-membered macrolides, and are active against both Gram-positive and Gram-negative bacteria such as *Haemophilus* spp., and *Moraxella* spp., including strains resistant to β-lactams and macrolides (Table 1). Ketolides are effective against macrolide-resistant bacteria due to their ability to bind to two sites on the bacterial ribosome and to structural modifications that reduce susceptibility to efflux-mediated resistance. The ketolide telithromycin is active against atypical organisms such as *Chlamydia* spp., *Mycoplasma* spp. and *Legionella* spp. Ketolides are not active against Enterobacterales or *Pseudomonas aeruginosa* (Zhanel et al., 2002).

Table 1. Classification of macrolides and derived compounds

Ring size	Compound class	Substances
12-membered ring	Macrolides	Methymycin**
14-membered ring	Macrolides	**Erythromycin***, Pikromycin, Lankamycin, **Clarithromycin**, **Roxithromycin**, Oleandomycin, Flurithromycin, Troleandomycin, Dirithromycin
	Ketolides	Telithromycin**, Cethromycin, Solithromycin
15-membered ring	Azalides	Tulathromycin*, Gamithromycin*, **Azithromycin**
16-membered ring	Macrolides	**Spiramycin***, Niddamycin, Carbomycin, Tylosin*, Josamycin, Midecamycin, Miocamycin, Rokitamycin, Tildipirosin*, Tilmicosin*, Tylvalosin*, Kitasamycin, Mirosamycin, Terdecamycin
17-membered ring	Macrolides	Sedecamycin

Bolded substances are authorised for human use in the EU/EEA or most EU/EEA countries.
*: Approved for veterinary use in one or more EU Member States (with marketing authorisation).
**: No longer authorised in the EU.

2.1.3. Pharmacodynamics

The mechanism of action of macrolides and their derivatives (azalides and ketolides) is based on the inhibition of protein synthesis, thereby stopping bacterial growth, with certain subclass-specific variations. As a result, they are active against actively dividing bacteria (Fohner et al., 2017). Macrolides generally exert bacteriostatic effects, for which the pharmacodynamic index correlating with *in vitro* and *in vivo* efficacy was initially identified as the time during which the antibiotic concentration remains above the minimum inhibitory concentration (MIC) (t > MIC) (Giguère, 2006a, 2006b). The newer generation macrolides, such as azithromycin, are significantly more potent against certain bacterial species and more bioavailable. Azithromycin is more powerful than erythromycin against *Haemophilus influenzae* and *Campylobacter* spp., and markedly more active than older-generation macrolides against many genera within the Enterobacterales order. In addition, bactericidal activity has been found *in vitro* for tulathromycin against *Actinobacillus pleuropneumoniae* in a porcine tissue cage infection model (Yao et al., 2022).

In addition to the desired direct antibacterial effects, other effects of macrolides and their derivatives may need to be considered for their contribution to clinical efficacy or selection of resistance:

Post-antibiotic effect (PAE): The PAE refers to the period following a decline of antimicrobial concentration below the MIC, during which bacterial growth remains suppressed. Macrolides and other antimicrobials demonstrate PAEs for Gram-negative bacteria (Craig, 1998; Dudley, 1991; Lutsar et al., 1998). For macrolides, the PAE is more pronounced with newer-generation agents, such as azithromycin or clarithromycin; however, the clinical implications of long PAEs remain unclear. This effect is mostly investigated *in-vitro,* and it would be particularly relevant for substances with an

intrinsic long half-life, such as third-generation macrolides. A long PAE may allow extended dosing intervals or shorter treatment regimens. Thus, additional investigations are needed to clarify the clinical relevance of PAE (Athamna et al., 2004), including for the selection of resistance.

Post-leucocyte enhancement (PALE): The PALE refers to the increased susceptibility of bacteria to phagocytosis and intracellular killing following exposure to an antimicrobial agent. Drugs that produce the greatest PAE also tend to produce the greatest PALE (Prescott et al., 2000).

Sub-MIC effects: *In vitro* studies have shown that concentrations below MIC of erythromycin can lead to *de novo* resistance selection in *Campylobacter coli* and *Enterococcus faecium*, and promote enrichment of resistance in *Escherichia coli* (EFSA, 2021b). Sub-MIC concentrations also contribute to mutagenesis (Gullberg et al., 2014; Stanton et al., 2020) as well as to horizontal gene transfer and virulence (Scornec et al., 2017; Stanczak-Mrozek et al., 2017).

Immunomodulatory effects: Macrolides can have significant immunomodulatory effects independent of their antimicrobial activity (Blondeau, 2022; Chin et al., 2000; Kricker et al., 2021; Tamaoki et al., 2004). Azithromycin, for example, has been shown to enhance the host pro-inflammatory reaction response, improve phagocytosis, and reduce local inflammation (Ribeiro et al., 2009; Zimmermann et al., 2018). For tulathromycin, immune-modulating and anti-inflammatory actions have been demonstrated in experimental studies. This has been demonstrated in both bovine and porcine polymorphonuclear cells (PMNs; neutrophils), where tulathromycin promotes apoptosis and the clearance of apoptotic cells by macrophages, reduces the production of pro-inflammatory mediators such as leukotriene B4 and CXCL-8, and induces the production of the anti-inflammatory and pro-resolving lipid lipoxin A4. Although immunostimulation has been demonstrated for several macrolides in rodents, pigs, and humans, immunosuppressive effects have been observed in poultry species for tylvalosin (El-Ela et al., 2016).

2.1.4. Pharmacokinetics

Macrolides are generally well absorbed (except for erythromycin base after oral administration, which is degraded in low-pH environments such as the stomach). Chemically, macrolides are weak bases with high lipid solubility. Once in the bloodstream, they preferentially bind to alpha-1-acid glycoprotein. They typically exhibit large volumes of distribution and good penetration into tissues, especially the lungs, liver, and kidneys (with concentrations 50 times higher than in plasma). Their weakly basic character results in diffusion trapping in acidic fluids, such as milk. Moreover, macrolides are present at high intracellular concentrations and accumulate in phagocytic cells. Recently, extracellular and intracellular activity of macrolides, namely clarithromycin alone, as well as azithromycin or gamithromycin in combination with doxycycline, has been demonstrated against *Rhodococcus equi* (Huguet et al., 2025). Furthermore, it has also been suggested that intracellular penetration and accumulation in neutrophils may contribute to killing or inhibiting intracellular pathogens. Elimination is slow. Macrolides are eliminated mainly by the liver, with a variable proportion excreted in bile as parent drug and/or metabolites, undergoing enterohepatic cycling and having a long terminal half-life. Thus, irrespective of whether administered orally or parenterally, macrolides exert microbiological effects on the intestinal microbiota.

It is essential to note that the pharmacokinetic parameters of these compounds vary substantially according to numerous factors that influence the drug's plasma concentration. These factors include the animal species (and breeds within a species), age, gender, bodyweight and body condition, physiological status, route of administration, vehicles or excipients used in commercial formulations, amount and type of nutrition, or concomitant administration of other drugs. The analytical methods employed (HPLC vs microbiological assay) and their respective sensitivity levels, as well as sampling

times, sample numbers, and data treatment (compartmental vs non-compartmental), can affect results; therefore, caution is required when comparing data.

2.2. Lincosamides

2.2.1. Structure and mechanism of action

Lincosamides constitute a group of antibiotics characterised by a chemical structure comprising an amino acid and sugar moieties. Natural lincosamides are produced by several *Streptomyces* species, mainly by *Streptomyces lincolnensis, Streptomyces roseolus* and *Streptomyces caelestis* and by *Micromonospora halophytica.* Lincomycin was first isolated from *Streptomyces lincolnensis* from a soil sample. Many semisynthetic derivatives of lincomycin have been developed.

Lincosamides are antibiotics that block microbial protein synthesis, which involves many steps, including the activation of amino acid monomers by aminoacyl-tRNA synthesis, chain elongation, and chain termination of the growing polypeptides on the ribosome. Antibiotics disrupt the timing and specificity of these steps, thereby slowing growth or causing lethal effects in the microorganism. The original lincosamide, lincomycin, has been superseded by clindamycin, which exhibits improved antibacterial activity.

Clindamycin disrupts protein synthesis by binding to the 50S ribosomal subunit of bacteria, thereby inhibiting the early stage of chain elongation. The antibacterial spectrum of activity of clindamycin is similar to that of macrolides and streptogramins (Johnson, 2021).

Pirlimycin is a lincosamide antimicrobial used in intramammary VMPs to treat intramammary infections. This semisynthetic lincosamide acts by binding to the 50S ribosomal subunit, preventing the binding of aminoacyl-tRNA and inhibiting peptidyl transferase activity, thereby blocking protein synthesis.

Although macrolides primarily bind to the nascent peptide as it enters the exit tunnel and block translation in a sequence-dependent manner, they compete with lincosamide which are considered to act closer to the peptidyl transferase center (PTC), directly interfering with the transpeptidation reaction (the formation of the peptide bond) and/or translocation. This direct action on the PTC is what distinguishes their primary mechanism from the exit tunnel-blocking action of macrolides, even though their binding sites overlap. Lincosamides share a common mechanism of action on sensitive microorganisms and exhibit a similar antibacterial spectrum.

2.2.2. Pharmacodynamics and spectrum of activity

The spectrum of lincosamides is more limited compared to macrolides (Roberts, 2008). The antibacterial spectrum includes Gram-positive cocci and anaerobic bacteria, but excludes Enterobacterales, *Pseudomonas* spp., and *Acinetobacter* spp. Indeed, lincosamides are mainly active against Gram-positive bacteria (e.g. staphylococci and streptococci, but not enterococci), many anaerobes (e.g. *Bacteroides fragilis*, *Fusobacterium* spp., *Dichelobacter nodosus, Clostridium perfringens*), and some *Mycoplasma* spp. (e.g., *Mycoplasma hyopneumoniae*). Lincosamides possess good anti-staphylococcal activity.

The two most widely used lincosamide substances in veterinary medicine are lincomycin and its derivative clindamycin. Since 2001, a VMP containing the lincosamide pirlimycin has been authorised for intramammary use. In humans, only clindamycin is currently used (in both oral and injectable forms) due to its higher antibacterial activity and bioavailability, as well as the adverse toxic effects associated with lincomycin.

Lincosamides can be bactericidal or bacteriostatic, depending on the drug concentration, bacterial species, and bacterial inoculum (Giguère, 2013).

2.2.3. Pharmacokinetics

Lincosamides are basic compounds with pK_a values of about 7.6; pirlimycin has a higher pK_a of 8.5. This makes pirlimycin more effective in acidic (lower pH) environments. They have high lipid solubility and consequently exhibit large volumes of distribution. After oral administration, they are well absorbed from the intestines of non-herbivores. They are primarily eliminated through hepatic metabolism, although approximately 20% is excreted in an active form in the urine. In monogastrics, approximately 90% of orally administered lincosamides are absorbed, with slight variations depending on the specific drug administered. Plasma concentrations via this route peak within 2 to 4 hours. Intramuscular administration of lincosamides yields good absorption, with peak plasma levels achieved within 1–2 hours. Clindamycin is hydrolysed in the liver into at least seven metabolites. Tissue concentrations consistently exceed serum concentrations several times because of the passage of substances across cell membranes. Around 90% of clindamycin is bound to plasma proteins, and it is generally more stable and rapidly absorbed than lincomycin. Extensive binding to plasma proteins and relatively rapid elimination prevent concentrations in cerebrospinal fluid from exceeding 20% of serum concentrations. Clindamycin achieves therapeutic concentrations in bone, although levels are relatively low, around 10–30% of serum concentrations (Giguère, 2013).

Lincosamides have a broad distribution in several tissues, except in cerebrospinal fluid. When administered intramuscularly to rats, lincomycin accumulated at the highest concentrations in the kidneys, compared to other tissues, while clindamycin accumulated at the highest concentrations in the lungs (Osono & Umezawa, 1985). Clindamycin accumulates in macrophages and other white blood cells, resulting in concentrations up to 50 times higher than plasma levels (Johnson et al., 1980).

2.3. Streptogramins

2.3.1. Structure and mechanism of action

Streptogramins are a group of natural (virginiamycin, pristinamycin, and mikamycin) or semisynthetic (quinupristin-dalfopristin) cyclic peptides. Streptogramins act by inhibiting translation via binding to the 50S subunit of the bacterial ribosome (Frasca, 2017a, 2017b).

Streptogramins consist of two structurally different components, A and B. The A components, such as pristinamycin IIA, virginiamycin M, mikamycin A, and dalfopristin, are polyunsaturated macrolactones. The B components, such as pristinamycin IB, virginiamycin S, mikamycin B, and quinupristin, are cyclic hexadepsipeptides.

2.3.2. Pharmacodynamics and spectrum of activity

The spectrum of activity of streptogramins includes a broad range of aerobic and anaerobic Gram-positive bacteria, such as staphylococci, including MRSA and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), and most *Enterococcus faecium* isolates, but excluding *Enterococcus faecalis*. This Gram-positive species is intrinsically resistant to type A streptogramins (phenotype 126 LSA, resulting in lincosamide and streptogramins A resistance) and to A+B streptogramin combinations, due to the presence of the *lsa(A)* gene. Streptogramins are also active against *Streptococcus pneumoniae*, β-haemolytic streptococci, viridans streptococci, *Corynebacterium* spp., and *Listeria monocytogenes*. In addition, streptogramins exhibit activity against most Gram-positive

anaerobes, such as *Actinomyces* spp., *Clostridium* spp., *Lactobacillus* spp., *Peptostreptococcus* spp., and *Cutibacterium acnes*. They are also active against *Mycoplasma* spp., *Ureaplasma urealyticum*, and *Chlamydia* spp. They exhibit good activity against fastidious Gram-negative bacteria, which are microorganisms that are difficult to grow in the laboratory due to their complex or restricted nutritional and environmental requirements, including *Moraxella catarrhalis*, *Neisseria* spp., and *Legionella pneumophila*. Streptogramins have variable activity against the *Bacteroides fragilis* group and other Gram-negative anaerobes. Finally, Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. have intrinsically low susceptibility even at high streptogramin concentrations.

The target of both components of virginiamycin is the 50S subunit of the bacterial ribosome. The A-component binds to the peptidyl transferase catalytic centre (PTC) of the ribosome, preventing the attachment of transfer RNA (tRNA) and thereby blocking the formation of peptide bonds between the growing peptide chain (attached to peptidyl-tRNA) and aminoacyl-tRNA, consequently stopping the elongation of the growing polypeptide chain. The B component does not affect the peptidyl transferase reaction but inhibits peptide elongation. Thus, the extension of the nascent protein chain is prevented, and, in addition, the peptidyl-tRNA is released from the ribosome, resulting in incomplete peptides. Overall, the B-component is structurally and mechanistically like macrolides (such as erythromycin) and lincosamides, as all three inhibit elongation or translocation via the exit tunnel.

The structural composition of the ribosomal complex is vital as virginiamycin S1 acts synergistically with the conformational change of the peptidyl transferase centre of the 50S-ribosome induced by virginiamycin M1. Individually, each component of virginiamycin exhibits only moderate bacteriostatic activity by binding to the 50S subunit of bacterial ribosomes, thereby blocking translational processes, whereas the combination of the two components leads to a synergistic effect with up to a hundred-fold higher activity, resulting in bactericidal effects (Speciale et al., 1999). It has also been suggested that the synergistic activity tends to reduce the emergence of bacterial resistance to either drug (Giguère et al., 2013).

2.3.3. Pharmacokinetics

Virginiamycin is the only approved streptogramin for veterinary medicine; however, it is not approved in the EU, although an MRL is established for poultry species. It is approved for swine and chickens in various countries outside the EU, including the USA, Canada, South America, South Africa, Australia, and New Zealand.

Giguère et al. (2013) state that there is little data available on the pharmacokinetic properties of virginiamycin in animals but note that the drug is poorly absorbed after oral administration. In contrast, the European public MRL assessment report on virginiamycin (EMA/CVMP, 2014) states that pharmacokinetic studies and residue depletion studies in chickens confirm a rapid depletion of 14C-virginiamycin factor S1 and M1 residues in tissues (liver, kidney, skin, fat, and muscle). This indicates that virginiamycin is absorbed to some extent in chickens.

3. Pharmacokinetics/Pharmacodynamics relationship of MLS

Unlike other antimicrobial families, the PK/PD properties of these three groups (macrolides, lincosamides and streptogramins) have been challenging to define. Historically, they were primarily considered to have time-dependent antibiotic activity, expressed as the time when the free concentration remains above the MIC (fT>MIC). This was especially true for short-acting macrolides such as erythromycin.

However, advances in PK/PD modelling have updated our understanding. The PK/PD index free area under the concentration-time curve (fAUC)/MIC is frequently used to reflect the co-dependence of concentration and time of exposure for efficacy. It has been notably investigated using a semi-mechanistic PK/PD model, which demonstrated that erythromycin (a macrolide), initially regarded as being best related to the fT>MIC, exhibited a stronger correlation with efficacy when fAUC/MIC was used as the PK/PD index (Craig et al., 2002; Nielsen et al., 2011). Nevertheless, it was also highlighted that no single PK/PD index can be relied upon as an indicator of efficacy across a drug class and among species, pathogens and dosing regimens (Nielsen et al., 2011).

For **macrolides**, the complexity was exemplified by Giguère and Tessman (2011), who demonstrated that plasma concentrations are considerably lower than the MIC of the pathogens for which they are approved for most or all of the dose interval, which prevents the use of fT >MIC for predicting efficacy. This is particularly true for the newer long-acting drugs. Nonetheless, multiple studies have demonstrated their efficacy against several diseases (DeDonder et al., 2016). To explain the clinical efficacy, it was first hypothesised that the higher drug concentrations at the infection site or relevant biophase should be considered rather than simply relying on plasma levels. Indeed, due to the lipophilic properties of macrolides, they can diffuse into tissue and cells and reach high intracellular concentrations (with varying degrees depending on the macrolide). However, Toutain et al. (2021) highlighted the methodological challenges in accessing these matrices for measuring effective concentrations (compared to blood/plasma samples). Recently, Huguet et al. (2025) confirmed the intracellular penetration within macrophage-like cells and bacterical activity of several macrolides (clarithromycin, azithromycin, gamithromycin) against *Rhodococcus equi*, by combining bacterial counts and optical microscopy.

Recently Luo et al. (2026) proposed a PK evidence-based approach to formulation optimization aimed at enhancing treatment efficacy. Their work demonstrated that differences in formulation can produce markedly improved pharmacokinetic profiles and tissue distribution patterns. Among evaluated preparations, Formulation I exhibited superior pharmacokinetic properties, including enhanced systemic exposure, higher bioavailability, prolonged mean residence time, slower elimination and optimal tissue distribution. It preferentially accumulated in respiratory and lymphoid tissues, correlating with tylvalosin's efficacy against respiratory pathogens. With lung concentrations of ~1000 $ng \cdot g^{-1}$ and high levels in lymph nodes, Formulation I offered a robust formulation for extended antimicrobial coverage in swine respiratory diseases.

However, this is not sufficient to explain the efficacy of macrolides against strict extracellular pathogens (such as *Pasteurella multocida* and *Mannheimia haemolytica*) (Toutain et al., 2021). It was thus demonstrated that the *in vivo* activity of macrolides may be increased in serum compared to the traditional microbiological media used for MIC determination. Indeed, Lees et al. (2017) demonstrated that for tulathromycin, a 50- to 80-fold increase in potency in serum (i.e., corresponding to a 50- to 80-fold lower MIC) was observed compared to Mueller-Hinton broth (MHB) for *Pasteurella multocida* and *Mannheimia haemolytica*. These findings were incorporated into a robust PK/PD analysis by Toutain et al. (2017), which highlighted that unbound tulathromycin concentrations levels in serum explain the efficacy of single (standard) dose of tulathromycin in clinical use against respiratory pathogens in calves, due to the significantly lower MIC values in serum (i.e., higher antimicrobial activity). As a result, a dosage regimen can be computed for tulathromycin using classical PK/PD concepts (with fAUC/MIC as the preferred index). Similar results were obtained for tulathromycin in pigs against *Pasteurella multocida* (Q. Zhou et al., 2017) and *Streptococcus suis* (Y.-F. Zhou et al., 2017b) but also for other macrolides like gamithromycin (Zhou et al., 2020) and tildipirosin against several pig pathogens (Lei et al., 2018).

Regarding the fAUC/MIC predictive target values to achieve therapeutic efficacy, a range of 25-35 h for macrolides is generally accepted (Finberg & Guharoy, 2012). However, no universal value likely exists; rather, drug and pathogen-specific values are more appropriate, depending on multiple factors such as the bacterial load (high vs low, which could correspond to a curative versus metaphylactic treatment), the expected efficacy (i.e. bacteriostatic vs bactericidal), among others (Toutain et al., 2021).

For tulathromycin, Toutain et al. (2017) reported an fAUC/MIC value of approximately 24 h, supporting a bactericidal effect in the treatment of pneumonia in calves. Later, Y.-F. Zhou et al. (2017a) proposed fAUC24h/MIC values for tulathromycin of 44.55, 73.19, and 92.44 h, corresponding to bacteriostatic, bactericidal, and virtual eradication activity, respectively, for the treatment of *Pasteurella multocida* in pigs.

Wang et al. (2022) investigated the PK/PD relationship of gamithromycin for *Streptococcus suis* infections in piglets. This study demonstrated that the dose-response relationship, expressed as the AUC/MIC ratio, was the predictive PK/PD index closely linked to antimicrobial activity. For this same active substance, and the same animal species infected by *Haemophilus parasuis*, Zhou et al. (2020) concluded that the AUC24h/MIC targets in serum associated with bacteriostatic, bactericidal, and eradication activities were 15.8, 30.3, and 41.2 h, respectively. The PK/PD-based population dose prediction indicated a probability of target attainment for a dose of 6 mg/kg against *Haemophilus parasuis* of 88.9%. Additionally, for gamithromycin, DeDonder et al. (2016) demonstrated that in cattle with bovine respiratory disease associated with *Mannheimia haemolytica* or *Pasteurella multocida*, the AUC/MIC associated with clinical success in these cases was 3.49 hours (*Mannheimia haemolytica*) and 3.21 hours (*Pasteurella multocida*).

For tylosin, in healthy pigs, AUC24h/MIC values for bacteriostatic activity were 0.98 and 1.10 h; for bactericidal activity, AUC24h/MIC values were 1.97 and 1.99 h for *Actinobacillus pleuropneu*moniae and *Pasteurella multocida*, respectively. For infected pigs, AUC24h/MIC values for bacteriostatic activity were 1.03 and 1.12 h; for bactericidal activity, AUC24h/MIC values were 2.54 and 2.36 h for *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*, respectively (Lee et al., 2023).

For **lincosamides**, most information available is related to clindamycin. According to Martinez et al. (2012), although clindamycin's effects are time-dependent, this alone is insufficient to support the use of T>MIC as the appropriate PK/PD index. Indeed, antimicrobial efficacy is more closely associated with the AUC/MIC ratio.

For **streptogramins**, studies have demonstrated that they exhibit concentration-independent killing properties and produce prolonged post antibiotic effects on Gram-positive organisms. The efficacy of antibiotics characterised by this pattern of activity is best correlated with AUC24h/MIC as a PK/PD parameter. This is supported by prior animal infection models, which have identified the AUC/MIC ratio as the principal PK/PD parameter predictive of the clinical efficacy of streptogramin (Andes & Craig, 2006).

As described in this chapter, the free-drug AUC to MIC ratio (fAUC/MIC) is the primary PK/PD index for many antimicrobial agents. The target fAUC/MIC should be selected according to the desired clinical and microbiological outcome. Notably, the required target increases substantially as the therapeutic goal shifts from bacteriostatic activity (inhibition of bacterial growth) to bactericidal activity (bacterial killing), and ultimately to near-complete bacterial eradication. Consequently, the choice of the fAUC/MIC target must be tailored to the clinical context, infection site, pathogen, and therapeutic objective. In PK/PD modelling and in a clinical context, the $AUC_{(24h)}$ represents a key therapeutic target, however, it must be interpreted in conjunction with the duration of treatment. Insufficient drug

exposure, resulting from inadequate dosing or suboptimal treatment duration, may lead to reduced clinical efficacy and promote the development of antimicrobial resistance.

4. Resistance mechanisms to macrolides, lincosamides and streptogramins

4.1. Acquired resistance phenotypes

Although they have distinct chemical structures, MLS_B group shares similar mechanisms of action (Table 2). They all have antibacterial effects by inhibiting protein synthesis, through binding to the bacterial 23S rRNA ribosomal subunits.

Therefore, genes causing resistance or reduced susceptibility to any MLS_B group antibiotics may lead to the development of cross-resistance to others. The resistance determinants include rRNA methylases, efflux systems, and inactivating genes such as esterases, lyases, phosphorylases, and transferases (Table 2). The most common mechanism of MLS resistance is due to the presence of rRNA methylases encoded by the *erm* genes. These enzymes methylate the adenine residue(s), resulting in MLS resistance. The methylated adenine prevents the drugs from binding to the 50S ribosomal subunit. The other two mechanisms efflux pumps and inactivating genes are encoded by the *msr* (for macrolides and streptogramin B) and *ere* determinants, respectively (Van Hoek et al., 2011).

Hereafter, are summarised the different resistance phenotypes among MLS:

- **M resistance phenotype:** This resistance phenotype is mediated by efflux pumps, which is due to the presence of the *mef* genes that encode Major Facilitator Superfamily efflux pumps confering low-level resistance typically restricted to macrolides.
- **MLS_B resistance phenotype:** Defined by resistance to macrolides, lincosamides, and streptogramin B. This phenotype can be inducible or constitutively expressed and has been well described in *Staphylococcus aureus*. The most common mechanism of resistance to MLS_B group antibiotics involves methylase enzymes encoded by *erm* genes. This resistance is often inducible by phenotypic expression of methylase enzyme ($iMLS_B$) but may also occur as constitutive resistance ($cMLS_B$).
- **(Macrolides, Lincosamides, Streptogramin B, Ketolides and Oxazolidinones) MLSKO expanded resistance phenotype:** Bacterial resistance to MLSKO antibiotics is known to be mediated by three mechanisms of resistance: (i) mono- or di-methylation of the A2058 residue located within the conserved domain V of 23S rRNA, impairing drug binding. This is the most widespread mechanism conferring cross-resistance to MLSKO antibiotics; (ii) drug inactivation by esterases, lyases, transferases, and phosphorylases conferring resistance to structurally related antibiotics; and (iii) antibiotic efflux by the resistance/nodulation/division (RND) family and the major facilitator superfamily (MFS) (Hurst-Hess et al., 2021). No single ABCF protein confers concomitant resistance to all MLSKO antibiotics; instead, they are grouped into three categories that mediate resistance to the following: (i) lincosamides and group A streptogramins (represented by *vmlR*, *vga*, *lsa*, and *sal* genes), (ii) macrolides, ketolides, and group B streptogramins (*msr*-type genes), and (iii) oxazolidinones (*optrA* genes). These proteins function by binding to the ribosome and actively displacing the antibiotic from its binding site, effectively "protecting" the ribosome from the drug.
- **Macrolides and Streptogramin B (MSB) resistance phenotype:** Defined by resistance to macrolides and streptogramins B. This phenotype is primarily induced by erythromycin and affects

only C14 or C15 macrolides and streptogramin B. The gene responsible is *msrA*, which codes for an efflux pump and shows a slightly broader spectrum of resistance than *mef* genes.

- **SA+B resistance phenotype**: The SA+B resistance phenotype refers to a resistance pattern in *Staphylococcus aureus* characterised by resistance to streptogramin A and streptogramin B, while lincosamides and macrolides may remain active. Strains expressing this phenotype are resistant to streptogramin A compounds (MIC for SgA $\geqslant$ 8 µg/mL) and their derivatives (e.g. pristinamycin IIA, virginiamycin M, or dalfopristin), without necessarily being resistant to streptogramin B compounds (e.g. pristinamycin IA or quinupristin). Resistance of this type in *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) is usually associated with the accumulation of multiple resistance mechanisms, including *vga* (ABC-F ribosomal protection), *vat* (streptogramin A acetyltransferases), and *vgb* (streptogramin B lyases). These genes are frequently plasmid-borne and may occur in combination with *erm* methylase genes (resulting in MLS B phenotype), contributing to complex resistance phenotypes.

- **L resistance phenotype:** Resistance to lincosamides alone is mediated by the *lin*A gene, a Lincosamide Nucleotidyl Transferase that enzymatically inactivates the drug, found in *Staphylococcus aureus* and in *Staphylococcus haemolyticus*. The incidence of staphylococci resistant to lincosamides alone, without resistance to macrolides or streptogramins, is less than 5% in France.

- **LS_A resistance phenotype**: Resistance to lincomides and streptogramin A, is mediated primarily by the *lsaA* gene (Lincosamide-Streptogramin A resistance). The mechanism is an ATP-binding cassette (ABC) efflux pump that simultaneously removes both lincosamides and streptogramin A from the cell

- **Other resistance phenotypes – phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (PhLOPSA) resistance phenotype:** The *cfr* gene encodes an RNA methyltransferase that modifies the adenine residue at position 2503 of the 23S rRNA gene, conferring combined resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics and decreased susceptibility to the 16-membered macrolides spiramycin and josamycin. These classes of protein biosynthesis inhibitors share overlapping binding sites at the peptidyl transferase centre of the ribosome. The *cfr* gene's presence, which is typically plasmid-borne and highly mobile, is often selected or co-selected by the extensive use of phenicols (florfenicol) and pleuromutilins in livestock. Its presence in food-producing animals poses a high risk because it threatens the efficacy of linezolid, reserved for human use only to treat life-threatening infections (e.g., MRSA and VRE).

Table 2 - Macrolides (and related classes), lincosamides and streptogramins - mechanisms of action and acquired resistance

	MACROLIDES AND RELATED CLASSES					
	MACROLIDES (14' lactone ring)	**KETOLIDES** (14' lactone ring)	**AZALIDES (15'** lactone ring)	**MACROLIDES** (**16**' lactone ring)	**LINCOSAMIDES**	**STREPTO-GRAMINS**
AUTHORISED IN EU/EEA COUNTRIES	**erythromycin*** **clarithromycin** **roxithromycin**	telithromycin** solithromycin	**azithromycin**	**spiramycin*** tilmicosin* tylvalosin* tylosin*	**clindamycin,** lincomycin	**pristinamycin**
MECHANISM OF ACTION	Protein synthesis inhibition by binding to 50S		Protein synthesis inhibition by binding to 50S		Protein synthesis inhibition by binding to 50S	Protein synthesis inhibition by binding to 50S
MECHANISMS OF ACQUIRED RESISTANCE						
ENZYMAMTIC DEGRADATION	**Kinases/ phosphotransferases:** *mhp*-genes **Esterases of lactone ring:** *ere*A, *ere*B genes	**Kinases/ phosphotransferases:** *mhp*-genes		**Kinases/ phosphotransferases:** *mhp*-genes	*lin*A/*Inu*A	Acetyltransferases: *vat*-genes
DRUG EFFLUX	Inducible ***Mef* pumps:** *mef*A, *mef*E genes,	Inducible *Mef* **pumps:** *mef*A, *mef*E genes			Inducible ***Mef* pumps:** mefA, mefE genes	ABC-transporters: *vga*, *lsa*
MODIFICATION OF TARGET SITE	Inducible **methylases of 23S rRNA of 50S**: *erm*-genes Mutations in rRNA and 50S ribosomal proteins				Inducible **methylases of 23S rRNA of 50S**: *erm*-genes Methyltransferase: *cfr*-gene	Inducible **methylases of 23S rRNA of 50S**: *erm*-genes Methyltransferase: *cfr*-gene
OTHER	Ribosomal protection: *msr*A, *msr*D	Ribosomal protection: *msr*A, *msr*D			*Vga*(A)-genes	

In bold: Authorised for human use in one or more EU/EEA countries
* Substances approved for veterinary use in one or more Member States in the EU (having marketing authorization, MA)
** Not any longer authorised in the EU/EEA

5. Consideration on susceptibility testing

Both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI) have guidelines for susceptibility testing of bacterial species against macrolides and lincosamides; however, there are variations in methodologies and breakpoints between the two organisations. From a technical point of view, it is worth noting that the pH value of the Mueller-Hinton medium should be precisely between 7.2 and 7.4; otherwise, the achieved MIC may be inaccurate. These challenges can be addressed by implementing a robust quality control scheme.

Both EUCAST and CLSI provide breakpoints for human pathogens. CLSI additionally provides veterinary breakpoints for dogs (clindamycin), swine (tildipirosin, tilmicosin, tulathromycin) and cattle (spectinomycin, tildipirosin, tilmicosin, tulathromycin) as well as for intramammary infections in bovine (pirlimycin). Although macrolides and lincosamides are commonly used in poultry species' production in the EU, no breakpoints are currently established. The majority of CLSI's veterinary-specific breakpoints are only for the dilution method (CLSI, 2024).

EUCAST has published ECOFF values for several macrolides, lincosamides and streptogramins (EUCAST). ECOFFs, when available, are used by EFSA for EU-wide indicator and zoonotic bacteria resistance monitoring. EUCAST has no clinical breakpoints for veterinary pathogens but a subcommittee of the EUCAST, called VetCAST, was founded in 2015, with the aim of contributing to global standards for susceptibility testing and setting breakpoints for different bacterial species of animal origin (VetCAST).

For a proper interpretation of AST results for macrolides, it is necessary to consider that only 14- and 15-membered macrolides can activate inducible resistance, but not 16-membered macrolides or lincosamides. The reason is that the 14- and 15-membered macrolides (like erythromycin) have a specific structure that allows them to bind to the ribosome and stall the translation of a short leader peptide that precedes the *erm* gene, thus *inducing* the production of the methylase enzyme. It should be noted that for MSB vs inducible MLSB (iMLSB) phenotypes, both appear erythromycin-resistant and clindamycin-susceptible on routine AST, but they arise from different mechanisms. MSB is caused by *mef*/*msr* efflux pump genes, which export macrolides and streptogramin B but do not affect clindamycin, so the D-test is negative and clindamycin remains reliably active. Inducible MLSB is caused by *erm*-mediated methylation of the 23S rRNA target site. The *erm* genes show inducible expression, so clindamycin tests susceptible initially, but a positive D-test reveals inducible resistance. Clinically, clindamycin can be used for MSB isolates but should not be used for iMLSB due to the risk of treatment failure.

Thus, AST should include testing of erythromycin (a 14-membered macrolide), a 16-membered macrolide (e.g., tilmicosin), and a lincosamide (e.g., clindamycin). If a bacterium has been tested susceptible to erythromycin, it will also be susceptible to all macrolides. Conversely, if a bacterium has been tested as erythromycin-resistant but tilmicosin- and clindamycin-susceptible, it could harbour an inducible resistance gene. In such a situation, use of tilmicosin and clindamycin can very quickly lead to mutations in genes regulating the expression of methylases, which results in an irreversible switch from an inducible to a constitutive expression type, leading to persistent resistance to all macrolides and lincosamides (Werckenthin, 2000).

Both constitutive and inducible resistance can also be detected using a simple disk diffusion test (D-test). The test allows for the identification of four different phenotypes:

- The inducible MLS_B phenotype (D^+): Resistant to erythromycin and susceptible to clindamycin with a D-zone of inhibition around the clindamycin disk, mediated by *erm* gene methylase.

- The constitutive MLS_B phenotype: Resistant to both erythromycin and clindamycin.
- The MSB phenotype: Resistant to erythromycin and susceptible to clindamycin, but the clindamycin zone of inhibition remains circular (no D-zone), indicating resistance is mediated by an efflux pump (*msr*) rather than methylase induction.
- The susceptible phenotype: Susceptible to both clindamycin and erythromycin (Seifi et al., 2012).
- Inducible resistance can also be detected by broth microdilution by testing a 14-membered macrolide and a 16-membered macrolide in parallel (D. J. Hardy et al., 1988).

Pirlimycin should be tested separately and only against isolates from bovine intramammary infections. There are only valid clinical breakpoints for the indication of intramammary infection for the bacterial species S*taphylococcus aureus and Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis*.

In the absence of veterinary clinical breakpoints, some laboratories use human-derived breakpoints. However, human-derived breakpoints are not considered pertinent, as they are based on human data (e.g., dosing regimens and PK). Thus, unless data are available to substantiate extrapolation from humans to animals, human-derived breakpoints cannot be considered appropriate for target animal species.

Alternatively, ECOFFs aim to detect bacteria with acquired resistance mechanisms. They are used for epidemiological monitoring of resistance development but can also serve as an indicator to the clinician that the pathogen under treatment may have reduced susceptibility compared with pathogens treated in the original clinical trials. At the European level, the stated goal of the Veterinary Subcommittee of EUCAST is to bridge this gap by establishing scientifically robust veterinary clinical breakpoints that incorporate animal-specific PK/PD data, moving beyond the exclusive use of ECOFFs to guide therapy.

6. Sales and use of macrolides and lincosamides in veterinary medicine

6.1. Use and indications in veterinary medicine in the EU

Macrolides and lincosamides are authorised for use in animals in the EU through national procedures, mutual recognition, or centralised procedures. By 2026, nine macrolides and three lincosamides have been authorised for veterinary use in some or all Member States: erythromycin, tildipirosin, tylosin, tylvalosin, spiramycin, tilmicosin, tulathromycin, gamithromycin, azithromycin (topical), lincomycin, clindamycin, and pirlimycin. They are available for parenteral, oral, or topical administration. To date, no streptogramins have been authorised for veterinary use within the EU.

Further background information on the permissible use outside the terms of the marketing authorisation of macrolides, ketolides and lincosamides can be found in the EMA 'Scientific advice under Article 107(6) of Regulation (EU) 2019/6 for the establishment of a list of antimicrobials which shall not be used in accordance with Articles 112, 113 and 114 of the same Regulation or which shall only be used in accordance with these articles subject to certain conditions' (EMA/CVMP, 2023). Based on this advice, the final list of antimicrobials, together with specific conditions for their use outside of the terms of marketing authorisation was established by Regulation (EU) 2024/1973 (European Union, 2024).

6.1.1. Use and indications in food-producing animals

The macrolides tilmicosin, tylosin and tylvalosin are widely used for the treatment of diseases that are common in food-producing animals. The most common indications for food-producing animals are the treatment of gastrointestinal infections and the treatment and metaphylaxis of respiratory infections (EMA/CVMP/SAGAM, 2011). In swine, specific indications also include arthritis. In cattle, in addition to all common infections such as respiratory and genital infections, foot lesions and intramammary infections are listed as principal indications.

No macrolides are authorised for use in aquaculture in the EU. However, erythromycin, lincomycin and tylosin were identified as necessary for the treatment of food-producing aquatic species, in the EMA 'Scientific advice under Article 114(3) of Regulation (EU) 2019/6 on veterinary medicinal products - List of substances used in veterinary medicinal products authorised in the Union for use in food-producing terrestrial animal species or substances contained in medicinal products for human use authorised in the Union, which may be used in food-producing aquatic species in accordance with Article 114(1)' (EMA/CVMP, 2025). The final aforementioned list of aquatic substances will be reflected in a Commission implementing Regulation according to Article 114(3).

Azithromycin and clarithromycin are also included in the list established by the Commission Implementing Regulation (EU) 2025/901 ('list of essential substances for equines'), for the treatment of *Rhodococcus equi* infections susceptible to azithromycin or clarithromycin, respectively, in equine species (European Union, 2025). However, it should be noted that only one topical VMP containing azithromycin is approved for dogs in the EU and that no VMPs containing clarithromycin are approved in animals in the EU. Thus, treatment in horses with such substances is mainly done outside of the terms of marketing authorisation with human medicinal products within the permissible framework.

The indications for the more recently approved macrolide-containing products are more restricted. New requirements include the definition of clinical disease(s) and causative organism(s) in the product indication, as requested in currently approved guidelines on antimicrobials, such as the 'Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP, 2021).

- In swine, tylvalosin is indicated for the treatment of porcine proliferative enteropathy caused by *Lawsonia intracellularis*, and for the treatment and metaphylaxis of swine dysentery caused by *Brachyspira hyodysenteriae* and swine enzootic pneumonia caused by *Mycoplasma hyopneumoniae*. Injectable products containing tylosin are also indicated for the treatment of swine enzootic pneumonia and respiratory infections caused by *Actinobacillus pleuropneumoniae, Pasteurella multocida* and *Glaesserella parasuis* (EMA/CVMP, 2022, 2023; EMA/CVMP/SAGAM, 2011).
- In cattle, tylosin and tilmicosin are approved for oral administration for treatment and metaphylaxis of pneumonia in calves, notably due to *Mycoplasma* spp. Furthermore, detailed indications for the injectable macrolides on centralised authorisation are, depending on the product, treatment of bovine respiratory infections caused by *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*, the treatment and metaphylaxis of bovine respiratory disease associated with *Mannheimia haemolytica*, *Mycoplasma bovis*, and infectious bovine keratoconjunctivitis associated with *Moraxella bovis* (EMA/CVMP/SAGAM, 2011).
- In poultry species, oral products containing macrolides (tylosin, tilmicosin or tylvalosin) are approved for the treatment and metaphylaxis of respiratory disease associated with *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

- In rabbits, oral products containing tilmicosin are approved for treatment and metaphylaxis of respiratory infections due to susceptible *Pasteurella multocida* and *Bordetella bronchiseptica*.

In the EU, lincomycin is authorised in combination with spectinomycin for the treatment of Gram-positive and anaerobic respiratory and enteric infections in livestock. Indications in authorised products include *Brachyspira hyodysenteriae*, *Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae*, *Brachyspira pilosicoli*, *Lawsonia intracellularis* and associated Enterobacterales (e.g., *Escherichia coli*) in pigs, and *Mycoplasma gallisepticum*, *Avibacterium paragallinarum* and *Escherichia coli* in poultry species. The main administration route for the above is oral, except for respiratory infections in large animals, where the primary administration route is intramuscular injection.

Lincomycin in combination with neomycin is also authorised for intramammary treatment of staphylococcal, streptococcal, and mycoplasma intramammary infections in cattle. In ruminants, lincomycin is approved for use against *Staphylococcus aureus*-associated arthritis, and as a topical treatment of foot lesions in cattle. Pirlimycin was authorised exclusively for the treatment of subclinical intramammary infections in lactating cows caused by staphylococcal or streptococcal infections, and since 2025 is no longer authorised.

Concerning streptogramins, and considering that virginiamycin possesses an MRL status in poultry species (EMA/CVMP, 2015), it could be used in veterinary medicine for food-producing animals in accordance with Article 113(2) of Regulation (EU) 2019/6, provided that these uses and indications are also compliant with the requirements of Article 107(3) and (4) of Regulation (EU) 2019/6. During the open call for data in the advice on the designation of antimicrobials or groups of antimicrobials reserved for the treatment of certain infections in humans (EMA/CVMP, 2022), there were reports of the use of virginiamycin for necrotic enteritis in fattening chickens and for laminitis in horses.

6.1.2. Use and indications in companion animals

Few macrolides have authorised indications in companion animals. In dogs, spiramycin is authorised, often in combination with metronidazole in oral formulations, for the treatment of Gram-positive and anaerobic infections of the oral cavity and sinuses. Products containing erythromycin for injection have limited availability in the EU for the treatment of respiratory and skin infections due to *Staphylococcus* spp. and *Streptococcus* spp.

Regarding horses declared as not being intended for slaughter for human consumption in the single lifetime identification document referred to in Article 8(4) of Regulation (EU) 2019/6, i.e., 'the passport', and other companion animals, theoretically, any substances can be used, as per Article 112 of Regulation (EU) 2019/6 (with the exception of antimicrobials reserved for the treatment of certain infections in humans, as established in Regulation (EU) 2022/1255) (European Union, 2022a). As indicated above, no macrolides are authorised for use in horses; however, they could be used in equidae species subject to certain conditions under Articles 112 and 113 as per Commission Implementing Regulation (EU) 2024/1973. Additionally, azithromycin and clarithromycin are listed in the Commission Implementing Regulation (EU) 2025/901, as substances which bring added clinical benefit for the treatment of equidae in the EU (European Union, 2025). Outside the terms of a marketing authorisation, under the permissible framework, macrolides (e.g., erythromycin, clarithromycin, azithromycin, gamithromycin and tulathromycin) are used in monotherapy or have been used in combination with rifampicin to treat severe and life-threatening cases of pneumonia in foals caused by *Rhodococcus equi*.

Macrolides are also part of the recommended treatment (in combination with, e.g., rifampicin and a fluoroquinolone) for cats and dogs for rare but serious, life-threatening infections caused by

Mycobacteria spp. (Ettinger, 2018; Möstl et al., 2015). One topical product containing azithromycin (in combination with miconazole and sulfamethoxazole) is authorised for dogs. Erythromycin may be used to treat enteritis due to *Campylobacter jejuni* in dogs, when treatment is warranted (CDC, 2021; Monfort et al., 1989).

Concerning lincosamides, in dogs and cats, clindamycin is authorised for oral treatment of infected wounds, abscesses, and oral and dental infections due to *Staphylococcus* spp., *Streptococcus* spp., *Bacteroides* spp., *Fusobacterium* spp., and *Clostridium perfringens*. In dogs, it is also indicated for superficial pyoderma caused by *Staphylococcus pseudintermedius* and osteomyelitis caused by *Staphylococcus aureus*. A topical formulation of clindamycin is on the EU market for the treatment of superficial wounds and superficial interdigital pyoderma in dogs.

Although lincomycin is also authorised for parenteral administration in dogs and cats, in practice, oral administration of clindamycin is the preferred option for treating infected wounds, abscesses, and oral and dental infections caused by *Staphylococcus* spp., *Streptococcus* spp., and anaerobic bacteria (*Bacteroides* spp. and *Fusobacterium* spp.), as well as *Clostridium perfringens*.

According to published literature, clindamycin is used in dogs and cats within the permissible framework for indications outside the terms of the marketing authorisation, e.g. osteomyelitis, prostatitis, in combination therapy for sepsis and acute pneumonia, and for protozoal infections (toxoplasmosis, neosporosis, babesiosis) (Giguère et al., 2013; Riviere & Papich, 2018; WSAVA, 2020).

Lincosamides are contraindicated for systemic treatment in horses, ruminants, or rabbits due to the potential to cause overgrowth of *Clostridium difficile*, which can result in severe fatal diarrhoea.

6.2. Sales of macrolides and lincosamides in veterinary medicinal products in the EU

Sales of antibiotic VMPs available in the ESVAC and ESUAvet databases[2] were used to analyse the aggregated sales of macrolides and lincosamides across 29 EU/EEA countries from 2017 to 2024. To conduct a trend analysis during this period of time, the data were analysed using the ESVAC methodology. In line with this methodology, sales are presented in two formats[3]:

- **mg/PCU** - for sales of antibiotic VMPs primarily used in **food-producing animals** (including horses)[4]. The quantities of active substances sold are normalised to the animal population biomass potentially treatable with them (Population Correction Unit)[5]. This indicator includes sales of the following product forms: boluses, injectable products, intramammary products (for lactating and for dry cow treatment), intrauterine products, oral solutions (including powders and concentrates for administration in drinking water), oral pastes, oral powders (administered in feed), and premixes (for medicated feed typically produced by feed mills).

[2] The latest ESUAvet annual surveillance report, as well as the ESUAvet Power BI public dashboard, containing data on sales and use of antimicrobial medicinal products in animals across Europe are available on the EMA website: https://www.ema.europa.eu/en/veterinary-regulatory-overview/antimicrobial-resistance-veterinary-medicine/european-sales-use-antimicrobials-veterinary-medicine-esuavet-annual-surveillance-reports

[3] For further information on ESVAC methodology, please refer to the last ESVAC report available on the EMA website : https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2022-trends-2010-2022-thirteenth-esvac-report_en.pdf. To be noted that antibiotic VMPs for topical use and HMPs are out of scope.

[4] Horses are considered food-producing animals, as per Regulation (EC) No 854/2004. For the purpose of this analysis, sales of VMPs authorised for horses not intended for slaughter are also included.

[5] For further information on PCU methodology, please refer to the first ESVAC report available on the EMA website: https://www.ema.europa.eu/en/documents/report/trends-sales-veterinary-antimicrobial-agents-nine-european-countries_en.pdf

- **Tonnes** - for sales of antibiotic VMPs mainly used in **companion animals**. These figures refer to sales of tablets since tablets are typically approved for companion animals only and are not normalised to PCU.

The results presented below refer to sales from 2017 to 2024 of the following active substances as reported to the ESVAC and ESUAvet databases (Table 3).

Table 3. Macrolides and lincosamides active substances reported to ESVAC and ESUAvet databases, between 2017 and 2024

Class	Substances
Macrolides	Erythromycin Gamithromycin Spiramycin Tildipirosin Tilmicosin Tulathromycin Tylosin Tylvalosin
Lincosamides	Clindamycin[1] Lincomycin Pirlimycin

[1] MRL not established for any food-producing animals.

Regarding streptogramins, no sales were reported in the ESVAC and ESUAvet databases from 2011 to 2024.

6.2.1. Sales for food-producing animals (in mg/PCU) in 2024 in 29 EU/EEA countries

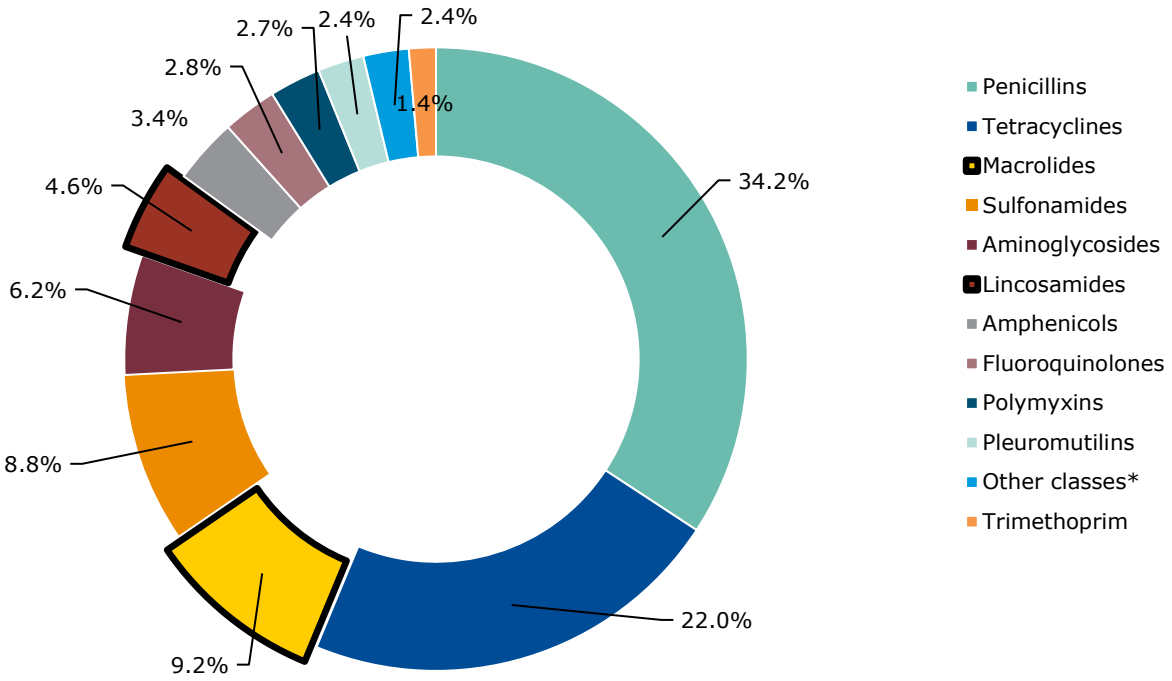
In 2024, macrolides were the third most-sold antimicrobial class for food-producing animals, representing 9.3% (7.8 mg/PCU) of total aggregated sales, while lincosamides were the sixth most-sold antimicrobial class, accounting for 4.6% (3.9 mg/PCU) (Figure 1). The spatial distribution varied considerably across the 29 EU/EEA countries, with macrolide sales ranging from 0.001 mg/PCU to 24.2 mg/PCU and lincosamide sales from 0.001 mg/PCU to 16.6 mg/PCU. One country reported no sales for both classes in 2024.

Regarding product forms in 2024, for macrolides, oral solutions were the highest-selling product form, accounting for 79.9% of the total sales (mg/PCU), followed by premixes (12.3%), injectable products (6.7%), oral powders (0.9%) and intramammary products (0.1%). When combining oral solutions, premixes and oral powders[6], 93.2% of total sales of macrolides for use in food-producing animals were VMPs predominantly intended for group treatment.

For lincosamides, a similarly high proportion of VMP sales was observed for group treatment, accounting for 94.5% of total sales. Oral solutions were again the leading product form (74.6%), followed by premixes (19.9%), injectable products (5.2%), and intramammary products (0.2%). For lincosamides, the proportion of oral powders is negligible (<0.01%).

[6] Oral powders that can be administered both via feed and as oral solution are reported in ESVAC and ESUAvet databases as oral powders. Although a small proportion of oral powders and oral solutions are suitable for the treatment of single animals or a very limited number of animals, the overall sales figures for these product forms, in addition to the sales of premixes, provide a reasonable estimate of sales for group treatment, including groups in one pen / farm.

Figure 1. Proportion of aggregated sales in 2024 (in mg/PCU), by antibiotic class, in 29 EU/EEA countries



* Other classes include cephalosporins, other quinolones, other antibacterials, nitrofuran derivatives and imidazole derivatives. Of note, some sales may involve non-food-producing animals, such as companion animals, fur animals, exotic birds, and racing pigeons.

Data source: ESUAvet database, EMA

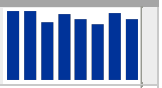
6.2.2. Sales (in mg/PCU) trends of macrolides and lincosamides for food-producing animals

Since 2017, sales of macrolides in the 29 EU/EEA countries have fluctuated, showing a modest overall decline of 8.6% by 2024 (from 8.6 mg/PCU in 2017 to 7.8 mg/PCU in 2024). The lowest value during this period was observed in 2022 (6.9 mg/PCU). Sales of lincosamides, compared to 2017, decreased by 56.1% in 2024 (from 8.9 mg/PCU in 2017 to 3.9 mg/PCU in 2024), with small year-toyear- variations observed during this period of time. (Figure 2).

At the substance level, tylosin, lincomycin, and tilmicosin were the three most-sold antibiotic substances during this eight-year analysis period, accounting for 94.1% of all macrolide and lincosamide sales in 2024. Tylosin, lincomycin and tilmicosin sales generally declined between 2017 and 2024, although the magnitude and pattern of change differed by substance. For tylosin, despite some year-to-year fluctuations, a moderate 12.0% reduction was observed (from 6.76 mg/PCU in 2017 to 5.95 mg/PCU in 2024), while for lincomycin sales declined by 56.1% (from 8.91 mg/PCU in 2017 to 3.91 mg/PCU in 2024). Tilmicosin showed small year-toyear- variations within a narrow range, with 2024 values similar to 2017 (from 1.20 mg/PCU in 2017 to 1.21 mg/PCU in 2024) (Table 4). Sales of other less-sold antibiotic substances have also decreased over this period, except for tylvalosin, tulathromycin (with an intrinsic long elimination shelf-life) and clindamycin, for which increases of 58.9%, 159.0% and 94.2%, respectively, were noted (Table 4).

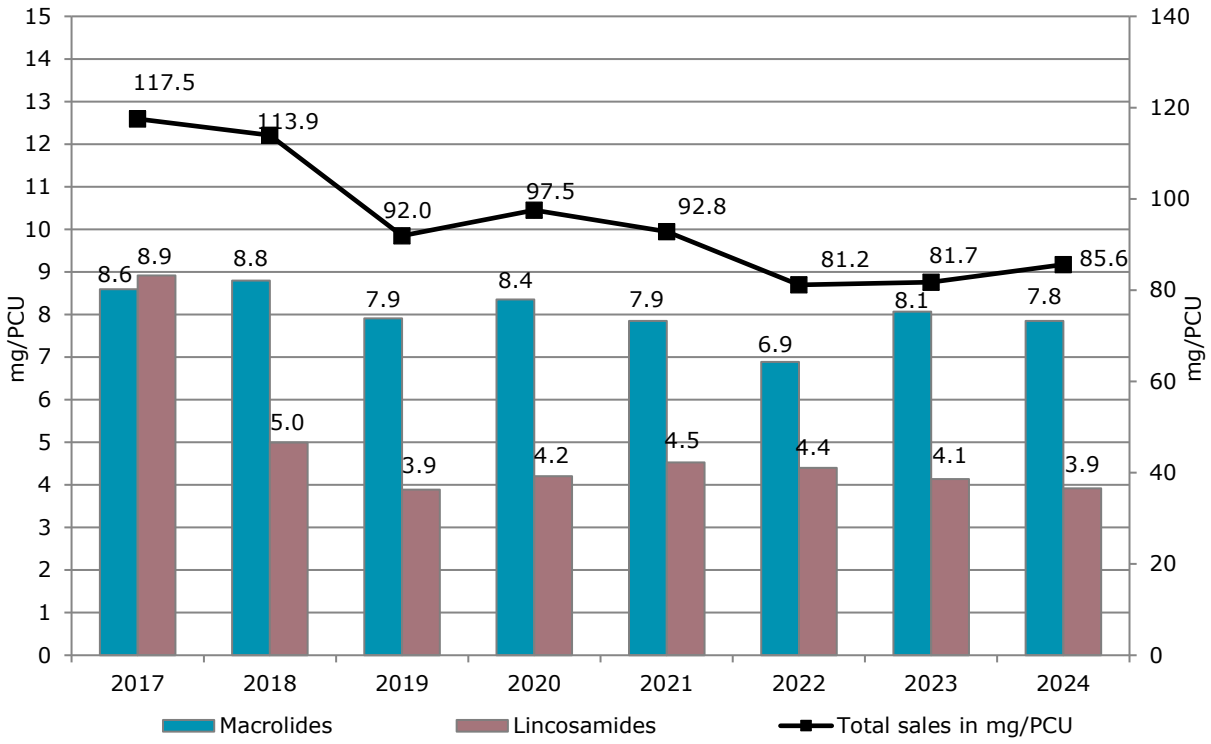
Regarding the product forms, in 2017, group treatment forms (oral solutions, premixes and oral powders) accounted for 93.6% of all sales, and in 2024 this proportion remained at 94.6%. Notably, from 2017 to 2024, sales of all product forms decreased, except for intramammary products (Figure 3).

Table 4. Trends of aggregated sales (mg/PCU) by antibiotic substance from 2017 to 2024, in 29 EU/EEA countries

Substance	2017	2018	2019	2020	2021	2022	2023	2024	Trend
Tylosin	6.76	6.76	5.67	6.47	5.94	5.44	6.60	5.95	
Lincomycin	8.91	4.99	3.88	4.20	4.52	4.40	4.13	3.91	
Tilmicosin	1.20	1.36	1.62	1.29	1.26	0.91	1.04	1.21	
Tylvalosin	0.22	0.24	0.27	0.31	0.33	0.23	0.15	0.35	
Tulathromycin	0.05	0.07	0.06	0.07	0.08	0.09	0.10	0.14	
Erythromycin	0.15	0.15	0.12	0.06	0.13	0.13	0.10	0.13	
Spiramycin	0.16	0.17	0.12	0.11	0.08	0.06	0.05	0.05	
Gamithromycin	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	
Tildipirosin	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.01	
Clindamycin	0.002	0.003	0.003	0.003	0.004	0.004	0.004	0.004	
Pirlimycin	0.0001	0.0001	0.0001	0.0001	0.00004	0.00001	0.0000004	0.0000002	

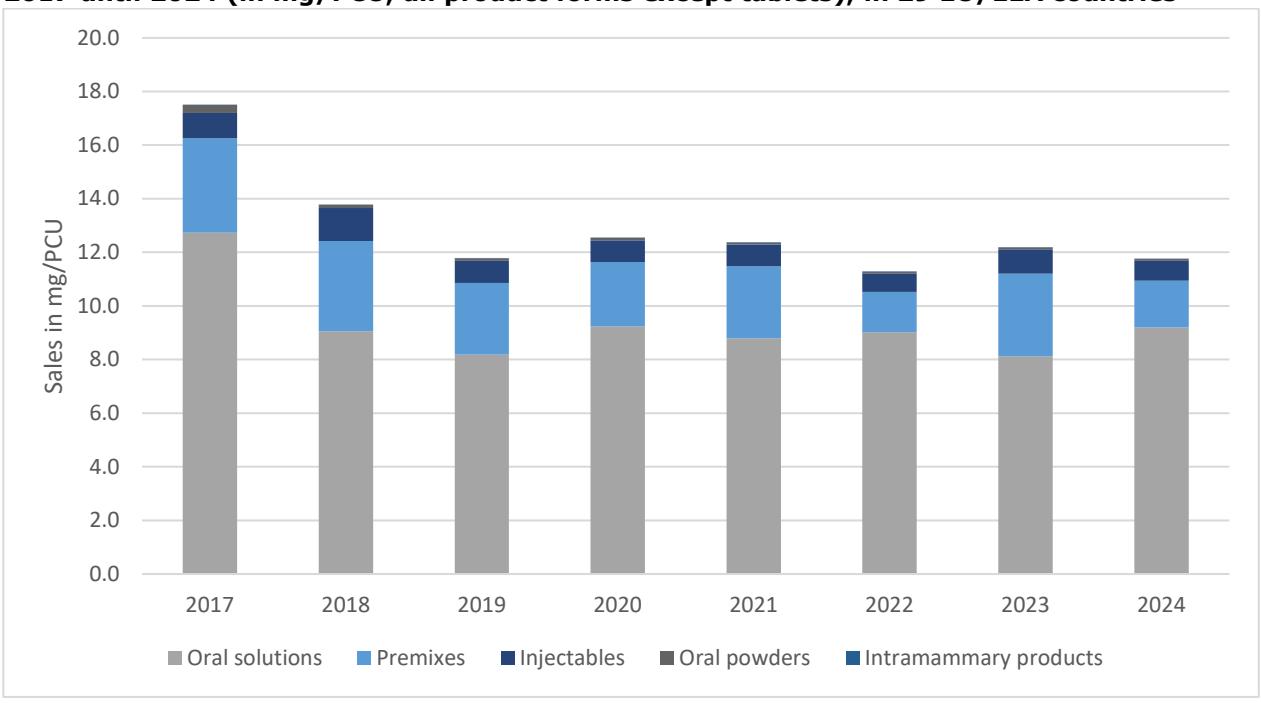
Data sources: ESVAC (2017-2022) and ESUAvet (2023-2024) databases, EMA

Figure 2. Trends of aggregated overall sales, macrolides and lincosamides, from 2017 until 2024 (in mg/PCU, all product forms except tablets), in 29 EU/EEA countries



Data sources: ESVAC (2017-2022) and ESUAvet (2023-2024) databases, EMA

Figure 3. Trends of aggregated sales of macrolides and lincosamides by product form from 2017 until 2024 (in mg/PCU, all product forms except tablets), in 29 EU/EEA countries



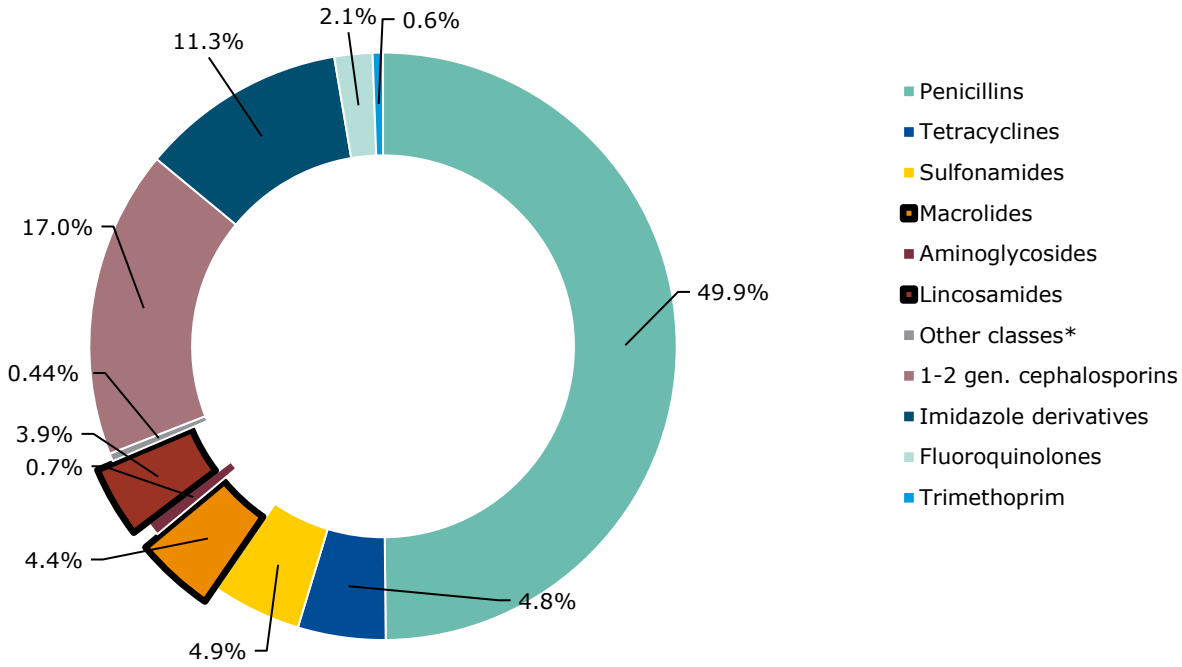
Data sources: ESVAC (2017-2022) and ESUAvet (2023-2024) databases, EMA

6.2.3. Sales of VMPs for companion animals (in tonnes) in 2024 for 29 EU/EEA countries

In 2024, macrolides and lincosamides were the fourth and the sixth most-sold antimicrobial classes for companion animals (tablet sales), representing 4.4% (2.8 tonnes) and 3.9% (2.5 tonnes) of the total aggregated sales, respectively (Figure 4).

The spatial distribution varied considerably across the 29 EU/EEA countries. Macrolide sales ranged from <0.0001 tonnes to 1.0 tonnes, with 7 countries reporting no sales in 2024. Lincosamide sales ranged from <0.0001 tonnes to 0.57 tonnes, with 3 countries reporting no sales in 2024.

Figure 4. Proportion of aggregated sales (in tonnes) in 2024, by antibiotic class in 29 EU/EEA countries



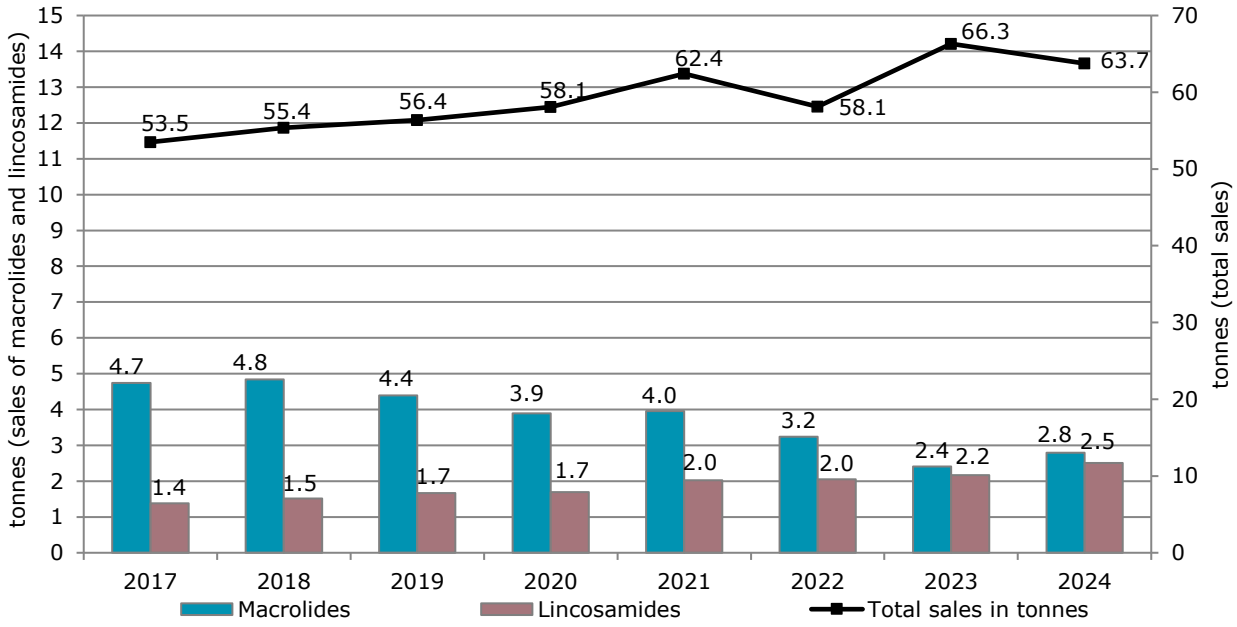
* Other classes include polymyxins, amphenicols, nitrofuran derivatives and pleuromutilins. Imidazoles derivatives refer to metronidazole. Notably, some sales may involve fur animals, exotic birds, and racing pigeons.

Data source: ESUAvet database, EMA

6.2.4. Sales (in tonnes) trends of VMPs containing macrolides and lincosamides for companion animals

Since 2017, sales of macrolides in the 29 EU/EEA countries have decreased by 41.1% (from 4.7 tonnes in 2017 to 2.8 tonnes in 2024), with the lowest value during this period observed in 2023 (2.4 tonnes) (Figure 5). However, sales of lincosamides have increased steadily, by 81.3% since 2017, peaking in 2024 (from 1.4 tonnes in 2017 to 2.5 tonnes in 2024) (Figure 5).

Figure 5. Trends of aggregated overall sales, sales of macrolides and lincosamides, from 2017 until 2024 (in tonnes, tablets, VMPs), in 29 EU/EEA countries



Data sources: ESVAC (2017-2022) and ESUAvet (2023-2024) databases, EMA

Note: This figure should be interpreted with caution, as it represents only tablet VMPs sales data. Furthermore, these data are not normalised to the companion animal population.

7. The use of macrolides, lincosamides and streptogramins in human medicine

7.1. Indications in human medicine

Macrolides and lincosamides are authorised for human use in most EU/EEA countries and play a critical role in the treatment of bacterial infections, particularly in respiratory and soft tissue indications. While these antibiotics are widely authorised and used, streptogramins and 16-membered macrolides other than spiramycin are authorised in only a few EU/EEA countries.

The 2017 EU Guidelines for the prudent use of antimicrobials in human health (European Union, 2017) are high-level recommendations to ensure that antimicrobials are used appropriately. They recommend that national strategies should include, among others, antimicrobial stewardship programmes at all levels of care.

Considering the differences in the prevalence of resistance between countries, all SmPCs of antibacterial agents in the EU include the following sentence in Section 4.1: *'Consideration should be given to official guidance on the appropriate use of antibacterial agents.'* Some countries have developed national guidelines for antimicrobial use, specifying indications, dosing regimens, and durations, given the prevalence of resistance and the need for treatment protocols. Other countries follow recommendations established by local and/or European scientific societies, and/or WHO (e.g. 'The WHO AWaRe antibiotic book) (WHO, 2022).

7.1.1. Indications for macrolides in human medicine

As indicated above, macrolides are generally bacteriostatic and characterised by a moderately broad spectrum of activity. In human medicine, macrolides are active against most Gram-positive (e.g., *Staphylococcus* spp., including beta-lactamase-producing strains, *Streptococcus* spp., *Enterococcus* spp., *Clostridium* spp.), selected Gram-negative organisms (e.g. *Neisseria gonorrhoeae*, *Helicobacter pylori* and *Campylobacter* spp., *Shigella* and *Salmonella* spp.), and several species responsible for intracellular infections, such as *Mycobacterium* spp., *Chlamydia* spp., *Mycoplasma pneumoniae*, and *Legionella* spp. (Gordon, 2018; Van Bambeke, 2018a, 2018b; van Ingen, 2018; Wenzler & Rodvold, 2018).

Macrolides are among the most used classes of antibiotics in humans. They are used in the management of Respiratory Tract Infections (RTIs), acute bacterial sinusitis, acute bacterial otitis media, pharyngitis, tonsillitis, mild to moderately severe Community Acquired Pneumonia (CAP), uncomplicated chlamydia infections, urethritis, cervicitis, acute exacerbation of chronic bronchitis (adequately diagnosed), Skin and soft tissue infections (SSTIs), campylobacteriosis and *Helicobacter pylori* infections. Macrolides are also an important treatment alternative for patients allergic to penicillin and cephalosporins (EMA/CVMP, 2022).

Clarithromycin has an orphan designation for the treatment of nontuberculous mycobacterial lung disease (European Union, 2022c). Azithromycin dihydrate has an orphan designation for the prevention of bronchopulmonary dysplasia (European Union, 2022b).

In 2025, EMA's human medicines committee (CHMP) recommended several changes to the use of the antibiotic azithromycin in the EU, including the removal of certain indications. As a result, most of the authorised uses in human medicine have been amended to be more precise. The dosing recommendations, including per age groups, have also been harmonised (EMA, 2025a).

This extensive review was carried out to promote a more rational use of this antibiotic based on current evidence and preserve its effectiveness, after recent data showing that AMR development against azithromycin has been increasing in recent years, and a recent DARWIN EU study showing a broad use of this antibiotic across the EU, both in adults and children (EMA, 2023).

7.1.2. Indications for lincosamides in humans

Lincosamides are represented mainly by two substances: clindamycin and lincomycin. Due to superior microbiological activity and bioavailability of clindamycin, lincomycin is rarely used clinically today. The antibacterial spectrum of activity of clindamycin is similar to that of macrolides, streptogramins, and chloramphenicol (Johnson, 2021).

Clindamycin is active against Gram-positive bacteria, e.g. *Staphylococcus* spp. (including many beta-lactamase-producing strains), *Streptococcus* spp., including penicillin-resistant *Streptococcus pneumoniae*, but it is not typically active against *Enterococcus* spp. or Gram-negative bacteria (Danziger, 2017). It has a potent activity against anaerobic bacteria such as *Bacteroides fragilis*,

Clostridium perfringens, *Fusobacterium* spp., *Prevotella melaninogenica* and *Peptostreptococcus* spp. (Johnson, 2021). Lincosamides also have activity against some protozoa (EMA/CVMP, 2022).

Currently, clindamycin is regarded as the first-line treatment for bacterial vaginosis. Other essential indications are the treatment of staphylococcal anaerobic infections, including mixed infections (for which they must be combined with an antibiotic with activity against aerobic Gram-negative bacilli) (Finch et al., 2010).

The high prevalence of clindamycin-resistant *Staphylococcus* spp., *Streptococcus* spp., and anaerobes in some geographic locations limit the clinical usefulness of this agent. Also, as a bacteriostatic antibiotic, clindamycin is not considered to be suitable to treat severe infections as monotherapy, especially in immunocompromised hosts (Danziger, 2017). To treat the above-mentioned infections, including staphylococcal infections, alternative antibiotic agents (e.g. penicillin–beta-lactamase inhibitor combinations, tetracycline, cephalosporins and metronidazole,) are available (Danziger, 2017).

Clindamycin is used in combination for the treatment of inhalational anthrax, however the burden of this disease is low (ECDC, 2022). For anthrax, there are alternatives to clindamycin, e.g. vancomycin or linezolid, that can be included as part of combination therapy (Wilson, 2021). The incidence of anthrax has declined over the last few decades and remains at a very low level. In 2020, there were only three confirmed cases reported in the EU/EEA area (ECDC, 2022). In the EU, clindamycin is authorised at national level and it is indicated for the treatment of serious infections caused by anaerobic bacteria, including intra-abdominal infections, SSTIs, tonsillitis and dental infection. As needed, clindamycin should be administered in conjunction with another antibacterial agent that is active against Gram-negative aerobic bacteria (EMA/CVMP, 2022).

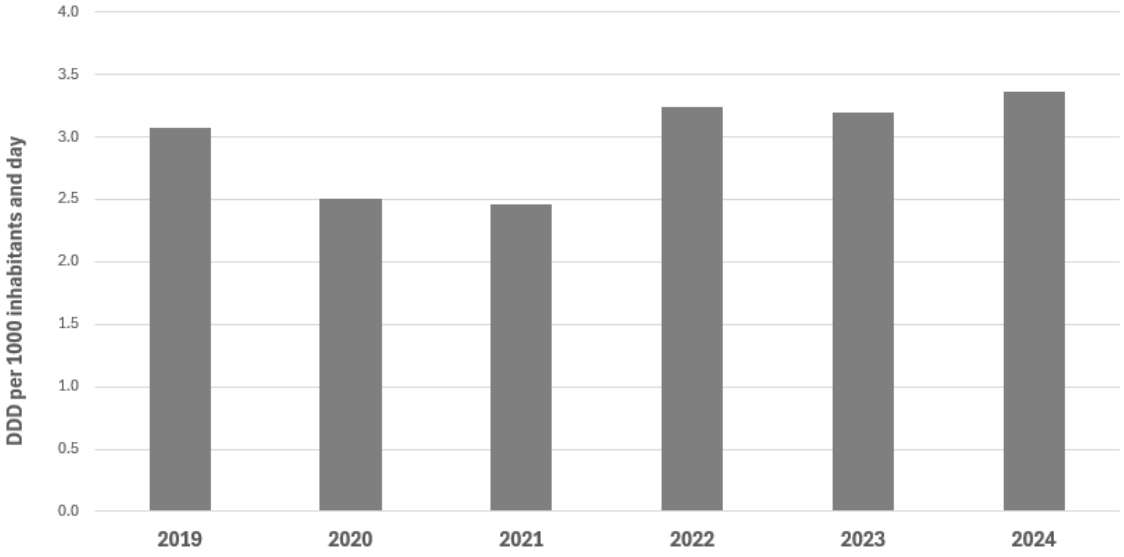
7.1.3. Indications for streptogramins in humans

Streptogramins are a group of natural (virginiamycin, pristinamycin) or semisynthetic (quinupristin-dalfopristin) cyclic peptides belonging to the macrolide–lincosamide–streptogramin group of antibiotics. France is the only European Member State in which streptogramins (i.e. pristinamycin) are authorised for human use. Pristinamycin is included in guidelines for the treatment of sinusitis, exacerbation of chronic bronchitis, pneumonia and skin infections, mainly in cases of penicillin allergy (ANSM, 2023).

7.2. Consumption of macrolides, lincosamides and streptogramins in humans in the EU/EEA (ESAC-Net)

The total (community and hospital sectors combined) EU/EEA population-weighted mean consumption of macrolides, lincosamides and streptogramins (J01F) has fluctuated between 2019 and 2024, reaching its lowest level in 2021 (Figure 6). The decrease observed in 2020 and 2021, during the pandemic, was transitional and no statistically significant trends were detected at the EU/EEA level during the period (ECDC, 2025b). In 2024, as in previous years, total consumption varied significantly across Member States, ranging from 0.7 to 6.3 DDD per 1,000 inhabitants per day.

Figure 6. Total (community and hospital sectors combined) population-weighed EU/EEA mean* consumption of macrolides, lincosamides and streptogramins (J01F) consumption trends, 2019-2024 (in DDD per 1000 inhabitants per day)



		2019	2020	2021	2022	2023	2024
EU/EEA *		3.1	2.5	2.5	3.2	3.2	3.4
Country range	Min	0.6	0.6	0.5	0.6	0.6	0.7
	Max	6.7	5.9	5.6	7.3	6.1	6.3

*) EU/EEA refers to a population-weighted mean based on reported and imputed data from 29 EU/EEA countries. Use of imputations has been described in 'European Centre for Disease Prevention and Control. Antimicrobial consumption in the EU/EEA (ESAC-Net)' - (ECDC, 2025b). Data source: ESAC-Net, ECDC

In 2024, at the EU/EEA level, the community consumption of MLS (J01F) came second only to penicillins (J01C), representing 18% of the total consumption of systemic antibiotics (J01) in the community (3.3 out of 18.8 DDD per 1,000 inhabitants per day). Significant differences were observed between Member States, as in 2024, the consumption in the community expressed in DDD per 1000 inhabitants per day varied by a factor of nine between 28 EU/EEA countries, ranging from 0.7 to 6.2 DDD per 1000 inhabitants per day in Norway and Slovakia, respectively. Additionally, when measured as a proportion of the total antibiotic consumption in the community, the percentage of MLS (J01F) consumption ranged from 5% (Norway) to 32% (Slovakia). In the hospital sector, also at EU/EEA level, MLS (J01F) accounted for 8% in 2024 and were the fifth most commonly consumed subgroup (after penicillins J01C; 34%, cephalosporins and other beta-lactams J01D; 28%, other antibacterials J01X; 11%, and quinolones J01M; 9%, respectively) (ECDC, 2025b).

The ESAC-Net study group reported that in 2017, three substances accounted for 90% of the consumption of MLS in the community expressed in DDD per 1000 inhabitants per day: clarithromycin, azithromycin and clindamycin (Adriaenssens et al., 2021). While lincosamide (J01FF; mainly clindamycin) consumption was reported in all countries, streptogramin (J01FG, pristinamycin) consumption in the community was reported only in France. Different European countries reported a distinct pattern of MLS use, illustrated by Norway which had the highest use of short-acting macrolides (i.e. erythromycin) relative to the total use of MLS (>40%), whereas certain European countries used intermediate-acting macrolides the most (i.e. clarithromycin, except Denmark, mainly roxithromycin),

and the others reported long-acting macrolides primarily, like azithromycin (Adriaenssens et al., 2021).

8. Occurrence of resistance in bacteria from animals and humans

8.1. Resistance in bacteria of animal origin

Resistance to MLS is widespread among bacteria isolated from animals. To date, harmonised and comparable resistance data remain limited. Although individual Member States have conducted studies, these are often based on different evaluation criteria and are therefore only partially similar. Thus, comparing prevalence data of resistance across time periods and geographical sites is challenging due to the origin of isolates, antimicrobial panels used, susceptibility testing methods and interpretation criteria for resistance may differ (Schwarz et al., 2010). For example, in some EU countries' surveillance data are available for decades, whereas for others, the epidemiological situation is unknown. This disparity could compromise the representativeness of available data. While isolates of major animal pathogen species have been collected through national monitoring programmes, bacterial species tested vary considerably between countries reporting such data. Additionally, published scientific studies can serve as valuable sources of information.

At the EU level, EFSA coordinates EU surveillance programs, enabling harmonised, comparable resistance data for zoonotic and indicator bacteria.

Currently, there is no harmonised European surveillance system for clinical isolates from animals. At the national level, some countries have developed their own surveillance systems for AMR in diseased animals, including Czechia, Denmark, Finland, France, Germany, Ireland, the Netherlands, Norway, Sweden and Switzerland. However, these systems do not consistently monitor the same animal species, bacterial species, or antimicrobials, nor do they use the same methods for susceptibility testing or for determining resistance breakpoints. Only a few monitoring programs routinely test bacteria for macrolide resistance. One reason is the lack of interpretation options: either as clinical breakpoints or as ECOFFs. Currently, there is also an initiative aimed at compiling data on antibiotic resistance in animal pathogens, food-producing, and companion animals across Europe (EU-JAMRAI). Also, an industry-driven programme produces harmonised AMR data in diseased food-producing and companion animals from some EU countries (i.e. CEESA Vetpath).

8.1.1. EU surveillance of zoonotic and indicator bacteria of animal origin

Data on AMR in zoonotic and indicator bacteria from humans, animals, and food are collected annually by EU Member States, jointly analysed by EFSA and ECDC, and reported in a yearly 'EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food'.

The annual monitoring of AMR in animals and food within the EU is targeted at selected animal species corresponding to the reporting year. The monitoring includes zoonotic bacteria (e.g. *Campylobacter* and *Salmonella* spp.) from humans, animals, and food. The isolates from animals and food are tested against a defined panel of antibiotic agents using harmonised methods. Contrarily, isolates from humans are tested from a national clinical perspective, as described in section 8.2.1. below. Additionally, resistance in enterococci and MRSA from animals and food sources is also addressed.

Importantly, for the EU surveillance, resistance is assessed using ECOFF values and erythromycin is considered an indicator of resistance for macrolides.

- ***Campylobacter* spp.**

In *Campylobacter jejuni,* resistance to erythromycin is generally detected at low levels in samples originating from food-producing animals in the countries reporting data to EFSA for 2022 and 2023, even if the prevalence of resistance up to around 15% occurs. In comparison, *Campylobacter coli* isolates displayed higher rates of resistance to erythromycin, with large dispersion between reporting countries (e.g. range of 0.0–75.9% for *Campylobacter coli* from calves, 0.0–62.8% from pigs, 0.0-36.1% from broilers, and 0.0-87.7% from turkeys) (ECDC/EFSA, 2025b).

- ***Enterococcus* spp.**

Transferable resistance genes have emerged in *Enterococcus* spp. of animal origin, and the occurrence of resistance against macrolides is high. However, *Enterococcus* spp. is not currently tested as an indicator species on a mandatory basis at the EU level. However, some countries report data on a voluntary basis. In 2022 and 2023, the occurrence of resistance to erythromycin among *Enterococcus faecium* varied from 8.9 to 72.2% and among *Enterococcus faecalis* from 0.0 to 83.3% (ECDC/EFSA, 2025b).

8.1.2. Resistance in target animal pathogens

- ***Mycoplasma* spp.**

Available information indicates that reduced susceptibility to macrolides is present among *Mycoplasma* spp., notably in pigs for *Mycoplasma hyopneumoniae and Mycoplasma hyosynoviae* but also in *Mycoplasma bovis* from cattle and *Mycoplasma gallisepticum* from poultry species (de Jong et al., 2021; Gautier-Bouchardon, 2018; Klein et al., 2019; Swedres-Svarm, 2024).

- ***Brachyspira* spp.**

Among *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* from pigs in the EU, resistance to macrolides and lincosamides are frequent (EFSA, 2021a, 2022a). For example, when assessed with suggested ECOFFs occurrence of resistance to tylosin and tylvalosin has been reported in *Brachyspira hyodysenteriae* ranging from 32% to 80% (EFSA, 2021a; Pringle et al., 2012).

Also in 2014, based on the frequent occurrence of resistance to tylosin among *Brachyspira hyodysenteriae* in many countries, the CVMP has omitted the indication for treatment of swine dysentery from tylosin products administered orally to pigs (EMA, 2014).

- ***Pasteurellaceae***

In the national European surveillance programs, as far as they include the determination of *Pasteurella multocida* and *Mannheimia haemolytica* at all, resistance to macrolides in these bacterial species is generally not very common (BVL, 2023; FINRES-Vet, 2023; Swedres-Svarm, 2024).

- ***Staphylococcus* spp.**

Occurrence of resistance among staphylococci (*Staphylococcus aureus*) isolated in intramammary infections in bovine against macrolides is low in most EU Member States where data are available. More precisely, up to around 5% resistance to macrolides and lincosamides have been reported (ANSES, 2023; BVL, 2022; Overesch et al., 2013; Swedres-Svarm, 2023). However, in Czechia resistance to lincosamides was detected in around 20% of isolates (SVU, 2024).

Among companion animals and horses, occurrence of resistance to macrolides and lincosamides in *Staphylococcus aureus* is low in Finland, Germany, and Sweden (BVL, 2023; FINRES-Vet, 2023; Swedres-Svarm, 2024). Contrary, among *Staphylococcus aureus* from skin infections in pigs in Germany, resistance to erythromycin was common (BVL, 2023).

EFSA identified high levels of resistance to lincosamides in *Staphylococcus pseudintermedius* isolates from cats and dogs in the EU (EFSA, 2022b). Among isolates from dogs, the occurrence of resistance to macrolides and/or lincosamides was around 20% in France, Germany, and Sweden (ANSES, 2023; BVL, 2023; Swedres-Svarm, 2024). However, among isolates from cats in Sweden, the occurrence of resistance was lower (SVA, 2024). For other staphylococci from dogs and cats in Sweden, the occurrence of resistance varied from 5 to 10% (Swedres-Svarm, 2024).

CNS isolated in intramammary infections in bovine have developed resistance to MLS antimicrobials and the percentage of isolates showing resistance rates to tylosin (≈10%) and lincomycin (≈20%) are higher than the one of coagulase-positive staphylococci (CoPS) (ANSES, 2022).

- ***Streptococcus* spp.**

According to the French monitoring programs, resistance proportion of *Streptococcus suis* isolated in pigs is established and up to 65% of the isolates were resistant to macrolides-lincosamides. High MIC is also seen in a large proportion of isolates in Germany (BVL, 2023).

In bovine, around 15% of *Streptococcus uberis* and *Streptococcus dysgalactiae* isolates are resistant to erythromycin, and thus cross-resistant to lincosamides (inducible or constitutive MLSb phenotype) (ANSES, 2023). Among streptococci from intramammary infections in cattle in Czechia and Sweden, resistance is rare although isolates with high MIC exist (SVU, 2024; Swedres-Svarm, 2023).

In dogs, the MLSb phenotype (resistance to macrolides, lincosamides, and streptogramines) is observed in 18% of isolates from otitis (ANSES, 2022). Among *Streptococcus canis* from dogs in Finland, resistance to macrolides and lincosamides occur in 10-20% of investigated isolates (FINRES-Vet, 2023).

- ***Enterococcus* spp.**

In France, a very large proportion of *Enterococcus hirae* from pigs are resistant to erythromycin and lincomycin (ANSES, 2023). Among *Enterococcus cecorum* from poultry species (hens and broilers) in France, occurrence of resistance to erythromycin and lincomycin is lower but still about 30-40% (ANSES, 2023). Among *Enterococcus faecalis* from poultry species in Czechia, 18% were considered resistant to erythromycin (SVU, 2024).

- ***Other pathogens***

Resistance data for *Glaesserella parasuis* and *Histophilus somni* are even rarer than those mentioned above. Limited data on *Histophilus somni* from cattle in Finland and Czechia showed no resistance to tulathromycin (FINRES-Vet, 2023; SVU, 2024). No resistance in *Actinobacillus pleuropneumonia* to tilmicosin was reported in Denmark since 2015 (DANMAP, 2024). No resistance was reported in Finland in 2022 or Sweden in 2023 (FINRES-Vet, 2023; Swedres-Svarm, 2024). In France, resistance against erythromycin is very low (ANSES, 2018).

Information on prevalence of resistance in anaerobes is limited, but high levels of resistance to lincomycin were detected in *Clostridium perfringens* from Belgian broilers (Gholamiandehkordi et al., 2009).

8.2. Resistance in target bacteria of human origin

Many of the bacteria causing human infections against which MLS compounds may be effective are mostly human pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Neisseria gonorrhoeae, Haemophilus influenzae, Helicobacter pylori, Shigella* spp. and *Salmonella* Typhi. At the EU/EEA level, though, only a few are monitored regarding resistance.

The European Antimicrobial Resistance Surveillance Network (EARS-Net), coordinated by the ECDC, gathers information on resistance in selected bloodstream infections, but only *Streptococcus pneumoniae* is monitored regarding resistance to macrolides. Between 2019 and 2023, the macrolide resistance for the EU/EEA overall varied between 15.9% and 18.4%, ranging from 4% to 53.8% across countries (ECDC, 2024b).

Moreover, the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) reports on resistance in *Neisseria gonorrhoeae* against azithromycin. In 2022, around 25.6% of isolates had an MIC>1 mg/L (EUCAST ECCOFF), which was a significant increase since 2018 where 7.6% of isolates had an MIC>1 mg/L, although a decrease to 23.2% was observed in 2023 (ECDC, 2025c).

Apart from the monitoring programmes in the EU, single studies covering different geographical areas and bacterial species also provide information. In summary, these studies indicate varying levels of resistance across Europe.

There appears to be a lack of data on the rate of resistance in *Haemophilus influenzae* to the macrolide class of antibiotics in the EU. This may be partially explained by the fact that susceptibility testing to macrolides in *Haemophilus influenzae* is not usually performed due to the lack of a good correlation between *in vitro* and *in vivo* results (i.e., *in vitro* resistance may not be synonymous with a lack of response *in vivo*).

Among *Streptococcus pyogenes*, macrolide resistance rates vary widely depending on geographic areas; rates tend to be very low (<4%) in some Northern European countries, while in Southern and Eastern European countries, the rate of resistance is higher (5–39%) (Berbel et al., 2022). In a 6-year study in Greece, the erythromycin and clindamycin resistance rates among *Streptococcus pyogenes* were 20.4% and 18.8% respectively (Meletis et al., 2023).

In Europe, the resistance rates of *Helicobacter pylori* for adults were 17.5% for clarithromycin, and were significantly higher in Western/Central and Southern Europe (>20%) than in Northern European countries (<10%) (Megraud et al., 2013). In a more recent study, the proportion of *Helicobacter pylori* antimicrobial resistance to clarithromycin in European countries in 2018 was 21.4% ranging from 4.8 to 36.9%. People born in Western/Central Europe, Southern Europe and outside Europe were shown in univariate and multivariate analysis to have a higher risk for resistance compared with those born in Northern Europe (Megraud et al., 2021).

The emergence of multidrug-resistant (MDR) *Salmonella* Typhi (defined as resistance to chloramphenicol, ampicillin, and trimethoprim) led to the use of ciprofloxacin as the first choice. However, with the rapid emergence of resistance against the fluoroquinolone class, third-generation cephalosporins, macrolides, and carbapenems have been used increasingly for the treatment of typhoid fever. Of specific concern is the emerging resistance against azithromycin, the only remaining oral drug to treat extensively drug-resistant (XDR) *S*. Typhi. Since the first report of azithromycin resistance from Bangladesh in 2019, cases have been reported from Nepal, India, and Pakistan. Travel-associated infections with XDR *Salmonella* Typhi have been reported in several European and non-European countries. Therefore, AMR in *Salmonella* Typhi is considered a global threat (Marchello et al., 2020).

8.2.1. MLS resistance in zoonotic bacteria – data from human medicine

As indicated in the 'The European Union One Health 2024 Zoonoses report', in 2024, the first and second most reported zoonoses in humans were campylobacteriosis and salmonellosis. Shiga toxin-producing *Escherichia coli* (STEC) was the third most reported zoonotic agent in humans, followed by

listeriosis (confirmed invasive human cases of *Listeria monocytogenes)* and echinococcosis (ECDC/EFSA, 2025a).

The Food and Waterborne Disease Network (FWD-Net) monitor resistance in *Salmonella* spp. and *Campylobacter* spp. isolated from humans. The revised 'EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates', requests monitoring resistance against erythromycin in *Campylobacter* spp., whereas monitoring resistance against azithromycin is optional. For *Salmonella* spp. monitoring against azythromycin is mandatory (ECDC, 2016, 2021). Due to that, in the 'The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022', resistance against azithromycin in *Salmonella* spp. was only tested by nine countries. Resistance was overall low (0.5%) (EFSA, 2024). The highest level of resistance was found in *Salmonella Infantis* and *Salmonella Derby* (both 2%); and *Salmonella Kentucky* (3.9%) (EFSA/ECDC, 2023).

The level of resistance to erythromycin in human *Campylobacter jejuni* isolates in the EU was overall very low (1.1%), except for Spain, where the results indicated a higher level of resistance (14.2%). In *Campylobacter coli*, however, the overall level was higher (12.6%), but highly variable between countries, ranging from 0% in Ireland and Slovenia to 55.3% in Portugal.

In the 'Campylobacteriosis - Annual Epidemiological Report for 2022', for macrolides, which is the class of agents used to treat children with severe Campylobacter infections, or adults if the bacteria are resistant to fluoroquinolones, resistance was detected in 0.9% of *Campylobacter jejuni*, but in 7.8% of *Campylobacter coli*, with the highest proportions of resistance in *Campylobacter coli* in Greece (38.5%, although few isolates were tested), Portugal (26.9%) and Spain (19.3%). Decreasing trends in macrolide resistance were observed in six of 18 countries for *Campylobacter jejuni* and in four of 13 for *Campylobacter coli*. The proportion of isolates resistant to both of the antimicrobial classes used as first line treatment, fluoroquinolones and macrolides, was similar to resistance to macrolides alone as most isolates resistant to macrolides were also resistant to fluoroquinolones (ECDC, 2024a).

English studies on AMR in both O157:H7 STEC and non-O157:H7 STEC showed rather low rates of determinants of resistance to macrolides (Gentle et al., 2020; Greig et al., 2023).

Data on AMR in *Yersinia* spp. and *Listeria monocytogenes* are not collected at the EU/EEA level, and single-study data on MLS resistance in clinical isolates in Europe are scarce.

8.2.2. MLS resistance in commensal bacteria with a zoonotic potential – data from human infections

For bacteria with intermediate host specificity that are monitored in EARS-net, such as *Escherichia coli*, *Klebsiella pneumoniae,* and *Acinetobacter* spp., MLS antibiotics are usually ineffective and are therefore not monitored. This is also the case for other bacteria, such as *Staphylococcus aureus* and enterococci, for which macrolide compounds may be effective. These bacteria are only monitored for resistance against oxacillin, gentamicin, and vancomycin, respectively (ECDC, 2024b). Data on the prevalence of resistance to lincosamides or streptogramins are not routinely collected under EARS-Net.

The zoonotic transmission potential of *Staphylococcus aureus* has been underlined by the emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). Resistance rates in LA-MRSA as a whole are not surveyed at the EU/EEA level, but there are indications of MLS resistance found in European reports on LA-MRSA (Ceballos et al., 2020; Karampatakis et al., 2021). There. However, MRSA resistance rates are closely monitored as one of the EU antimicrobial resistance targets, for which 15% reduction at EU/EEA level should be achieved by 2030 in comparison with 2019, as established in the 'Council recommendations on stepping up EU actions to combat

antimicrobial resistance in a One Health approach' (European Union, 2023). In 2024, this value had already been exceeded, reaching 20.4% (ECDC, 2025a).

Although the data on resistance to quinupristin-dalfopristin among commensals that have caused human infections is limited, surveillance data on resistance to quinupristin-dalfopristin among commensals have been reported in the SENTRY Antimicrobial Surveillance Program (JMI Laboratories). It evaluated the susceptibility of *Staphylococcus aureus* and enterococci clinical isolates from hospitals and medical centres worldwide (overrepresented by the US and Europe) over 20 years, from 1997 to 2016. Resistance to quinupristin-dalfopristin was found in 0.2% of MRSA isolates and 0.9% of vancomycin-resistant *Staphylococcus aureus* (Diekema et al., 2019). Under the same surveillance programme, 83.5% of Vancomycin-resistant Enterococcus (VRE) *faecium* isolates from Europe were susceptible to quinupristin-dalfopristin. Oxazolidinones, daptomycin, oritavancin and tigecycline were more active against enterococci than quinupristin-dalfopristin (Pfaller et al., 2019). Almost all antibiotics with activity against MRSA and VRE are included in the list of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, as per Commission Implementing Regulation (EU) 2022/1255 (European Union, 2022a).

9. Possible links between the use of macrolides, lincosamides and streptogramins in animals and resistance in bacteria of animal origin

The link between the use of macrolides in animals and the development of macrolide resistance in animal populations has been documented in several published studies.

The studies reviewed provide a comprehensive overview of the link between antimicrobial use and resistance in various livestock production systems. The findings highlight the critical role of AMU in shaping the resistome, with macrolides and tetracyclines emerging as key drivers of resistance. Biosecurity measures, while essential for disease control, may also contribute to resistance selection. The observed co-selection and cross-resistance mechanisms underscore the need for a more integrated approach to AMU reduction, balancing disease prevention with antimicrobial stewardship strategies to mitigate the spread of resistance.

In 2024, the 'fourth joint inter-agency report on integrated analysis of consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the EU/EEA' (JIACRA) was published by ECDC, EFSA and EMA (ECDC/EFSA/EMA, 2024). While, in the 3rd report, macrolide resistance in *C. jejuni* in humans could be related to the resistance in food-producing animals (poultry species) (ECDC/EFSA/EMA, 2021), in the fourth report, no such association was seen. Still, in pigs, macrolide consumption was positively associated with resistance to the respective groups in *Campylobacter coli* (ECDC/EFSA/EMA, 2024). This is possibly because the occurrence of resistance in food-producing animals is generally low. Instead, while resistance to macrolides is rare in *Campylobacter jejuni*, it is more frequently observed in *Campylobacter coli*. Therefore, the variation is bigger, allowing for associations to be discovered.

A study conducted on by the Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) consortium characterised the antimicrobial resistome in relation to antimicrobial use (AMU) and biosecurity in pig farming across nine European countries (Van Gompel et al., 2019). Using univariate meta-analysis, the authors identified a positive association between total AMU during the fattening phase and the presence of ARG in pigs. However, no such correlation was found for AMU during other life stages. The primary resistance determinants associated with AMU were macrolides and tetracyclines, with molecular characterisation revealing that predominantly *erm* genes and related

clusters were present in various isolates. Regarding biosecurity, internal and total biosecurity scores were assessed, showing a positive association between biosecurity measures and macrolide resistance. A more refined analysis revealed that internal biosecurity measures, such as cleaning and disinfection, were specifically associated with increased macrolide and vancomycin resistance, which might potentially be explained by high use of specific types of disinfectants. The study also suggested that cross-resistance mechanisms, such as bacterial efflux pumps induced by exposure to both antimicrobials and biocides (e.g., quaternary ammonium compounds), along with co-resistance due to genetic linkage of ARGs with biocide and metal resistance genes, may play a role (Van Gompel et al., 2019).

Nikolaisen et al. (2020) investigated the relationship between antibiotic consumption and resistance in Danish mink production. The most frequently used antibiotics were aminopenicillins, tetracyclines, and macrolides. Notably, in 2018, macrolides, primarily tylosin, were the second most prescribed antibiotic group on Danish mink farms. MIC determinations of Danish isolates revealed lower proportions of benzylpenicillin and erythromycin non-wild-type (NWT) MICs compared to tetracycline. A significant association was found between non-wild-type erythromycin-resistant strains of *Staphylococcus delphini*, a pathogen causing extraintestinal infections in mink, and macrolide use during both the weaning and growth periods. Additionally, farms with long-term macrolide use were found to be 18.2 times more likely to harbour erythromycin NWT *Staphylococcus delphini* during the weaning period (Nikolaisen et al., 2020).

Mencía-Ares et al. (2021) examined the role of production systems and biosecurity measures in the development of AMR in commensal *Escherichia coli* and *Enterococcus* spp., identifying AMU as the primary differentiating factor. A total of 148 isolates were obtained from feces, slurry, and environmental samples from 37 farms, with four isolates per herd. No association between macrolide usage and *Escherichia coli* resistance was found. However, in *Enterococcus* spp., the most frequent MDR combination involved tetracyclines, macrolides, and streptogramins (23.8%). MIC analysis indicated that non-WT phenotypes were most prevalent for tetracycline (78.8%), followed by erythromycin (39.0%). Erythromycin resistance was more frequently detected in enterococcus isolates from slurry samples and was linked to increased use of lincosamides, penicillins, and phenicols. Notably, lincosamide use was associated with a nearly twofold increase in erythromycin resistance (Mencía-Ares et al., 2021).

Duarte et al. (2023) conducted a long-term study monitoring AMR in Danish swine production using phenotypic methods and metagenomics from 1999 to 2018. *Escherichia coli* and *Enterococcus faecalis* isolates were obtained from caecal samples, with phenotypic resistance assessed via broth microdilution and genotypic resistance analysed through shotgun metagenomics. Among *Escherichia coli* isolates, resistance was most frequently observed for sulfonamides, tetracyclines, beta-lactams, and trimethoprim, whereas *Enterococcus faecalis* exhibited the highest resistance to tetracyclines, followed by macrolides. The study noted a decline in tetracycline use across all swine production age groups after 2013, while macrolide use increased after 2016. Statistical correlations indicated that increased AMU significantly elevated the relative abundance of resistance genes in the *Escherichia coli* resistome, particularly those linked to sulfonamides, but not to macrolides (Duarte et al., 2023).

Andersen et al. (2017) examined the association between AMU and resistance in the faecal microbiota of finisher pigs in Denmark. AMU was calculated relative to the rearing period. While no significant correlation was found between AMU and phenotypically measured resistance, a strong association was observed between AMU and resistance measured via metagenomics. Regression analysis indicated significant effects of macrolides, broad-spectrum penicillins, sulfonamides, and tetracyclines on their respective resistance genes (Andersen et al., 2017).

In a follow-up study, Andersen et al. (2023) quantified the impact of AMU in Danish pig farms on ARG abundance in slaughter pigs. The study identified a decline in tetracycline use alongside an increase in macrolide use. Using ResFinder, a total of 232 different ARGs were detected, with tetracycline and macrolide resistance genes consistently present across all farm visits. Over the study period (2015–2016), the overall abundance of ARGs decreased; however, macrolide resistance genes showed a slight increase, whereas aminoglycoside and lincosamide resistance genes declined. These changes coincided with Denmark's Yellow Card Scheme, which aimed to reduce tetracycline use but inadvertently led to increased macrolide consumption, potentially explaining the rise in macrolide resistance. Additionally, co-selection effects were observed, with macrolide use appearing to increase ARG abundance across multiple antimicrobial classes (Andersen et al., 2023).

A 2023 Spanish study conducted a genetic comparison of *Pasteurella multocida* strains from both healthy (n=74) and diseased (n=32) animals affected by bovine respiratory disease (BRD) during the fattening period. Whole-genome sequencing of 14 *Pasteurella multocida* isolates from clinically affected and unaffected calves was performed, followed by *in silico* analysis of multilocus sequence types, virulence-associated genes, and ARGs. No significant differences were found in virulence-associated gene content. However, in clones commonly linked to BRD cases, such as ST79, ARGs conferring resistance to macrolides and tetracyclines were identified. These findings suggest that the historical use of these antimicrobial classes in BRD treatment may have contributed to the selection and spread of resistant strains across herds (Calderón Bernal et al., 2023).

10. MLS residues, antimicrobial resistant bacteria (ARB) and antimicrobial-resistant genes (ARGs) in the environment

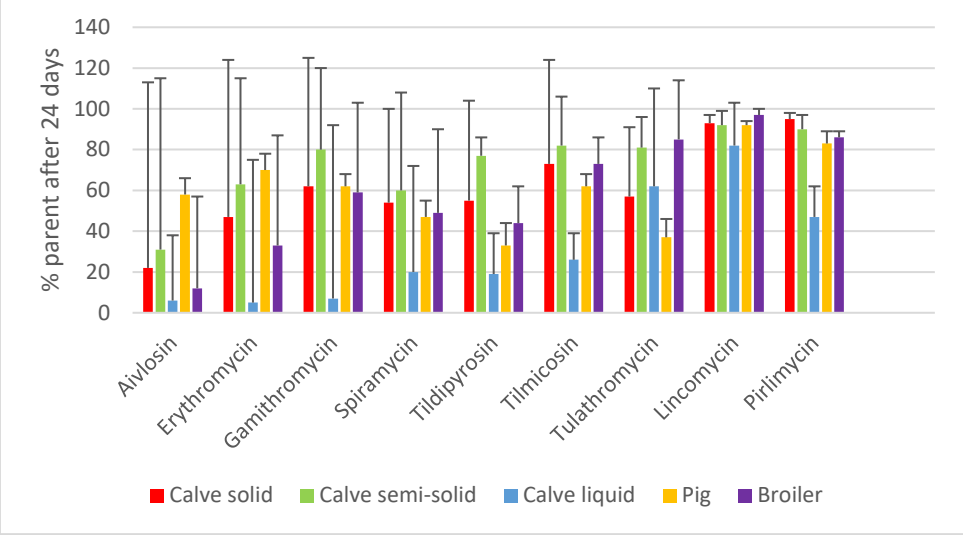
Within the EU, for all new VMPs, an environmental risk assessment (ERA) has been required as part of the authorisation process since 1993, with the relevant VICH GLs in force since 2000 (VICH GL 6 – 'Environmental impact assessment (EIAS) for veterinary medicinal products - Phase I') and 2005 (VICH GL 38 – 'Environmental impact assessments for veterinary medicinal products - Phase II'). This ERA covers the emission to the environment, fate of the active ingredient in the ecosystem and assessment of ecotoxicological risks for the ecosystem structure and functioning (EMA, 2016b). For antimicrobial VMPs for food-producing animals, depending on the estimated emission, fate aspects like sorption to soil and biodegradation are examined experimentally in the frame of a so-called Phase II ERA. Where available, this information is considered for this section. According to publicly available information stemming from authorisation procedures performed by EMA (EPARs), as well as the Dutch and Swedish national competent authorities, a Phase II assessment has been performed for products containing the macrolides tylosin, tilmicosin and tylvalosin and for the lincosamide lincomycin. Streptogramins are not authorised as VMPs in the EU and as such no environmental information is available from EU authorisation procedures. In the available EPARs for the macrolide-containing products, a risk to the environment (ecosystem structure and functioning) was not quantified (CBG-MEB, 2021a, 2021b; EMA, 2011, 2016a, 2017, 2020). For a VMP containing lincomycin, a risk for toxicity to terrestrial plants and cyanobacteria was identified that could not be mitigated (AEMPS, 2017). For VMPs used in companion animals, the exposure to the environment has historically been assumed to be limited and not to cause a risk to the environment. Thus, in the frame of the application procedures, no full ERA is currently requested for VMPs used in companion animals. Consequently, for VMPs containing macrolides and lincosamides that have been authorised only for companion animals, no environmental information is available.

10.1. Emissions of antimicrobial VMPs and their fate within the environment

Pathways for emission of antimicrobials to the environment have been described in the 'Reflection paper on antimicrobial resistance in the environment: considerations for current and future risk assessment of veterinary medicinal products' (CVMP, 2021). Although this paper was developed under the previous legal framework for VMPs, the scientific reflections in the aforementioned reflection paper remain relevant and will be considered for the development of a new 'reflection paper on the assessment of public health risks related to antimicrobial resistance acquired via the environment, resulting from the use of a veterinary medicinal product', to reflect the legal framework and requirements included in Regulation (EU) 2019/6.

This paper also covers the pathways for MLSb antimicrobials. In brief, for performing an ERA, initially, a total residue approach is applied, meaning that it is presumed that all the substance applied will be excreted and enters the environment without transformation, for instance through the spreading of manure as a fertiliser. Whether this is the case depends partly on the rate of degradation during storage of the manure. To examine this, Berendsen et al. (2018) applied a method that can be used to estimate the degradation of antibiotics during manure storage. The test conditions were supposed to mimic the general storage conditions in a slurry pit where the largest part is anaerobic with the top layer in contact with air. Calve, pig and broiler manure were tested at room temperature, with calve manure investigated in three different forms, i.e. as solid, semi-solid and liquid manure. The results showed that the macrolide tylvalosin is one of the MLS substances that degraded most rapidly, with half-lives of 5 days or less for calve and broiler manure but with slower degradation in pig manure (as demonstrated by a half-live of 35 days). Much slower degradation was observed for the macrolide tilmicosin, with a half-life up to 104 days, and for the two lincosamides tested, lincomycin and pirlimycin, with half-lives up to 269 and 699 days, respectively. Especially for the latter two, most of the parent substance was still present after 24 days. However, it should be mentioned that manure from different stables was used and, in some cases, a high variation of up to 90% was observed regarding degradation rates of the substances in manure samples from different barns. Notwithstanding this, Figure 7 illustrates the variability in removal efficiency not only across different substances, but also among different types of manure.

Figure 7. Levels of macrolides and lincosamides in manure after 24 days under storage conditions. The bars indicate the standard deviation. (Based on data from Berendsen et al. 2018)



The observation that the above-mentioned substances cannot be expected to be fully degraded after storage is also confirmed by Huygens et al. (2021), who detected lincomycin, tilmicosin, gamithromycin and tylvalosin in calf manure collected from nine Belgian farms at the time of spreading to agricultural land as fertiliser. Lincomycin was detected in all samples at concentrations ranging from 9 to 141 µg/kg and tilmicosin in eight out of the nine samples at concentrations ranging from 8 to 1149 µg/kg. Gamithromycin and tylvalosin were only detected in the manure from one farm at levels of 6 and 44 µg/kg, respectively. Similarly, Oliver et al. (2020) reported the presence of erythromycin (<1000 µg/kg), tilmicosin (0-29 µg/kg) and tylosin (0 – 115500 µg/kg) in manure from either the USA, Europe or China. Tylosin is considered to be significantly degraded during manure/slurry storage but was nevertheless detected in trace concentrations in samples of manured soils (Boxall et al., 2004). Altogether, these data indicate that, after the use of VMPs containing macrolide and lincosamide, the respective active substances are likely to reach the environment through the spreading of manure. However, the fraction of the dose that actually reaches the environment depends on the substance, type of manure and storage conditions. Furthermore, it should also be noted that degradation products may also pose similar effects as their parent substances (Berendsen et al., 2021).

Composting is often mentioned as a method to reduce the concentrations of antibiotics in manure, and Sun et al. (2022) reported half-lives for macrolides (azithromycin, erythromycin and tylosin) of 1.1 and 3.3 days under temperature-controlled and normal composting conditions, respectively. Zhang et al. (2019) also reported high removal of macrolides (clarithromycin, erythromycin, leucomycin, oleandomycin, roxithromycin and tylosin) and lincomycin.

Despite the potentially high removal rate of residues during storage and composting, an efficient removal is not assured, and MLS substances can reach soil by application of (composted) manure. Once an antimicrobial reaches the soil, its fate is determined by degradation and its mobility. Available information for tylosin shows that it is not persistent in soil, but that it might have a high potency to sorb strongly to soil, as indicated by K_{oc} values of up to 17,000 l/kg (CBG-MEB, 2021b). The rapid degradation of tylosin was also confirmed by Iverson et al. (2022), who reported half-lives in the range of 5 to 20 days. In contrast, tilmicosin was reported to be persistent in soil (CBG-MEB, 2021a) a finding confirmed by Perruchon et al. (2022), who also showed that tilmicosin persistence is increased if the substance is introduced in soil with manure, which is likely the case after use as a VMP. For tylvalosin, publicly available information only reports that it is not a Persistent, Bioaccumulative and Toxic (PBT) substance (EMA, 2020). Estimated data available for certain MLS substances (US EPA, 2012) indicate that none of them will be rapidly degraded, but, where it concerns hydrophobicity and mobility, this can differ highly between the individual compounds, with (estimated) log Kow values ranging from 0.56 for lincomycin to 7.2 for quinupristin (BioByte, 2006). In addition, as evidenced by Koc values of 108 l/kg, no mobility in soil is estimated for the latter two substances. In contrast to that, other substances such as erythromycin and clindamycin appear to be very mobile, with estimated Koc values of 25 and 13 l/kg, respectively. However, the Koc may not always be a reliable predictor of MLS substance mobility in soil, as, for instance, Koc values ranging from 300 to 17,000 l/kg were reported in the EPAR for tylosin depending on the soil tested. This indicates that the sorption of the substance is not always correlated to the organic matter content in the soil. For lincomycin, its mobility was tested in an experimental set-up by (Domínguez et al., 2014) who reported a recovery of 30% in leachates of soils amended with spiked pig slurry.

Field monitoring remains the most reliable approach for determining the environmental fate of a substance, as it provides direct evidence of its persistence, mobility, and transformation under real-world conditions. To be sure that the detection of a substance is linked to its use as a VMP, monitoring should be performed on agricultural land that is fertilised with manure. Zhou et al. (2010) reported

detection of lincomycin in soil samples from one farm where it was used, while tiamulin, tilmicosin and tylosin were not detected in samples from farms where they were used. Similarly, tylosin A was detected in soils fertilised with pig manure (Heuer et al., 2011), but it was also reported that in other occasions tylosin was not detected in soils amended with pig manure (Zhou et al., 2010). These findings are in contrast to tetracyclines, which were detected more consistently and also in cases where the substances were not known to be used on the farms (Zhou et al., 2010). This could either indicate a slower degradation of tetracyclines or other sources to the environment than their use as a VMP. Nevertheless, macrolides are reported as the most frequently quantified antibiotics in soil, organic waste products (including sewage sludges and animal faecal waste) and water in France (Haenni et al., 2022). In relation to the low detection levels of some of the MLS antimicrobials, Heuer et al. (2011) stated that detection levels are strongly affected by the binding of the antibiotic compound to the soil matrix (ageing), and the extraction procedure. Therefore, it is likely that the actual amount of antibiotics in soil microhabitats may be largely underestimated (Haenni et al., 2022).

In another study (Hou et al., 2015), tylosin and erythromycin were detected in manure samples and samples from soils amended with manure. Tylosin occurred in about 60% of the soil samples and erythromycin in about 20% of the soil samples, albeit soil concentrations were relatively low at 1.4 µg/kg. Li et al. (2022) presented an extensive review identifying eight sources of macrolides found in the aquatic environment. Three of these sources are related to VMP use, i.e. livestock farms (e.g. leakage from manure storage), agriculture (e.g. use of manure as fertiliser) and aquaculture (medicated feed and direct splashing). The main sources of macrolides in the water environment in rural areas were considered to be animal breeding and agricultural farmlands. Although many of the available studies on MLS substances and environmental exposure have been performed in China, the general picture obtained from these studies is at least partially applicable for Europe. Li et al. (2022) also collected data on water concentrations of macrolides published globally in scientific literature. For Europe, most reported concentrations were in the range of 0 to 50 µg/l (Li et al., 2022), but also higher concentrations with levels exceeding 500 µg/l were reported. These higher concentrations would result in a risk for aquatic plants. However, in Europe, these high-water concentrations of macrolides are mainly found in urban areas and thus, they are probably less related to the use of VMPs used in food-producing animals rather than to the use in companion animals or use in human medicine. Similarly, Haenni et al. (2022) reported on the detection of erythromycin in wastewater, surface and groundwater, and sediment samples in France indicating their use in humans and companion animals.

In conclusion, although residues and AMR are detected in surface and groundwater, a clear link between these findings and the use of antimicrobials as VMPs has not been established. A generalised understanding of the environmental emissions and fate of MLS antimicrobials remains elusive, as degradation rates and mobility vary significantly across individual substances and under differing exposure conditions. Even where low concentrations are reported, it is to be examined whether this is due to degradation or to ageing (dissipation). In accordance with the above, and as outlined in publicly available EPARs available for VMPs containing macrolides and lincosamides, a risk to the functioning of the ecosystem was only quantified in a single case for lincomycin (AEMPS, 2017).

Nevertheless, with the current ERA methodology, effects of antibiotic concentrations on individual bacteria in regard to induction or selection of AMR (Dong et al., 2022) cannot be investigated. Globally, no official guidelines to estimate the risk of AMR development in the environment are available. However, as indicated above, EMA recently has taken the first steps towards that direction, with the publication in 2025 of a ‘concept paper for the development of a reflection paper on the assessment of public health risks related to antimicrobial resistance acquired via the environment, resulting from the use of a veterinary medicinal product’ (EMA/CVMP/ERA, 2025). Therefore, it remains unclear whether

and to what extent the reported concentrations related to the use of VMPs might promote AMR development in the environment.

10.2. Emissions of ARB and ARGs and their fate within the environment

As indicated in the 'Reflection paper on antimicrobial resistance in the environment: considerations for current and future risk assessment of veterinary medicinal products' (CVMP, 2021) spreading of manure from food producing animals as fertiliser is considered a potential pathway for emission of resistant bacteria and resistance genes related to the use of antimicrobials in animals, including MLS. The MLSb resistance phenotype is currently mostly reported as indicator of MLSb resistant bacteria in manure and the environment. For the phenotypes MLSKO and PhLOPSA, no relevant publications were found. Apart from the MLSb phenotype, several studies are available that report on specific resistance genes used as indicators for MLS-resistant bacteria. Many resistance genes coding for MLSb resistance are known (Huber et al., 2020; Qin et al., 2022; Roberts, 2011), but mostly *erm* (B, C and F) and *ere*A genes are used for monitoring of MLSb resistance in the environment (Lu et al., 2022; Luiken et al., 2022; Weinroth et al., 2019; Yang et al., 2022). These genes have been detected in dairy, poultry species and pig manure (Jindal et al., 2006; Shen et al., 2022; Wang et al., 2021; Zhou et al., 2009), and are also found in soils from horse breeding farms (Huber et al., 2020).

Notably, MLSb-resistant bacteria and related resistance genes do occur naturally in the environment (Navarro et al., 2023) and it has also been reported that the use of MLS in veterinary medicine is not the only cause of MLSb resistance or specific macrolide ARGs in the environment (Koike et al., 2010; Perera et al., 2020; Zhao et al., 2021). Nevertheless, there is a clear link between the use of MLS in VMPs and the abundance of MLSb resistance in the environment, as shown by Li et al. (2020) and Li et al. (2020); W. Liu et al. (2021), who detected higher levels of MLSb resistance genes in soils amended with animal manure (poultry species, cattle, pig) than in soils unamended or amended with a chemical fertiliser. Similarly, Zhou et al. (2009) reported an increase of MLSb resistance in soil, albeit this increase was transient in time and over a period of months to years no increase MLSb resistance in soil was detected. Although specifically targeted in the research *Clostridium* spp. were reported as a major class of resistant micro-organisms in the environment examined. In the study of Zhou et al. (2009), the soil concentrations of MLS substances were always below detection limits. In the studies of Li et al. (2020) and W. Liu et al. (2021), no detection of substances was performed. Thus, the effect of emissions of manure-born ARGs cannot be disentangled from the contribution of MLS residues present in the manure to the environmental resistome. A difference between the studies of Li et al. (2020), W. Liu et al. (2021) and that of Zhou et al. (2010) is that the first two studies investigated the presence of DNA sequences through PCR analysis. The latter study examined the presence of resistance genes through fluorescence *in-situ* hybridisation, and it is unsure if another method could have detected the presence of resistance for a longer period. However, Macedo et al. (2021), who have been monitoring *erm*(B) genes, also observed a temporal increase of MLS ARGs after the spreading of manure. Irrespective of the methodological constraints, these studies show a contribution of MLS antimicrobials included in VMPs to the abundance of MLSb resistance in the environment. Whether this is a temporal or long-term presence is unsure and potentially depends on multiple factors.

The resistance genes detected in soil do not only represent living resistant bacteria, a correlation to mobile genetic elements (MGEs) has also been reported (W. Liu et al., 2021; Shen et al., 2022; Wang & Chai, 2022), indicating the potential of horizontal gene transfer of MLSb genes between bacteria in the environment. The presence of antimicrobial residues in the environment, as discussed above, is likely to support the expression and selection of these genes through horizontal gene transfer (Huber et al., 2020). Even at levels well below the inhibitory concentration, pre-existing resistance in a population can be enriched and *de novo* resistant mutants can be selected (Gullberg et al., 2011).

Manure is not the only pathway for emission of MLSb ARGs from farms. These genes have also been detected in farm air conditioning systems (Li et al., 2019) as well as in farm dust (Luiken et al., 2022), suggesting airborne emission from farms. Farms are, however, not the only source for spreading of ARGs related to veterinary use of MLS, since a poultry species meat processing plant has also been reported to spread MLSb genes to the environment (Semedo & Song, 2023).

Fate

After the application of manure, ARBs and ARGs present in the manure are likely to spread into the environment. This spreading is potentially affected by the timing of the manure application. Barrios et al. (2020) studied how the timing of pig manure application relative to rainfall events impacts the fate and transport of antibiotics and ARGs in surface runoff and manure-amended soil. The results showed that the concentration of *erm*(A) and *erm*(C) in the runoff decreased with increasing time of draught (> 2 weeks) after application of manure. Wu et al. (2020) also showed that *ere*A was detected in the wet season in the Maozhou river in China, but not during the dry season. Apart from moisture, there are multiple factors that can influence the fate of ARGs in the environment. Although not specifically for MLSb ARBs and AGRs, Han et al. (2022) has reviewed the following potential factors: microbial community structure, type and concentration of antibiotic, soil physicochemical properties (e.g.: pH, humidity, nutrition and temperature), spatial factors (e.g.: temperature and rainfall), plants and the presence of heavy metals, polycyclic aromatic hydrocarbons (PAHs) and pesticides. J. J. Zhang et al. (2022) reported a seasonal dependency of the concentrations of macrolide ARBs and ARGs in river water. A potential influence of use patterns was, however, not investigated. The general impression from the available scientific literature is that the effects of manure spreading on levels of resistance genes in soil are temporal (Macedo, 2021). Nevertheless, movement from soil into agricultural products has been demonstrated, i.e. by Mei et al. (2021), who studied the uptake of ARGs in carrots grown in soil amended with pig manure and showed the uptake of MLSb ARGs in the produce. In conclusion, these data indicate a potential pathway for exposure of humans to MLSb ARBs and ARGs.

Treatment

In order to be able to consider risk mitigation measures (RMM) that reduce the spreading of MLS resistance, it is important to know the fate of ARBs and MGEs during manure storage, biogas production from manure and other manure treatment processes. Where it concerns storage, it was shown that the prevalence of the MLSb ARGs *erm*(B) and *erm*(F) in dairy farm manure and showed that the manure management methods like liquid solid separation, piling or lagoon storage practice have little effect on reducing (MLSb) ARGs (Wang et al., 2021) and ARBs (Wang et al., 2015). Anaerobic conditions, however, have been reported to be suitable to reduce the levels of MLSb ARGs, as shown by Pu et al. (2018), whose study demonstrated that, under anaerobic fermentation conditions for biogas production, the levels of MLSb resistance genes in pig manure declined but at the same time, the number of erythromycin resistant bacteria was increased after anaerobic digestion. Angenent et al. (2008); K. Zhang et al. (2022) also reported a significant decrease of MLSb ARGs during treatment of livestock wastewater in an anaerobic microbial fuel cell. Similarly, Xu et al. (2021) showed that MLSb resistance genes were more dominant in aerobic soils than anaerobic soils. Chen et al. (2010) investigated the fate of three *erm* genes (B, F and X) in pig waste during treatment procedures at three different farms. It was shown that the levels of *erm* resistance genes varied in different waste treatment systems. It is nonetheless important to mention that the levels of these genes could increase at any stage after a reduction in a preceding stage. The presence of residues can affect the levels of ARBs as shown by Angenent et al. (2008) as in their experiment the levels of MLSb resistant bacteria increased from 18% in the input to an average of 45% in the reactor when it was fed with tylosin containing manure.

In addition, it should be noted that the reductions reported do not apply to resistance genes of all classes of antimicrobial substances. Pu et al. (2018) reported that e.g. aminoglycoside and florfenicol resistance genes and relevant bacteria enriched under anaerobic conditions, and K. Zhang et al. (2022) also reported an increase of abundance of beta-lactamase genes against a decrease of more than 50% for MLSb resistance genes in a microbial fuel cell. Therefore, when applying anaerobic conditions as RMM for MLSb ARGs, it should be considered that ARGs of other antimicrobial classes may increase in abundance and as such shifting the risk of resistance to other substances.

Composting is also often considered for the treatment of manure. B. Liu et al. (2021) published a review on the factors influencing the fate of resistance genes during aerobic composting; *erm*(B) genes were thereby reported to decrease during composting of chicken manure, while an increase of *erm*(A) and *erm*(B) was reported during the composting of pig manure. Additions to the manure to be composted could improve the removal of ARGs during the composting process. For instance, it was reported for *erm*(X) that the addition of superabsorbent polymers could improve removal of this gene. Altogether, the general impression is that manure treatment reduces the levels of MLSb resistance genes (Macedo, 2021; Youngquist et al., 2016). But although composting could reduce the level of MLSb resistance genes and the fact that increased composing or storage time can also significantly decrease the relative quantities of these genes (Wang et al., 2012), it has also been shown that MGEs are still transferable after six weeks of composting (Le Devendec et al., 2016). Another method that has been examined is aerobic thermophilic biotreatment but although the proportion of tylosin resistant bacteria decreased by treatments up to 60°C and gene diversity was reduced (*erm*(AB) to *erm*(B)), full dissemination of resistant bacteria could not be achieved with this method (Chénier & Juteau, 2009). In contrast, in an anaerobic thermophilic digester the level of *erm* B and F genes increased (Ma et al., 2011).

In conclusion, it can be stated that MLS-resistant bacteria and genes are emitted to the environment by treated animals, particularly through manure application, but that there are also other sources of environmental emission. After entering the environment, these genes can be detected in the environment by use of current technologies. There are many factors influencing their fate, and although the effect of the spreading of manure is probably only temporal, knowledge of these factors could serve to propose risk mitigation measures. Regardless, risk mitigation measures should always be considered for a range of antimicrobials wider than MLS antimicrobials, as conditions reducing MLS resistance genes could increase the levels of resistance genes for other classes of antimicrobials.

11. Transmission of antibiotic resistance or resistance determinants between animals, humans and the environment

11.1. Transmission of resistant bacteria

The prevalence of MLS resistance mechanisms in commensal bacterial species from both humans and animals is increasing. Understanding the transmission routes between animals and humans remains complex. Zoonotic pathogens such as *Salmonella* spp. and *Campylobacter* spp., which have well-established reservoirs in food-producing animals, are commonly transmitted to humans (ECDC/EFSA/EMA, 2015; EMA/EFSA, 2017). The emergence of multidrug-resistant strains, including those with MLS_b resistance phenotypes in livestock, raises concerns about animals as potential reservoirs of resistant bacteria or resistance determinants for humans. LA-MRSA is another major concern, as it is resistant to multiple antibiotics, including macrolides. LA-MRSA, particularly sequence type ST398, has been identified in pigs, cattle, companion animals, and humans, with direct and indirect transmission documented. For example, occupational exposure to livestock increases the risk

of human colonization (Aires-de-Sousa, 2017). Molecular studies reveal that while some MRSA lineages are host-specific, others can infect a broad range of hosts. Furthermore, LA-MRSA CC398 isolates exhibit diverse resistant determinants including resistance genes such as *cfr*, *vga*(C), and *erm*(54). While the *cfr* gene is a major resistance determinant frequently found in LA-MRSA of the clonal complex CC398 (often isolated from pigs), it is not exclusive to this lineage or even to *Staphylococcus aureus* itself. Indeed, the *cfr* gene has also been identified in LA-MRSA CC1 from livestock (Iurescia et al., 2023); also in Methicillin-Susceptible *Staphylococcus aureus* isolates (Fan et al., 2017; Ruiz-Ripa et al., 2020) and in other Staphylococcal species such as *Staphylococcus sciuri*, *Staphylococcus warneri* due to its location on mobile genetic elements like plasmids and transposons (Tn558 variants), which facilitate horizontal gene transfer (Kehrenberg et al., 2007). The *cfr* gene has spread beyond staphylococci and been detected in other bacterial genera, including *Enterococcus* spp., *Bacillus* spp., and *Escherichia coli* (Morroni et al., 2018).

Although food products of animal origin can be contaminated with LA-MRSA, community-associated, and hospital-associated MRSA strains, there is no direct evidence linking food consumption to an increased risk of MRSA colonization or infection in humans (Aires-de-Sousa, 2017; Larsen et al., 2016). However, MRSA and MRSP can be transferred between companion animals and humans, with MRSP strains implicated in human infections (Chanchaithong et al., 2014; Paul et al., 2011; Rodrigues et al., 2017; Zomer et al., 2017).

The transmission of antibiotic-resistant bacteria between animals and humans presents a significant public health challenge. Evidence suggests that food-producing animals, companion animals, and occupational exposure contribute to the spread of resistant pathogens. Generally, resistance differed greatly between reporting countries and antimicrobials. A high proportion of *Salmonella* spp. and *Campylobacter* spp. isolates from humans and animals were resistant to commonly used antimicrobials (ampicillin, tetracycline and sulfonamides) in human and veterinary medicine. Some cases of CP-producing *Salmonella* were reported in 2022 and 2023 (the majority harbouring blaOXA- 48 or blaOXA-48 -like genes). Detection of CP-producing *Escherichia coli* isolates (carrying blaOXA-48, blaOXA-181, blaOXA-244, blaNDM-5 and blaVIM-1 genes) in broilers, fattening turkeys, fattening pigs, cattle under 1 year of age and meat from pigs by seven Member States in 2022 and 2023, requires a thorough follow-up. The temporal trend analyses in key outcome indicators (complete susceptibility and prevalence of ESBL-/AmpC- producing *Escherichia coli*) showed an encouraging progress in reducing AMR in food-producing animals in several EU Member States over the last 10 years (ECDC/EFSA, 2025b).

While the extent of foodborne transmission remains unclear, the growing prevalence of resistant strains in animal populations underscores the need for continued surveillance, responsible antimicrobial use, and enhanced biosecurity measures to mitigate the risk of transmission to humans.

11.2. Transmission of resistance determinants

A variety of mobile *erm* genes, responsible for 14- (e.g., erythromycin), 15- (e.g., azithromycin), and 16-membered (e.g., josamycin) macrolides and clindamycin resistance via ribosomal target site methylation, have been identified in *Staphylococcus* spp. Notably, *erm*(54) was recently discovered in MRSA ST398 in Germany on the plasmid pHKS3860 (Krüger et al., 2022). While *erm* genes are widespread in *Staphylococcus*, they are less commonly found in *Streptococcus* species, though they have been detected in *Streptococcus suis*, *Streptococcus gallolyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*. Among these, *Streptococcus suis* is a significant zoonotic pathogen and antimicrobial resistance gene reservoir, with *erm*(B) frequently

detected worldwide, often co-existing with *tet*(O) on various genomic islands and integrative conjugative elements (ICEs).

The *erm*(T) gene, first identified in *Lactobacillus reuteri*, has since been reported in multiple Gram-positive and Gram-negative bacteria, including *Glaesserella parasuis*. These genes are often plasmid-borne, facilitating horizontal transfer between bacterial populations. Likewise, *erm*(T) has been identified in *Streptococcus suis*, residing on plasmid pSC262 or ICESsuSC117, both capable of interspecies transfer. Other mobile *erm* variants, including *erm*(42), *erm*(44), *erm*(46), *erm*(48), *erm*(51), and *erm*(X), have been found in diverse bacterial species such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Rhodococcus equi*, and *Cutibacterium acnes*, often associated with plasmids, transposons, or genomic islands, promoting horizontal gene transfer and resistance spread (Kostova et al., 2024; Wipf et al., 2017). The *cfr* gene encodes a 23S rRNA methylase, conferring resistance to multiple antibiotic classes, including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A compounds, as well as decreased susceptibility to the 16-membered macrolides. Since its discovery in *Staphylococcus sciuri* from bovine origin, *cfr* has spread globally across numerous bacterial genera and environments, frequently associated with mobile genetic elements such as transposons, plasmids, and integrative conjugative elements, enhancing its dissemination. Similarly, *vga* genes, encoding ATP-binding cassette transporters (efflux pump), mediate resistance to streptogramin A, lincosamides, and pleuromutilins. Various *vga* gene variants have been identified, predominantly in *Staphylococcus* species, with some facilitating resistance spread among livestock-associated MRSA isolates (Berbel et al., 2022; Feßler et al., 2018).

The widespread of mobile resistance determinants, such as *erm*, *cfr*, and *vga* genes, highlights the dynamic nature of AMR dissemination. The ability of these genes to transfer across bacterial species via plasmids, transposons, and genomic islands accelerates resistance spread in both human and veterinary settings. This underscores the necessity for stringent antimicrobial stewardship, continued genomic surveillance, and biosecurity measures to mitigate the risk of further resistance transmission.

12. Impact of resistance on animal and public health

12.1. Animal health

Macrolides are essential in veterinary medicine, classified as VCIAs by the WOAH due to their broad-spectrum applications (WOAH, 2025b). They are categorised as AMEG Category C (Caution), which refers to alternatives in human medicine that may only be used under specific indications when there are no clinically effective antibiotic alternatives in Category D (EMA/CVMP/CHMP, 2019).

Macrolide resistance is of particular concern due to the presence of transferable resistance genes (*erm*-genes), notably in *Campylobacter* spp., which increases the risk of resistance emergence and dissemination. Although the prevalence of *erm* genes in animal isolates remains low in the EU, high levels of macrolide resistance have been reported in MRSA from pigs in several EU countries. High levels were specifically observed in MRSA from pigs in Belgium and Portugal, and from calves in Belgium and Switzerland. In *Streptococcus suis* proportion of resistance to macrolides-lincosamides is up to 65% of isolates from pigs in France and high MICs are also seen in a large proportion of isolates in Germany. Also, the occurrence of macrolide resistance is reported as high in *Enterococcus* spp. of animal origin, which often harbour transferable resistance genes like *erm* (EFSA, 2024).

The loss of macrolide efficacy would significantly restrict treatment options for key bacterial infections in food-producing animals, including Mycoplasma infections in pigs and poultry species, *Lawsonia intracellularis* in pigs, *Fusobacterium necrophorum* in cattle, and respiratory infections in cattle.

Alternative treatments to macrolides vary by species:

Pigs: Alternatives for respiratory pathogens include amoxicillin-clavulanic acid, amphenicols, and pleuromutilins (all AMEG Category C). For Mycoplasma infections, tetracyclines or fluoroquinolones (AMEG Category B) may be used. Alternatives for *L. intracellularis* are more limited, primarily consisting of tetracyclines (AMEG Category D) and pleuromutilins (AMEG Category C).

Cattle: Respiratory pathogens can be treated with amoxicillin-clavulanic acid, amphenicols, trimethoprim-sulfonamides, and tetracyclines (depending on susceptibility). For Mycoplasma infections, tetracyclines are alternatives, though increasing resistance has reinforced the importance of macrolides, which remain essential for bovine respiratory disease and enzootic pneumonia in calves. Fluoroquinolones and amphenicols may be required for complicated cases.

EFSA/ECDC surveillance 2023 and 2024 data indicates that resistance to erythromycin was low or absent in *Campylobacter jejuni* from humans, poultry species, and calves, but higher and highly variable between countries in *Campylobacter coli* from humans and all targeted animals, with highest levels observed in calves. *Salmonella* spp. and *Escherichia coli* generally show low azithromycin resistance. However, most MRSA isolates subjected to antimicrobial susceptibility testing were multidrug-resistant with high levels of erythromycin resistance reported. Additionally, the *erm*(B) gene has been detected on isolates in *Campylobacter coli* from pigs and fattening turkeys, suggesting potential for resistance spread due to macrolide use in food-producing animals (ECDC, 2026).

Macrolide resistance also affects specific pathogens in other species (EMA/CVMP, 2023):

Pigs: High resistance (32–80%) to tylosin and tylvalosin has been observed in *Brachyspira hyodysenteriae*, resulting in the withdrawal of tylosin for swine dysentery treatment.

Rabbits: Macrolides are used to treat respiratory diseases (*Pasteurella multocida*, *Bordetella bronchiseptica*) and epizootic rabbit enteropathy.

Companion animals: Approved uses are limited, but spiramycin (often combined with metronidazole) is used for oral infections in dogs. Human-authorised macrolides (e.g., azithromycin, clarithromycin) are occasionally used off-label for respiratory infections, *Borrelia burgdorferi*, *Helicobacter* spp., *Chlamydophila felis,* and *Lawsonia intracellularis* in horses. Tylosin is used in dogs for antibiotic-responsive diarrhoea and is listed as essential by WSAVA for severe Campylobacter infections and chronic enteric diseases.

Macrolide resistance poses a significant threat to veterinary medicine, particularly for infections with few or no alternative treatments. While surveillance indicates overall low to moderate resistance levels in key foodborne pathogens, resistance in respiratory and enteric pathogens of livestock continues to rise. Given their importance in veterinary medicine, macrolide use should be strictly regulated to preserve efficacy while ensuring effective disease management. Continued monitoring, responsible antimicrobial stewardship, and the development of alternative therapies are essential to mitigate the impact of resistance on both animal and public health.

Lincosamides, including lincomycin and clindamycin, are important antibiotics in veterinary medicine for treating infections caused by Gram-positive bacteria and anaerobes. They are classified as VHIA by WOAH due to their broad applications in food-producing and companion animals (WOAH, 2025). According to AMEG categorization, lincosamides are in Category C (Caution), meaning that they should only be used under specific conditions when no Category D alternatives are effective. Alternatives treatment exists but the emergence of resistance to lincosamide would limit treatment options for several important infections in food-producing animals.

Pigs: Lincomycin-resistant *Streptococcus suis* could lead to persistent meningitis and septicaemia, complicating treatment. Clostridial enteritis becomes harder to control, increasing morbidity and mortality. Alternatives treatment option includes Tetracyclines (Category D) and pleuromutilins (Category C) as well as beta-lactams notably for respiratory and enteric infections.

Cattle: Resistance in anaerobic infections impedes effective treatment of footrot, liver abscesses, and metritis. Alternatives treatment option includes Tetracyclines (Category D), amphenicols (Category C) as well as beta-lactams.

Companion animals: Clindamycin-resistant *Staphylococcus* pseudintermedius and *Staphylococcus aureus* lead to chronic skin and soft tissue infections, requiring longer or more complex therapies. Alternatives treatment option includes potentiated Beta-lactams (Category C).

12.2. Human health

Macrolides are classified in the 'Watch' category of the WHO's AWaRe classification, indicating their higher resistance potential and critical role in treating specific infections (WHO, 2025). These antibiotics, including erythromycin, azithromycin and clarithromycin, are commonly used as first-line treatments for respiratory tract infections, skin infections, and certain sexually transmitted infections.

In 2024, the WHO reclassified macrolides from HPCIA to (CIA, as they did not meet the criteria for frequent causes of invasive and life-threatening infections (WHO, 2024). However, macrolides remain essential for the treatment of *Legionella* spp., *Campylobacter* spp., and multidrug-resistant *Salmonella* and *Shigella* spp. infections. While Campylobacter infections are widespread, most cases are self-limiting and require treatment only in severe cases, particularly in vulnerable populations such as children.

The rise in fluoroquinolone-resistant *Campylobacter* spp. has made macrolides the preferred alternative, yet resistance remains a concern. At the EU level, resistance to macrolides in *Streptococcus pneumoniae* increased from 16.8% to 19% between 2020 and 2024, according to EARS-Net data (ECDC, 2025a). Additionally, *Neisseria gonorrhoeae* resistance to azithromycin has become a growing concern. The percentage of isolates with azithromycin MICs above the epidemiological cut-off (ECOFF, MIC >1 mg/L) gradually increased from 7.6% in 2018 to 25.6% in 2022 but decreased to 23.2% in 2023 (ECDC, 2025c). Currently, azithromycin monotherapy is not recommended unless the isolate is first shown to be susceptible.

Increasing macrolide resistance not only threatens treatment efficacy but also limits options for patients allergic to beta-lactams, where macrolides serve as alternatives. As resistance continues to rise, preserving their effectiveness requires prudent use, enhanced surveillance, and the development of alternative treatments.

Macrolides play a crucial role in human medicine, yet their rising resistance rates pose a growing challenge to public health. While their recent reclassification by the WHO reflects a reassessment of their criticality, they remain essential for treating several bacterial infections. The increasing resistance in key pathogens, including *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, underscores the urgency of antimicrobial stewardship. Prudent use, continuous surveillance, and updated treatment guidelines are vital to preserve their efficacy and to mitigate the risk of resistance development.

Lincosamides are classified in the 'Watch' category of the WHO's AWaRe classification, which highlights their higher resistance potential and their critical role in treating specific infections (WHO, 2025). This class, primarily represented by clindamycin (lincomycin is in the 'Access' category), is commonly used to treat skin and soft tissue infections, anaerobic infections, and certain infections caused by Gram-

positive cocci, including methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Streptococcus pyogenes*. Although lincosamides are not classified as highest-priority, their use is essential notably in cases where first-line beta-lactams cannot be used. At the EU level, clindamycin resistance among *Staphylococcus aureus* isolates has been relatively stable but remains clinically significant, particularly in MRSA, where inducible resistance via the *erm* gene complicates therapy. In *Streptococcus* spp., lincosamide resistance is often associated with cross-resistance to macrolides, which limits treatment options.

13. Discussion

MLS antimicrobials represent a diverse pharmacologically related group of antimicrobials whose clinical utility and resistance risks are inextricably linked across the human-animal-environment interface. The evidence gathered across this reflection paper reveals a complex 'feedback loop' where pharmacological characteristics, usage patterns, and environmental fate form a unified driver of AMR.

In the veterinary domain, the WOAH (formerly OIE) maintains macrolides as VCIA. Within the EU, they are placed in Category C ('Caution') of the AMEG, indicating they should be considered only when Category D alternatives are clinically ineffective. Macrolides remain among the few viable options for the treatment of porcine proliferative enteropathy caused by *Lawsonia intracellularis* and footrot in small ruminants. Lincosamides are classified as VHIA, while streptogramins, though not authorized for veterinary use in the EU, are classified as VIA by WOAH.

It should be noted that in 2024, the WHO published the Medically Important Antimicrobials List. Macrolides were reclassified from the HPCIA category to the broader CIA category, reflecting revised assessments of their role in treating invasive, life-threatening human infections and the availability of alternative therapies (WHO, 2024). This revision, which moved macrolides out of the highest-priority tier in the WHO classification, underscores the evolving evidence base for risk prioritisation and highlights the need for updated stewardship strategies across human and animal health sectors that align with One Health principles.

This dual importance for animal and human health positions MLS antimicrobials at the centre of AMR risk management considerations.

The widespread use of MLS antimicrobials in veterinary medicine in the EU, including group treatments (Figure 3) and metaphylactic use (please refer to section 6.1.1. , creates substantial selection pressure for resistance. Additionally, recent data placed MLS as the second-most-used group in community treatment for human medicine in the EU (please refer to section 7.2.

Resistance to macrolides is well documented in several bacterial species relevant to both animal and human health, including *Campylobacter* spp., *Enterococcus* spp., and *Staphylococcus* spp. For example, *Campylobacter coli* resistance to erythromycin has reached up to 75.9% in calves (please refer to section 8.1.1. .). Livestock-Associated MRSA (ST398) is a major concern, as it is resistant to macrolides and poses a risk to occupationally exposed humans. The emergence and dissemination of resistance in these organisms is of particular concern, given their zoonotic potential and their role as reservoirs of resistance genes. Resistance in bacteria to substances within the MLS group is characterised by a high degree of cross-resistance due to overlapping binding sites. Genes conferring resistance are frequently mobile. The MLS_B resistant phenotype is mediated by rRNA methylases encoded by *erm* genes. This phenotype confers resistance to macrolides, lincosamides, and Type B streptogramins. The M-phenotype is mediated by *mef* genes encoding efflux pumps, restricted to 14- and 15-membered macrolides. The MS_B-phenotype is mediated by *msr* genes, conferring resistance to macrolides and Type B streptogramins. Lastly, the PhLOPSA-phenotype conferred by the *cfr* gene,

providing resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A. This co-selects for resistance to the human last resort drug linezolid. To detect resistant phenotypes requires standardisation of in vitro methods. Currently, many veterinary clinical isolates are tested against human-derived breakpoints, which are unsuitable for veterinary medicine as they do not account for the extreme tissue accumulation seen in animals. The 'D-test' remains a vital tool to identify inducible MLSB phenotypes (please refer to section 5.

An additional concern relates to cross-resistance within and beyond the MLS group. Use of macrolides in animals may select for resistance (*erm*, *cfr*, and *vga* genes) that compromises the efficacy of related substances used in human medicine, including ketolides such as telithromycin. This interconnectedness reinforces the need to consider MLS antimicrobials within a One Health framework, recognising that resistance selected in animals can have downstream consequences for human therapeutic options.

Historically, certain macrolides, notably tylosin, were also used at subtherapeutic doses for growth promotion in food-producing animals. While such uses have been banned in the EU, the United States, and many other regions, they may still occur in countries with less stringent regulatory frameworks. Even where growth promotion is prohibited, prolonged administration at low doses for prophylactic or metaphylactic purposes may generate similar resistance selection pressures. Recent international and EU/EEA data demonstrate marked differences in antimicrobial usage patterns between countries, including for macrolides, indicating that substantial reductions in use are achievable without compromising animal health or welfare (EMA, 2025b; WOAH, 2025a). These observations underline the importance of promoting prudent use principles globally.

In the 29 EU/EEA countries, macrolides and lincosamides were the third and the sixth most-sold antimicrobial class for food-producing animals in 2024, representing 9.3% and 4.6% of the total sales, respectively (Figure 1). While lincosamide sales saw a 56% decrease from 2017 to 2024, macrolides had a moderate reduction of 8.6% (Figure 2). In 2024, over 93% of MLS sales were for group treatments (oral solutions and premixes) (Figure 3), which results in significant exposure of the intestinal microbiota. For companion animals, while macrolide sales decreased by 41.1% from 2017 to 2024, lincosamide sales increased by 81.3% over the same period, peaking in 2024 at 2.5 tonnes (Figure 5). There is also a temporal association between increases in lincosamide sales data in pets with the emergence of resistance in *Staphylococcus pseudintermedius*, a common companion animal pathogen that can colonise humans. For example, *Staphylococcus pseudintermedius* resistance to lincosamides is increasing, with MDR observed in up to 96–99% of MRSP isolates. The frequent discovery of the *vga* and *cfr* genes in these isolates suggests that lincosamide use in pets may be a contributing factor to the environmental and community pool of genes that confer resistance to human last-resort drugs. Prudent use of MLS antimicrobials is therefore essential. While acknowledging that macrolides and lincosamides are essential for the treatment of certain animal diseases, their use should be limited to situations where there is a clear clinical indication supported by diagnosis and evidence of likely benefit. Routine and standardised implementation of susceptibility testing, using adequate veterinary clinical breakpoints specific to animal species and infections for macrolides and lincosamides, is deemed necessary to ensure adequate use of these important antimicrobials in veterinary medicine. However, currently there is a concerning lack of specific veterinary clinical breakpoints, which calls for urgent action and further investigation in this field, as human-derived breakpoints are unsuitable and ECOFFs are more appropriate for surveillance purposes.

Routine prophylactic use, treatment of self-limiting conditions, or use in the absence of a defined bacterial aetiology should be avoided. Treatment duration should be carefully considered. While it should be restricted to the minimum necessary to achieve clinical cure, as unnecessarily long courses

increase selection pressure without additional therapeutic benefit, it should also be ensured that the minimum treatment duration is applied, to avoid the selection pressure for resistance strains.

For many macrolides, plasma concentrations are often significantly lower than the MIC of target pathogens. Efficacy is explained by the drugs' high lipophilicity, allowing them to reach intracellular concentrations up to 50 times higher than plasma levels, particularly within phagocytic cells. As such, our understanding of the PK/PD relationship of MLS antibiotics has shifted from a time-dependent model (t > MIC) to the area under the concentration-time curve relative to the MIC (AUC/MIC), which better reflects efficacy for newer long-acting macrolides. However, a fundamental paradox exists between clinical efficacy and resistance risk. The extraordinary capacity to accumulate in different lung tissue compartments (bronchoalveolar cells and pulmonary epithelial lining fluid) (please see section 3. justifies the use of macrolides for respiratory infections where plasma levels would otherwise be insufficient. However, this benefit is counterbalanced by the pharmacological drivers of resistance. The long terminal half-lives of third-generation macrolides create a prolonged "tail" of sub-inhibitory concentrations. As evidenced by the 2025 azithromycin CHMP referral in human medicine (please refer to section 7.1.1. these long-lasting, declining concentrations provide the primary selection pressure for *de novo* resistance and the enrichment of resistant subpopulations. This risk is further amplified by the fact that for orally administered macrolides and lincosamides, which account for over 93% of sales considered for group treatment in food-producing animals, (Figure 3), a substantial proportion of the administered dose reaches the intestinal microbiota at varying concentrations.

Dose selection is another critical factor influencing resistance development. Suboptimal dosing, particularly with older VMPs, may favour the selection of resistant subpopulations. Where evidence suggests that authorised doses are too low or treatment durations too long or not indicated, a review of SPCs is warranted. In many cases, dose optimisation within existing safety margins may be possible without the need for new tolerance or residue studies, particularly where different doses are already authorised for similar indications. Additionally, optimisation of dosage regimens can be enhanced by further research in the field, using a more accurate representation of the biophase in which the target bacteria are located.

The scope and wording of indications for use also require scrutiny. Indications should be restricted to diseases for which efficacy has been adequately demonstrated. Broad or poorly defined indications lacking a solid clinical basis risk inappropriate use and increased selection of resistance.

Furthermore, specific consideration should be given to third-generation macrolides, which are characterised by their unique PK/PD characteristics, such as long half-lives and tissue concentrations, increasing the risk of development of AMR when misused. During recent years, sales of tulathromycin in the EU/EEA countries have been increasing (Table 4) and it is expected that this increase will continue over the next few years, with the arrival to the market of generic products. As such, to preserve their efficacy and reduce the potential for AMR, alignment of their SPCs with the recommended standard sentences for antimicrobials available in the 'Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances', is considered advisable.

Finally, more data regarding resistance and further exploration of potential correlations between AMU and AMR are needed, especially for lincosamides and third-generation macrolides, which are not currently included in the implemented European surveillance systems. This monitoring should facilitate early detection of emerging trends and spread of macrolide- and lincosamide-resistant bacteria and ARG in animal commensals and zoonotic pathogens.

Regulation (EU) 2019/6 introduced the consideration of the risks of the environment acting as a potential vehicle for spreading AMR to humans as part of the benefit-risk evaluation for new antimicrobial VMPs. This reflection paper provides an environmental perspective on the role of MLS-resistant bacteria and genes emitted into the environment by treated animals, particularly through manure application, highlighting the importance of their early detection using current or novel technologies. It calls for further exploration on different risk mitigation measures that could be applied routinely, as well as for an investment in fundamental research on the spread of resistance via the environment and the associated quantitative risk of back-transmission to humans and animals. The MLSB phenotype is the primary indicator found in environmental manure samples, demonstrating the direct fallout of veterinary AMU. Interconnectedness is most apparent in environmental persistence. While some macrolides residues degrade relatively quickly, lincosamides like lincomycin and pirlimycin exhibit very high persistence in manure, with half-lives exceeding 200 to 600 days (please see section 10.1. This creates a chronic AMR selection pressure in agricultural soils long after the initial treatment. Residues remain active, potentially favouring the long-term stabilisation of mobile resistance genes like *erm(F)* and *cfr* in the soil resistome. The resulting 'back-transmission' risk to humans via food crops or groundwater represents a critical gap in current risk assessment models that necessitate further environmental research.

Loss of macrolide efficacy would severely restrict treatment options for *Mycoplasma* and *Lawsonia intracellularis* in swine and respiratory disease in cattle. In humans, rising macrolide resistance in *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* (25.6% in 2022) (please refer to section 8.2.) threatens first-line therapy options. Regulatory experience in the European Union illustrates how targeted risk management measures can mitigate AMR concerns associated with MLS antimicrobials. Several referral procedures under Articles 34 and 35 of Directive 2001/82/EC have led to the optimisation of indications and treatment duration, dose harmonisation, and the withdrawal of the marketing authorisations for certain formulations. Examples include the removal of claims for swine dysentery due to *Brachyspira hyodysenteriae* for tylosin- and lincomycin-containing products following the emergence of widespread resistance, the restriction of prolonged oral treatments in pigs and poultry species, and the withdrawal of premix formulations associated with low-dose, long-term exposure. These regulatory actions demonstrate the practical application of AMR risk assessment and highlight the importance of continuously reassessing the benefit–risk balance of authorised products in light of evolving resistance patterns. Notwithstanding these measures, antimicrobial resistance cannot be addressed in isolation. A holistic approach is required that combines prudent antimicrobial use with improved biosecurity, vaccination strategies, enhanced husbandry practices, and disease prevention measures. Such integrated approaches reduce the overall need for antimicrobial treatment, thereby lowering the selection pressure driving resistance.

14. Conclusions

Macrolides and lincosamides are important in human and veterinary medicine and are extensively used to treat a variety of infections. Streptogramins are also important in human medicine but are not used in veterinary medicine in Europe. Since the first publication of this reflection paper in 2011, the WHO classification of macrolides has been downgraded from HPCIA to CIA, whereas lincosamides and streptogramins remain HIA. This new classification is now more closely aligned with the EMA's AMEG Category C classification for macrolides, which highlights their importance in treating severe infections in veterinary medicine, including situations where they are the only option. However, contrary to the general sales of antimicrobial VMPs trend, sales of macrolides for food-producing animals have not declined significantly in recent years. Moreover, product forms intended for group treatment continue to represent the primary sales format for both macrolides and lincosamides. The clinical necessity of

MLS drugs in human and veterinary medicine is currently in a 'fragile balance' with the rising threat of AMR. While macrolides and lincosamides remain critically important for animal health, the emergence of resistance in pathogens like *Campylobacter* and the persistence of resistance genes in the environment necessitate shifting from routine use to more targeted therapy.

Additionally, since the first publication of this reflection paper in 2011, new knowledge and data on the resistance mechanisms for MLS have become available, including information on the widespread presence of the mobile resistance determinants, such as *erm*, *cfr*, and *vga* genes, as well as insights into the role of the *msrD* gene.

Macrolides and lincosamides accumulate in tissues ensuring efficacy in respiratory and intramammary infections, yet this same feature drives resistance through prolonged sub-inhibitory exposure. The evidence reviewed in this reflection paper highlights that suboptimal dosing, prolonged treatment durations, and lack of *in vitro* MIC standardisation and veterinary-specific clinical breakpoints all constitute barriers to prudent use and contribute to increased AMR risk. Conversely, international usage data and regulatory experience demonstrate that meaningful reductions in use and risk are achievable without compromising animal health and welfare. Without these, clinicians cannot uniformly distinguish susceptible from resistant isolates, leading to inappropriate use and selection pressure.

Following the One Health approach, this reflection paper has examined new environmental factors that enable better characterisation of the potential for MLS resistance to develop. It also highlights the importance of considering appropriate risk mitigation measures to reduce the potential spread of MLS resistance genes via the environment.

In conclusion, while macrolides and lincosamides remain indispensable in veterinary medicine, their use must be judicious and evidence-based. The CVMP advocates for a harmonised One Health approach, which integrates regulatory oversight, veterinary stewardship, surveillance of resistance and use, and preventive animal health measures to safeguard their efficacy and limit the spread of resistance. Due to their unique characteristics, the MLS group requires a harmonised regulatory approach across the EU/EEA to mitigate the risk of cross-resistance to human-critical macrolides. Such a comprehensive strategy is essential to preserve the therapeutic value of these antimicrobials for both current and future use in animals and humans.

15. Abbreviations

ABCs: Active Bacterial Core surveillance

ADRA: Dosage review and adjustment of selected veterinary antibiotics

ADRA tWP: Dosage Review and Adjustment of Established Veterinary Antibiotics temporary Working Party

AGP: Antimicrobial Growth Promoters

AMEG: Antimicrobial Advice ad hoc Expert Group

AMU: Antimicrobial Use

ARB: Antimicrobial-Resistant Bacteria

ARGs: Antimicrobial Resistance Genes

AST: Antibiotic Susceptibility Testing

AUC: Area Under the Curve

AWP: Antimicrobial Working Party (CVMP's working party)

BRD: Bovine Respiratory Disease

CAP: Community-acquired Pneumonia

CDC: Centers for Disease Control and Prevention

CDI: *Clostridioides difficile* Infections

CHMP: Committee for Human Medicinal Products

CIA: Critically Important Antimicrobials

CLSI: Clinical Laboratory Standards Institute

CNS: Coagulase-negative Staphylococci

CoPS: Coagulase-positive Staphylococci

CP: Carbapenemase

DNA: Deoxyribonucleic Acid

EARS-Net: European Antimicrobial Resistance Surveillance Network

ECDC: European Centre for Disease Prevention and Control

ECOFF: Epidemiological Cut-off Value

EEA: European Economic Area

EFFORT: Ecology from Farm to Fork of Microbial Drug Resistance and Transmission

EFSA: European Food Safety Authority

EPARs: European Public Assessment Reports

ERA: Environmental Risk Assessment

ERAWP: Environmental Risk Assessment Working Party (CVMP's working party)

ESAC-Net: European Surveillance of Antimicrobial Consumption Network

ESUAvet: European Sales and Use of Antimicrobials for Veterinary Medicine

ESVAC: European Surveillance of Veterinary Antimicrobial Consumption

EUCAST: European Committee on Antimicrobial Susceptibility Testing

EU-JAMRAI: EU Joint Action Antimicrobial Resistance and Healthcare-Associated Infections

Euro-GASP: European Gonococcal Antimicrobial Surveillance Programme

fAUC: free Area Under the Concentration-Time Curve

fT: free Concentration

FWD-Net: Food and Waterborne Disease Network

HIA: Highly Important Antimicrobials

HPCIA: Highest Priority Critically Important Antimicrobials

HPLC: High-Performance Liquid Chromatography

ICEs: Integrative Conjugative Elements

IDWP: Infectious Diseases Working Party (CHMP's working party)

JIACRA: Joint interagency Report on the Integrated Analysis of Consumption of Antimicrobial Resistance

MDR: Multidrug-resistant

MFS: Major Facilitator Superfamily

MGE: Mobile Genetic Elements

MHB: Mueller-Hinton Broth

MIC: Minimum Inhibitory Concentration

MLS: Macrolides, Lincosamides and Streptogramins

MLSKO: Macrolides, Lincosamides and Streptogramins knockout

MRL: Maximum Residue Limits

MRSA: Methicillin-resistant *Staphylococcus aureus*

MRSP: Methicillin-resistant *Staphylococcus pseudintermedius*

MSB: Macrolides and Streptogramin B

NWT: Non-wild-type

PAE: Post-antibiotic Effect

PAHs: Polycyclic Aromatic Hydrocarbons

PALE: Post-leucocyte Enhancement

PBT: Persistent, Bioaccumulative and Toxic

PCU: Population Corrected Unit

PD: Pharmacodynamics

PhLOPSA: Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins and Streptogramin A

PK: Pharmacokinetics

PMNs: Polymorphonuclear Cells

PTC: Peptidyl Transferase Catalytic Centre

RNA: Ribonucleic Acid

RMM: Risk Mitigation Measures

RND: Resistance/Nodulation/Division

RTIs: Respiratory Tract Infections

SA: Staphylococcus aureus

SB: Streptogramin B

SmPC: Summary of Product Characteristics (human medicines)

SPC: Summary of Product Characteristics (veterinary medicines)

SSTI: Skin and Soft Tissue Infections

STEC: Shiga Toxin-producing *Escherichia coli*

SWP-V: Safety Working Party CVMP's working party)

VCIA: Veterinary Critically Important Antimicrobials

VetCAST: Veterinary Committee on Antimicrobial Susceptibility Testing

VHIA: Veterinary Highly Important Antimicrobials

VIA: Veterinary Important Antimicrobials

VMPs: Veterinary Medicinal Products

VRE: Vancomycin-resistant Enterococcus

WHO: World Health Organization

WOAH (formerly known as OIE): World Organisation for Animal Health

WSAVA: World Small Animal Veterinary Association

XDR: Extensively drug-resistant

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