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

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

## CVMP recommendations for action

Macrolides and lincosamides are used for treatment of diseases that are common in food producing animals and for medication of large groups of animals (mass medication). They are critically important for animal health and therefore it is highly important that they are used prudently to contain resistance against major animal pathogens. In addition, MLS are listed by WHO (AGISAR 2009) as critically important for the treatment of certain zoonotic infections in humans and risk mitigation measures are needed to reduce the risk for spread of resistance from animals to humans.

Macrolides have been used for group and flock medication since several decades. Before the authorisation of growth promoters expired in EU these molecules were added in low doses in animal feed to increase feed conversion. Such use is not allowed in EU today but there are products approved for preventive treatment using low doses for long time.

Data recently published shows great differences between different countries on the use of antimicrobials in general - including macrolides - which indicates that there might be options to reduce use of these antimicrobials that are available without compromising animal health and welfare.

The recommendations below have been prepared following SAGAM's review on macrolides, lincosamides and streptogramins.

For veterinary medicinal products for food producing animals the CVMP concluded that the following recommendations are for consideration by Competent Authorities:

- Prudent use of antimicrobials should be strongly promoted. It is acknowledged that macrolides are first line treatment against a number of animal diseases but still there is a need to avoid overuse, for e.g. general prophylaxis where no specific diagnose is evident or where the disease in question would self cure without antimicrobials.
- Duration of treatment should be limited to the minimum required time for treatment of diseases. There might be a need to review certain SPCs to reduce the approved treatment duration in cases where it is found unnecessarily long in relation to the severity of the disease.
- Doses should preferably be selected considering AMR related risks. In case of old products where data on dose selection are sparse doses should anyway be reviewed and in case they are obviously too low (e.g. compared to other products containing the same active substance) this should be addressed. Notably there are often several different doses approved for different indications and thus there is an option to increase doses where relevant without asking for new tolerance or safety data.
- Indications for use should preferably be restricted to those for which efficacy has been proven and general indications without a solid clinical basis should be avoided. In case of old products where data are sparse indications should be reviewed and revised where appropriate to be as accurate as possible. In particular, combination products are of concern as there seems to be

products on the market for which the choice of included active components cannot be justified as their combined use lacks scientific rational.

Notwithstanding the list of recommendations above, the CVMP is of the opinion that antimicrobial resistance should not be considered in isolation but a global approach to the problem is needed. Implementation of prudent use principles remains a cornerstone to contain resistance together with biosecurity and other measures to promote animal health and thereby reduce the need for treatment.

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# 1. Mandate

The Scientific Advisory Group on Antimicrobials (SAGAM) was mandated to give advice to the CVMP on the need to exercise control on those classes of compounds of greater importance to human medicine in particular fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

The CVMP published a concept paper recommending the preparation of a Reflection Paper (concept paper on the use of macrolides, lincosamides and streptogramins in food-producing animals in the European Union: development of resistance and impact on human and animal health (EMEA/CVMP/SAGAM/113420/2009-CONSULTATION). The comments received supported the preparation of this reflection paper, and as a result the CVMP mandated the SAGAM to prepare a draft of the reflection paper.

This document discusses macrolides, lincosamides and streptogramins, with emphasis on macrolides and their use in food producing animals, excluding aquaculture and apiculture and its impact on human and animal health.

# 2. Introduction

Macrolides are antibacterial substances which have a central lactone ring as their basic structure. Lincosamides are structurally different from macrolides, but their binding sites overlap. Streptogramins consist of two types of molecules, A and B, acting in synergy. The binding site of streptogramin B overlaps that of macrolides and lincosamides. Modification of the bacterial target site of these molecules typically leads to cross-resistance between macrolides, lincosamides and streptogramin B (MLSB resistance phenotype).

Macrolides are used for treatment of diseases that are common in food producing animals and for medication of large groups of animals (mass medication). Lincosamides are more limited in indications, and the number of products is lower. Macrolides have been categorised as critically important and lincosamides as highly important for veterinary medicine in the list of antimicrobials of veterinary importance (OIE 2007). Streptogramins are currently not authorised for use in food producing animals in the EU. Macrolides and streptogramins are classified as critically important in human medicine (WHO 2007). Prioritization of classes of antimicrobials to be addressed most urgently in terms of risk management strategies for non-human use of antimicrobials has recently resulted in the selection of three groups: quinolones, 3rd/4th generation cephalosporins, and macrolides (WHO 2007).

Resistance to macrolides and lincosamides has emerged in common animal pathogens such as Brachyspira as well as staphylococcal and streptococcal species. Resistance to macrolides has also emerged in zoonotic pathogens such as *Campylobacter spp*. Erythromycin is the macrolide far mostly used in humans, and the increase of resistance against erythromycin is well documented. Resistance has also appeared among enterococci residing in animals, and can potentially be transferred to bacteria colonising or infecting humans. Macrolides and lincosamides have not been the sole alternatives for treatment of any infections in food animals, but are alternative choices for many common diseases. Because of increased resistance, they have become the only choice in some situations. Differences in the use of macrolides and lincosamides for humans and animals, as well as in the resistance situations exist between continents.

# 3. Objective

The objective of this document is to critically review recent information on the use of macrolides, lincosamides and streptogramins in food producing animals in the EU, its effect on development of resistance to these classes of antimicrobial agents in bacterial species that are of importance for human and animal health, and the potential impact on human and animal health.

# 4. Classification, mechanism of action, spectrum of activity and pharmacokinetics

## *4.1. Classification*

Macrolides are classified according to the number of atoms which comprise the lactone ring, reaching from 12 to 16 members (Yao and Moellering 2007). To this ring, two or more sugar moieties can be attached. Macrolides with a 12-member ring are no more in use. The first macrolide discovered in the early 1950ies was erythromycin, which is an organic substance produced by the actinomycete *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) (Zhanel, Dueck et al. 2001). The first macrolide intended for animal use was spiramycin, which was introduced in the early 1960ies, followed by erythromycin and tylosin (Prescott 2008). A chemically modified tylosin, tylvalosin (acetylisovaleryltylosin), was authorized for pigs in the EU in 2004.

In early 1990ies the semisynthetic, new generation macrolides were introduced into human medicine. Azalides, like azithromycin, have nitrogen atom(s) inserted into the lactone ring (Ballow and Amsden 1992; Bryskier and Butzler 2003). The first azalide approved for animal use in the EU in 2008 was gamithromycin. Ketolides such as telithromycin and cethromycin are a macrolide group developed only recently (Bryskier 2000; Hamilton-Miller and Shah 2002). Ketolides are 14-membered macrolides which have the L-cladinose moiety in position 3 replaced with a keto function (Xiong and Le 2001; Bryskier and Butzler 2003). They have activity against macrolide-resistant streptococci (Shain and Amsden 2002; Pfister, Jenni et al. 2004). New macrolides have also been developed for animal use. Tulathromycin authorized for use in cattle and swine in the EU is a semi-synthetic macrolide with three amine groups; it is a mixture of a 13 and 15-membered ring macrolide. Macrolides with this structure are termed triamilides.

Lincomycin and its semi-synthetic derivatives clindamycin and pirlimycin, belong to the lincosamides. Streptogramins are a unique group of antimicrobials as all of them consist of two structurally unrelated cyclic peptides, streptogramin A and B (Edelstein 2004). Among streptogramins, virginiamycin and pristinamycin are organic compounds; quinupristin/dalfopristin is a semisynthetic streptogramin derived from pristinamycin. The only streptogramin used for animals is virginiamycin, which until 1998 was approved as a feed additive for growth promotion.

**Table 1.** Classes of macrolides and related compounds (Bryskier and Butzler 2003; Giguère 2006a).

| **Macrolides** | | | **Lincosamides** | **Streptogramins (A and B)** |
|---|---|---|---|---|
| 14-membered ring | 15-membered ring | 16-membered ring | • Clindamycin* | • Pristinamycin |
| • Clarithromycin | • Azithromycin | • Josamycin | • Lincomycin* | • Quinupristin/Dalfopristin |
| • Erythromycin* | • Gamithromycin* | • Mideacamycin | • Pirlimycin* | • Virginiamycin** |
| • Oleandomycin | • Tulathromycin* | • Miocamycin | | |
| • Roxithromycin | | • Rokitamycin | | |
| • Telithromycin | | • Spiramycin* | | |
| | | • Tildipirosin*** | | |
| | | • Tilmicosin* | | |
| | | • Tylosin* | | |
| | | • Tylvalosin* | | |

* Substances approved for veterinary use (having marketing authorization, MA)
** Not any longer authorised in the EU
*** MRL set, no MA

## *4.2. Mechanism of action and spectrum of activity*

Macrolides inhibit protein synthesis of bacteria by binding to 50S subunit of the ribosome. Macrolides have their binding sites on the 23S rRNA of the 50S subunit, overlapping those of lincosamides and streptogramin B, but are different from those of phenicols like chloramphenicol. Macrolides, lincosamides and streptogramins generally have a bacteriostatic action, which is mainly time-dependant (Giguère 2006a; Giguère 2006b). Bactericidal activity has been found for some new generation macrolides against defined bacterial species in certain experimental conditions *in vitro* although the extent is limited compared to other classes (Seral, Van Bambeke et al. 2003). The clinical relevance of possible concentration-dependent action or post-antibiotic effects (PAE) of some new macrolides against certain pathogens detected in experimental conditions *in vitro* (Munckhof, Borlace et al. 2000; Jacobs, Bajaksouzian et al. 2003) has not been demonstrated. It is unlikely that e.g. possible PAE would contribute to the clinical efficacy of molecules with slow elimination, such as those in the most recent macrolide products authorized for animal use.

Macrolides are active against important human and animal pathogens, and their spectrum in general covers Gram-positive bacteria such as *Streptococcus, Staphylococcus, Enterococcus* and *Arcanobacterium pyogenes*, Gram-negative bacteria like *Actinobacillus pleuropneumoniae*, *Histophilus somni, Mannheimia haemolytica, Pasteurella multocida*, and *Campylobacter*, many anaerobic bacteria like *Brachyspira, Fusobacterium, Bacteroides* and *Clostridium* species, and other organisms such as *Lawsonia, Mycoplasma, Chlamydia, Bordetella, Moraxella, Leptospira* and *Spirocheta* species. However, marked differences exist between macrolides in their relative activity against different organisms (Hardy, Hensey et al. 1988; Bryskier and Butzler 2003). Furthermore, calibration of susceptibility testing for macrolides is difficult for many species, as guidelines for determination of minimal inhibitory concentrations (MIC) do not cover all micro-organisms listed, mainly because of culture conditions deviating from those for fastidious growing organisms (Schwarz, Silley et al. 2010).

In general, *Enterobacteriacea* are resistant to macrolides and lincosamides (Vaara 1993). Opposite to erythromycin or other 14-membered macrolides, azithromycin has activity against these Gram-negative bacteria, because it can penetrate their outer wall (Jones, Felmingham et al. 1988; Vaara

1993; Rise and Bonomo 2007). Azithromycin has moderate in vitro activity against *Salmonella* Typhi (Metchock 1990; Butler and Girard 1993); intracellular activity against non-typhoid Salmonella was also demonstrated (Chiu, Lin et al. 1999). Macrolides also have significant immunomodulatory effects independent of their antimicrobial activity (Chin, Lee et al. 2000; Tamaoki, Kadota et al. 2004). Azithromycin for example has been shown to enhance pro-inflammatory reaction of the host, to improve phagocytosis and to reduce local inflammation (Ribeiro, Hurd et al. 2009).

Lincosamides are structurally very different from macrolides, but share a similar mechanism of action. The spectrum of lincosamides is more limited as compared to macrolides, and e.g. enterococci are resistant (Roberts 2008). Streptogramins are active against Gram-positive bacteria, in particular aerobic, Gram-positive cocci. Group A and B streptogramins bind to separate sites of the bacterial ribosome. Group B streptogramins share an overlapping binding site with macrolides and lincosamides. Streptogramins are bacteriostatic, but the synergistic combination quinupristin/dalfopristin has shown bactericidal action against certain bacterial species (Speciale, La Ferla et al. 1999).

### *4.3. Pharmacokinetics*

As a class of antimicrobials, macrolides typically exhibit large volumes of distribution and a wide penetration to tissues. Chemically macrolides are weak bases, with high lipid solubility. Their activity is highly dependent on pH (Bryskier and Butzler 2003), with an optimal activity at pH higher than 7. Macrolides and lincosamides produce high intracellular concentrations and are known to accumulate in phagocytic cells. The actual efficacy of bacterial killing within the cells however has not been documented (Madgwick, Mayer et al. 1989; Barcia-Macay, Seral et al. 2006). Macrolides have an incomplete absorption after oral administration and they are eliminated mainly by liver, with a variable part of drug excreted in bile as parent drug or metabolites. These properties lead to entero-hepatic cycling and long terminal half-lives. Used by oral or parenteral route, macrolides have microbiological effects on the intestinal microbiota. One problem common for all macrolides is severe tissue irritation when given as injections, causing pain and inflammation. Erythromycin causes the most severe pain and irritation (Giguère 2006a). Lincosamides are absorbed well after oral administration to monogastric animals.

The more recently developed semisynthetic macrolides have a low clearance; the elimination half-life of tulathromycin in cattle and swine is close to 4 days and that of gamithromycin in cattle over 2 days. They are absorbed rapidly from the injection site, with bioavailability over 90%.

## 5. Use of macrolides, lincosamides and streptogramins

### *5.1. Use in human medicine*

Total consumption of MLS antimicrobials for humans in the EU (29 countries) in 2007 was 434 tons of active substance. MLS comprised in average 9.5 % of the total consumption, ranging from 2% to 27% (ESAC 2008). Outpatient use of MLS greatly differs between EU countries. In a survey in 2002 it varied by a factor of 26.9 between countries with the highest and lowest consumption (Goossens, Ferech et al. 2005). In 2005, consumption of MLS in the ambulatory care, expressed as DDD/1000 inhabitant days, was from less than 2 to 10.1, depending on the member state (ESAC 2008).

In humans, macrolides are used primarily to treat respiratory infections, skin infections, or infections of the genital tract. They are drugs of choice to treat human campylobacteriosis, in cases requiring antimicrobial therapy. Macrolides, mainly azithromycin, telithromycin or clarithromycin, are alternative drugs for treatment of pneumonia, sinusitis and otitis and the recommended choices for patients allergic for penicillins. Lincosamides (clindamycin) are used as an alternative to penicillin G to treat

infections caused by anaerobic bacteria, and in treatment of staphylococcal and streptococcal infections.

Streptogramins (quinupristin/dalfopristin) are authorized for use in infections caused by Enterococcus faecium. Quinupristin/dalfopristin is one of the few potential substances for the treatment of infections due to multi-resistant *Enterococcus faecium*, particularly in cases of vancomycin and linezolid-resistant strains, as well as to treat infections caused by multi-resistant staphylococci in humans (WHO 2007). It thus belongs to the last resort reservoir drugs.

Macrolides belong to the few available substances for treatment of serious *Campylobacter* infections. Macrolides (azalides) have also limited use in the treatment of *Legionella* and multi-resistant *Salmonella* infections (WHO 2007). Azithromycin is not authorized for treatment of *Salmonella* infections, but there is some published evidence on its clinical efficacy (Parry, Ho et al. 2007; Parry and Threlfall 2008).

## *5.2. Macrolides, lincosamides and streptogramins authorised for animals in the EU*

Macrolides, lincosamides and streptogramins have been authorised for use in food producing animals in the EU via national procedures, mutual recognition or centralised procedures. By the end of 2009, 7 macrolides and 2 lincosamides have been authorized for veterinary use in some or all Member States of the EU: erythromycin, tylosin, tylvalosin, spiramycin, tilmicosin, tulathromycin, gamithromycin, lincomycin and pirlimycin (Table 2). They are available either for parenteral administration by injection or for peroral use as premix formulations, or both (Figures 1 and 2). Pirlimycin is available for intramammary use only.

**Table 2.** Macrolides and lincosamides authorized in the European Union, status and year of first authorization, and animal species for which MRLs have been established.

| **Antimicrobial** | **Route of administration** | **Status and year of first authorisation (if available)** | **Species with MRL** |
|---|---|---|---|
| **Macrolides** | | | |
| Erythromycin | Injection, oral, intramammary2 | National[1] | All food animals |
| Gamithromycin | Injection | Centralized (2008) | Bovine |
| Spiramycin | Injection, oral, intramammary[2] | National | Bovine, porcine and chicken |
| Tilmicosin | Injection | National | All food animals |
| Tulathromycin | Injection | Centralized (2003) | Bovine and porcine |
| Tylosin | Injection, oral, intramammary[2], intrauterine[3] | National | All food animals |
| Tylvalosin | Oral | Centralized (2004) | Porcine and poultry |
| **Lincosamides** | | | |
| Lincomycin | Injection, oral, intramammary[2] | National | All food animals |
| Pirlimycin | Intramammary | Centralized (2001) | Bovine |

[1] Includes also mutual recognition procedures
[2] Occasional products in a few countries
[3] One product

Figure 1. Number of macrolide products per antimicrobial substance and Member State (data from 2009).

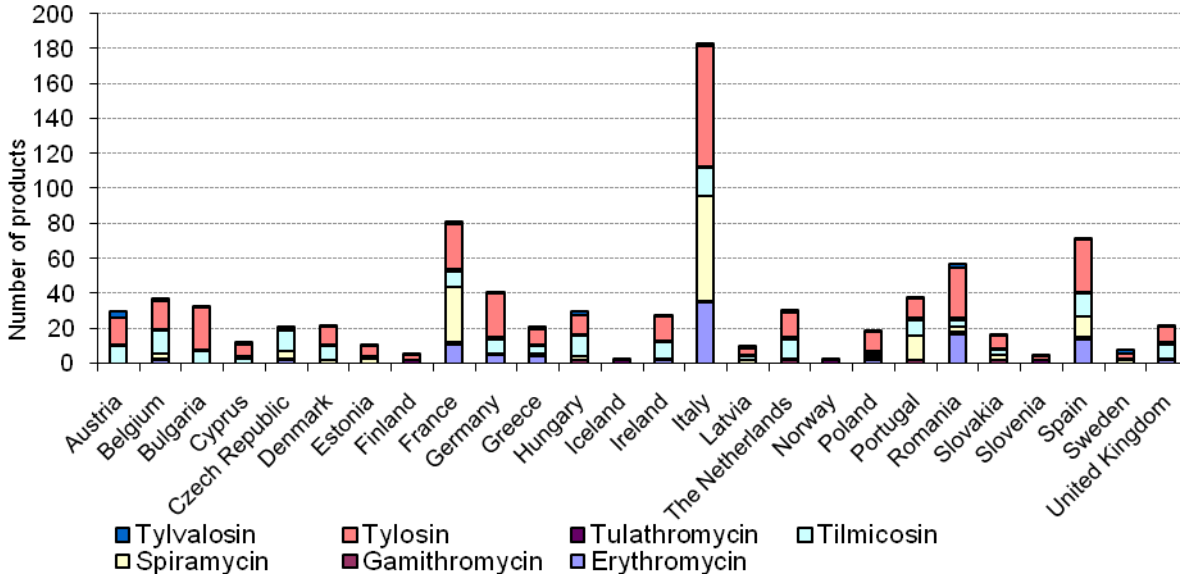
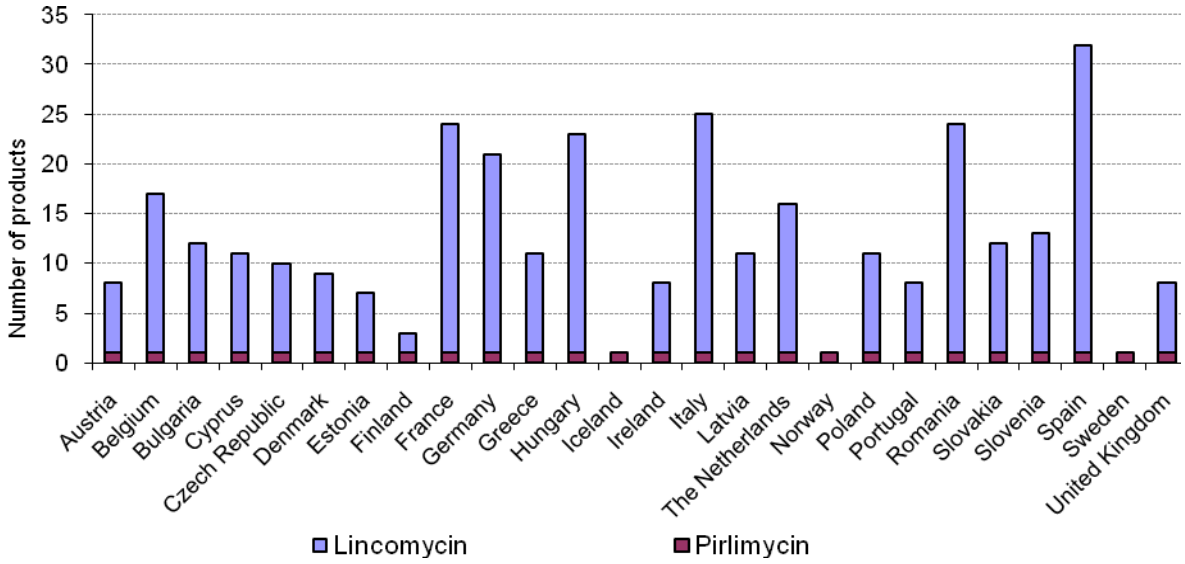



Figure 2. Number of lincosamides products formulated per antimicrobial substance and Member State (data from 2009).




## *5.3. Use of macrolides, lincosamides and streptogramins for animals in the EU*

Macrolides are widely used for treatment of diseases that are common in food producing animals. This class has also been categorised as critically important for veterinary medicine in the OIE list of antimicrobials of veterinary importance (Collignon, Powers et al. 2009). The first macrolide introduced for animal use was spiramycin, which was taken into use during early 1960'ies. In early 1970'ies, erythromycin and tylosin followed. Use of macrolides for growth promotion as feed additives began at the same times as the therapeutic use, and spiramycin and tylosin were used for growth promotion in food animals until withdrawn in the EU in 1998 (Council Regulation (EC) No 2821/98 of 17 December). The concept of so-called long-acting treatment (48 hours activity) was already introduced for food

287 animal therapy during late 1970'ies, when parenteral oxytetracycline products formulated in slow-
288 release bases were brought into market. Later for macrolides, the prolonged effect (>48 hours activity)
289 was achieved using molecules with a low clearance. The first macrolide introduced into veterinary
290 medicine with one-dose only posology was tilmicosin in the early 1990ies. The next macrolide
291 authorized with this regimen was tulathromycin in 2003, followed by gamithromycin. Some macrolides
292 and lincosamides are also used by the intramammary route, erythromycin and lincomycin on national
293 authorization and pirlimycin on centralized authorization. In this document, main attention is focused
294 on the systemic use.

295 At the moment, seven macrolides and two lincosamides (Table 2) are authorized for food animal use in
296 the European Union. The total number of products in Member States varies; from five to 183 products
297 containing macrolides and from one to 32 products containing lincosamides (Figures 1 and 2). In some
298 countries, the same macrolide product mostly aimed for medicated feed typically appears in as many
299 as 4-5 different strengths.

300 Consumption data for all animal use are available from 10 countries (Table 3). In a recent study, large
301 differences between countries in use of antimicrobials including MLS group in relation to slaughtered or
302 live food animals were found (Grave, Torren-Edo et al. 2010). The percentage of use of macrolides and
303 lincosamides in relation to the total use in kg for animals varies between member states and is in
304 average 8 %, ranging from 4 % to 13 %. Some countries report lincosamides together with
305 macrolides.

306 **Table 3.** Overall national sales, in tons of active substance, of use of macrolides and lincosamides and
307 total use of veterinary antimicrobials in 10 European countries (from 2007). Data were retrieved from
308 the latest report from the various national surveillance programs (European Medicines Agency
309 EMEA/CVMP/447259/2009).

310

| Country | Macrolides and lincosamides (% of total) | Macrolides | Lincosamides | Total |
|---|---|---|---|---|
| Czech Republic | 6.97 (8.8) | 6.51 | 0.46 | 79.36 |
| Denmark | 16.54 (13.5) | 13.30 | 3.24 | 123 |
| Finland | 0.62 (4.4) | - | - | 14 |
| France | - | 94.88 | 8.94 | 1349 |
| Germany* | 64.70 (8.3) | 52.60 | 12.10 | 784 |
| The Netherlands | 58.00 (9.8) | 58.00 | - | 590 |
| Norway | 0.02 (3.3) | Not given | 0.02 | 6 |
| Sweden | 1.52 (8.9) | - | - | 17 |
| Switzerland | 3.70 (5.1) | 3.70 | - | 72 |
| United Kingdom | 33.00 (8.6) | 33.00 | - | 382 |

311 *data from 2005

312 The nationally authorised macrolide products are mostly old, and their indications and posologies show
313 a great variation. For the initial macrolide products, indications were not very specific, but the products
314 were just aimed for treatment and prophylaxis of bacterial infections susceptible for these substances.
315 The main indications in swine are pneumonia, enteritis and arthritis, in cattle all common infections
316 such as respiratory and genital infections, foot lesions and mastitis, and in poultry respiratory
317 infections and necrotic enteritis. Products for in-feed medication containing macrolides or lincosamides
318 in combination with other antimicrobials are common. Most often macrolides are combined with colistin
319 or aminoglycosides, but also with sulphonamides, trimethoprim, oxytetracycline, or ampicillin. More
320 than 60 combination products containing macrolides with other antimicrobials are available in the EU;

in addition, numerous lincomycin products in combinations exist. The indications for combination products can be particularly broad. The approved duration of treatment for some products is long, e.g. for some tylosin containing premixes from 4 to 5 weeks. Based on the regimens with long duration of treatment it cannot be excluded that some ML products are probably used as feed additives for pigs and calves. Deviations from indicated dosages and treatment lengths of peroral products are possible (Samson, Godinho et al. 2006; Timmerman, Dewulf et al. 2006; Catry, Dewulf et al. 2007).

The indications for the recently approved macrolide and lincosamide products are more restricted, with listing of the target pathogens. The most common indications in all food animals are respiratory and gastro-intestinal infections. In cattle, detailed indications for the injectable macrolides on centralized authorization are, depending on the product, treatment and prevention of bovine respiratory infections caused by *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*, treatment and prevention of bovine respiratory disease associated with *Mannheimia haemolytica*, and *Mycoplasma bovis*, and infectious bovine keratoconjunctivitis associated with *Moraxella bovis*. In swine, injectable macrolides are indicated for treatment and prevention of swine enzootic pneumonia caused by *Mycoplasma hyopneumoniae*, and respiratory infections caused by *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Haemophilus parasuis*.

Tylvalosin is centrally authorized for oral administration and indicated in swine for treatment and prevention of porcine proliferative enteropathy caused by *Lawsonia intracellularis*, swine dysentery caused by *Brachyspira hyodysenteriae*, and swine enzootic pneumonia. The product is also authorized for poultry for the treatment and prevention of respiratory disease associated with *Mycoplasma gallisepticum*. Pirlimycin is authorized in the EU for treatment of bovine subclinical mastitis caused by common Gram-positive mastitis causing agents.

Macrolides and lincosamides are recommended in the textbooks and national treatment guidelines for many indications in food animals (Anonymous 2003; Giguère 2006a; Burch, Duran et al. 2008; Constable, Pyörälä et al. 2008). Macrolides are recommended, often as first choices, for treatment of respiratory infection in cattle and swine and for porcine proliferative enteropathy. They are alternative drugs for treatment of mastitis caused by Gram-positive bacteria and for some infections in poultry. Lincosamides are alternative substances for treatment of respiratory and gastro-intestinal infections in swine and poultry, as well as for treatment of bovine mastitis caused by Gram-positive bacteria; in addition they are used as alternatives for necrotic enteritis and mycoplasmosis in poultry. Use of erythromycin, azithromycin or clarithromycin (off-label) in combination with rifampicin has been suggested for treatment of *Rhodococcus equi* infections in foals (Giguère 2006a; Weese, Baptiste et al. 2008).

# 6. Mechanisms of resistance to macrolides, lincosamides and streptogramins

## *6.1. Natural resistance*

Naturally or intrinsically MLS resistant bacteria are macrolide-producing *Streptomycetes*, harbouring genes which provide a self-protective mechanism, as well as the naturally macrolide resistant *Mycobacterium tuberculosis* complex (Andini and Nash 2006) and several rapidly growing mycobacteria (Nash, Andini et al. 2006) that carry unique erm genes (erythromycin ribosomal methylase). Some of these mycobacterial innate methylase genes confer ML resistance, but not resistance to streptogramins (Roberts 2008). Equally, innate resistance genes (like *mrs*(C) for macrolide streptogramin resistance) coding efflux proteins have been described in enterococci (Roberts 2008).

Enterobacteriaceae such as *E. coli, Salmonella* and other Gram-negative bacilli have generally a low susceptibility to macrolides, because of the poor permeability of these hydrophobic substances across their bacterial wall (Vaara 1993). Azithromycin shows nevertheless activity against *Salmonella* (Jones, Felmingham et al. 1988; Capoor, Rawat et al. 2007).

## *6.2. Acquired resistance*

The first bacterial species with acquired resistance to macrolides described was a Staphylococcus showing resistance to erythromycin (Zhanel, Dueck et al. 2001; Roberts 2008). Later, more than 67 different genes, hosted by more than 58 different bacterial species, have been described in the context of MLS resistance (Roberts).

## *6.3. Horizontally transferable resistance*

The most common resistance mechanism is a target site modification mediated by at least 32 different rRNA methylases (*erm* genes) described in 34 bacterial genera (Leclercq and Courvalin 1991; Diner and Hayes 2009) (table 1). This mechanism was the first described and is due to a posttranscriptional modification of the 23S rRNA by adenine-methyl-transferases (methylases), adding one or two methyl groups to the same adenine residue (Roberts, Sutcliffe et al. 1999; Douthwaite, Hansen et al. 2000). This modification reduces the binding of the MLSB antimicrobials to the ribosomal target site.

The *erm* genes can be expressed constitutively or inducibly (Stepanovic, Martel et al. 2006; Giguère 2006a). When the gene is constitutively expressed, the bacterial strain harboring the gene will be phenotypically resistant to all or most MLSB antimicrobials. However, some of the genes are inducibly regulated by different mechanisms and, in absence of inducers, the enzyme is not produced and the corresponding strain shows a phenotype resistant to the inducing group of molecules only. Induction is generally triggered by exposure of the microorganism to 14-member or 15-member ring macrolides (due/related to a cladinose sugar moiety), but not by the 16-member ring macrolides. Inducibly expressed genes can convert to constitutively expressed resistance by deletions or mutations in the regulatory gene.

In bacteria isolated in humans, inducible resistant strains (e.g. *Staphylococcus* species) predominated in the 1960s to 1970s (Roberts, Sutcliffe et al. 1999). However, constitutive *erm* genes, associated with structural alternation in the attenuating mechanisms, have since been increasing. These strains show a stable resistant phenotype regardless of previous induction.

Many of the *erm* genes can be horizontally transferred because they are associated with conjugative or non-conjugative transposons, which tend to reside in the chromosomes (Roberts, Sutcliffe et al. 1999), but can also be located on plasmids. For instance, the conjugative transposon Tn1545, first described in 1987 by Courvalin and Carlier (Courvalin and Carlier 1987), carries many different antimicrobial resistance genes including erm(B) (Roberts 2008).

The *erm* genes have been identified in multiple bacterial genera, including Gram-negative and Gram-positive as well as aerobic and anaerobic bacteria (Edelstein 2003; Roberts 2008). In particular, *erm*(B) has the widest host range, that can be due to its frequent association with mobile elements, like transposons (Tn1545, Tn5384,Tn2009, or Tn2010), and its linkage to different genes conferring resistance to other antimicrobials, especially for tetracyclines (*tet*M, *tet*Q), or other substances (mercury, copper). Among animal pathogenic bacteria, *erm*(B) has been detected e.g. in streptococcal species such as *Streptococcus suis*, *S. uberis*, *S. dysgalactiae, S. agalactiae*, *Staphylococcus pseudintermedius, S. hyicus*, *S. aureus,* enterococci, and *Listeria monocytogenes* (Jensen, Frimodt-Moller et al. 1999; Boerlin, Burnens et al. 2001; Martel, Baele et al. 2001; Martel, Devriese et al. 2003; Culebras, Rodriguez-Avial et al. 2005; Loch, Glenn et al. 2005; Palmieri, Ratsch et al. 2007;

Schmitt-Van de Leemput and Zadoks 2007; Luthje, von Kockritz-Blickwede et al. 2007b; Haenni, Saras et al. 2010). Different erm genes including *erm*T have been found in the emerging meticillin resistant *S. aureus* ST398 in livestock (Fessler, Scott et al. 2010).

The second most common resistance mechanism is due to active expulsion of the antimicrobial from the bacteria mediated by efflux pumps. At least 16 different genes have been identified in relation to this mechanism. In Gram-positive bacteria, two classes of efflux pumps are implicated in acquired macrolide resistance: members of the ATP-binding-cassette (ABC) transporter superfamily, encoded by the mef (for macrolide efflux pump) genes, and members of the major facilitator superfamily, like that encoded by the msr genes (for macrolide and streptogramin B resistant efflux pump). Many of the *mef* genes are associated with conjugative elements located in the chromosome, whereas msr genes are mainly located on plasmids. The *msr*(D) gene, which is always downstream of the *mef*(A) gene, is the most prevalent gene of this group. Among animal pathogenic bacteria, *mef*(A) has been detected in S. suis (Martel, Devriese et al. 2003). Recently, a novel macrolide efflux gene (mef(B)) was detected in porcine isolates of *E. coli* (Liu, Keelan et al. 2009). In addition, efflux pumps of the Cme-ABC system also contribute to macrolide resistance in *Campylobacter* (Gibreel and Taylor 2006).

Although less common, resistance due to enzymatic inactivation of some members of the MLS antimicrobials has also been described, and currently there are 19 inactivating enzymes involved (table 1). At least two of the corresponding genes have linkage to integrons *ere*(A) (for erythromycin esterase), *lnu*/*lin*(F) (for lincomycin nucleotidyl transferase; (Roberts, Sutcliffe et al. 1999)) and *mph*(C) (for macrolide phosfotransferase) and one to insertion sequences (*mph*(C)), that can be in favour or their horizontal spreading. These genes have been detected in animal pathogens, like *mph*(C) in *S. aureus* and *lnu*/*lin* in *S. hyicus* (Luthje, von Kockritz-Blickwede et al. 2007b). *Streptococcus uberis* has been shown to express several genes such as *mph*(B) or *lin*(B) to confer resistance to macrolides or lincosamides (Schmitt-Van de Leemput and Zadoks 2007; Achard, Guerin-Faublee et al. 2008; Haenni, Saras et al. 2010).

The highly diverse resistance mechanisms described above also differ in their ability for eliciting cross-resistance to all or some members of the MLSB group. The rRNA methylases confer a MLSB resistant phenotype (resistance to macrolides, lincosamides and streptogramin B), whereas efflux pumps have usually a more narrow cross-resistance profile resulting in different resistance phenotypes (table 1). For instance, *mef* genes lead to the M phenotype characterized by resistance to 14 and 15-member ring macrolides and susceptibility to 16-member ring macrolides as well as to lincosamides and streptogramin B.

A new gene *cfr* for chloramphenicol and florfenicol resistance, which code for an unusual rRNA methylase, conferring a novel multidrug resistance phenotype (including resistance to lincosamides, streptogramins A, phenicols, pleuromutilins, and oxazolidinones), was detected in a bovine isolate of *S. sciuri* (Schwarz, Kehrenberg et al. 2002), and later also in other animal isolates like porcine *S. aureus* and bovine S. simulans (Long, Poehlsgaard et al. 2006). This gene has also been detected in human isolates of linezolid-resistant *S. aureus* (Arias, Vallejo et al. 2008). A novel transporter gene *vga*(C) mediating resistance to pleuromutilins, lincosamides and streptogramins A was found in porcine MRSA isolates of type ST398 (Kadlec and Schwarz 2009), and more recently vga(A) in bovine ST398 isolates (Fessler, Scott et al. 2010).

Finally, the most narrow resistance phenotypes are those elicited by inactivating genes, like phosphorylases (*mph* genes) conferring resistance only to macrolides, or transferases that render bacteria resistant only to streptogramin A (table 1). The plasmid-borne *mph*(A) gene that confers resistance to azithromycin and has emerged in *Shigella* is also present in human *E. coli* isolates, illustrating the possibility of transfer of resistance genes between bacterial species (Phuc Nguyen, Woerther et al. 2009).

## 6.4. Non-horizontally transferable resistance

Resistance mechanisms due to mutations in ribosomal RNA and ribosomal proteins conferring reduced macrolide susceptibility were first identified for proteins L4 and L22 in the 50S subunit of the ribosome (Lovmar, Nilsson et al. 2009). From the MLS resistance perspective, the most important are mutations in genes coding for 23S rRNA (domain V), whereas the role of mutations affecting the genes coding for ribosomal proteins L4 and L22 have been less studied.

Mutational events introducing base substitutions at position A2058 (or neighboring nucleotides) of the 23S rRNA confers MLS resistance (Vester and Douthwaite 2001), being the most prevalent or the only resistance mechanism in certain animal pathogens like *B. hyodysenteriae*, *B. pilosicoli*, and *Mycoplasma hyopneumoniae* (Karlsson, Fellstrom et al. 1999; Karlsson, Fellstrom et al. 2004b; Stakenborg, Vicca et al. 2005), as well as in the zoonotic *C. jejuni* and *C. coli* (Gibreel and Taylor 2006; Alfredson and Korolik 2007; Caldwell, Wang et al. 2008). These non-horizontally transferable resistance genes in animal pathogenic bacteria are less relevant in terms of spreading antimicrobial resistance in relation to public health, but remain of interest from the animal health perspective. Nevertheless, mutational changes in the zoonotic campylobacter bacteria warrant interest for public health.

Contrary to the resistance mechanisms that can be horizontally transferred, mutational changes are normally passed vertically to daughter cells during replication and generally not passed between bacterial strains or between different genera (Roberts 2008). However, after exposure to macrolides, these mutations can rapidily dominate bacterial populations in which the individual cells possess only one or two rRNA operons (Vester and Douthwaite 2001).

**Table 4.** Resistance genes and mechanisms of resistance for macrolides, lincosamides and streptogramins.

| Resistance phenotype | Genes | Characteristics | HGT* |
|---|---|---|---|
| $MLS_B$ | *erm* (A to Z and 30 to 41) | rRNA methylases that confers resistance to macrolides, lincosamides and streptogramins B. Can be either inducible or constitutive | + |
| $M(E)S_B$ | *msr* (A, C and D) | Efflux pumps (ATB-binding transporter) that confers resistance to macrolides (erythromycin only?) and streptogramins B | + |
| M | *mef* (A and B) | Efflux pump (major facilitator) that confer resistance to 14- and 15-member ring macrolides | + |
| LS | *Cfr* | rRNA methylases that confer resistance to lincosamides and streptogramins A. In addition, this enzyme confers resistance to phenicols, pleuromutilins, and oxazolidinones | + |
| M | *mph* (A to D) | Phosphorylases that confers resistance to macrolides | + |
| E | *ere* (A and B) | Esterases that confers resistance to erythromycin | + |
| $S_AL$ | *vga* (A to C) | Efflux pumps (ABC transporter proteins) that confers resistance to streptogramins A, lincosamides and pleuromutilins | + |
| $S_A$ | *lnu/lin* (A to F) | Transferases that confers resistance to lincosamides? | + |
| $S_A$ | *vat* (A to F) | Transferases that confers resistance to streptogramins A | + |
| L | *lsa* (A and B) | Efflux pumps that confers resistance to lincosamide | + |
| L | *car* (A) | Efflux pumps (ATB-binding transporter) that confers resistance to lincomicyn | + |

| Resistance phenotype | Genes | Characteristics | HGT* |
|---|---|---|---|
| | | | |
| L | *lmr* (A) | Efflux pumps (major facilitator) that confers resistance to lincomycin | + |
| O | *ole* (B and C) | Efflux pumps (ATB-binding transporter) that confers resistance to oleandomycin | + |
| S | *srm* (B) | Efflux pumps (ATB-binding transporter) that confers resistance to spyramicin | + |
| T | *tlr*(C) | Efflux pumps (ATB-binding transporter) that confers resistance to tylosin | + |
| MLS | rRNA operon | Mutations in nucleotide A2058 (or neighboring nucleotides) of 23S rRNA t confers resistance to macrolides, lincosamides and streptogramines | - |
| S | L4/L22 ribosomal proteins | Mutations, substitutions and delections on different positions of L4 and L22 ribosomal proteins confers resistance to streptogramins (L22) and reduced susceptibility to macrolides and lincosamides(L22, L4) | |

*HGT: horizontal gene transfer documented

## *6.5. Resistance in bacteria from food producing animals*

Resistance against MLS among animal pathogens as well as zoonotic bacteria has emerged, and is now common in different bacterial species. It is apparent that situations in different EU member states greatly differ, regarding the susceptibility of animal pathogens for antimicrobials of the MLS group. In general, it is difficult to compare prevalence data of resistance between different time periods and geographical sites, because origin of isolates, panels of antimicrobials used, methods used for susceptibility testing and cut-off values for resistance differ (Schwarz, Silley et al. 2010). For some EU countries, surveillance data for decades exists, but in some other, almost nothing is known. This may imply a selection bias which can compromise the representativeness of data as Pan European. Comparable data are available for zoonotic bacteria, as coordinated by the EU wide surveillance programs (EFSA 2010). For animal pathogens, uniform data are so far not available. Isolates of major animal pathogen species have been collected in national monitoring programmes, but bacterial species tested vary widely between countries reporting such data. In addition to these data, published scientific studies are available and can be used as sources for information. Despite these limitations, certain trends for MLS resistance among animal pathogens and zoonotic bacteria are apparent.

## *6.6. Emergence of resistance among animal pathogens*

### *6.6.1. Brachyspira*

High levels of resistance *in vitro* are reported for tylosin and in most EU countries, 90-100 % of the *Brachyspira* isolates are resistant (FINRES-Vet 1999; SVARM 2002-2009; Vyt and Hommez 2006; MARAN 2008; Hidalgo, Carvajal et al. 2009). Data on *in vitro* susceptibility of tylvalosin are scarce and no cut-off value is available, but isolates resistant to tylosin have generally slightly increased MIC values (Karlsson, Aspan et al. 2004a). Resistance of *B. hyodysenteriae* for lincomycin is close to that for tylosin (SVARM 2002-2009; FINRES-Vet 2007) (ITAVARM 2003), due to complete cross-resistance. Resistance among *B. pilosicoli* to tylosin has been reported to be 50% - 100%; also occasional high MICs for tylvalosin have been reported (SVARM 2002-2009; Karlsson, Fellstrom et al. 2004b; Pringle, Aarestrup et al. 2006a). Multiresistant isolates have also been found, with simultaneous resistance against lincomycin, tylosin, tylvalosin and tiamulin (Duinhof, Dierikx et al. 2008).In a field study on spontaneous infection of pigs caused by *Brachyspira hyodysenteria* it was concluded that *in vitro*

susceptibility testing of *B. hyodysenteriae* (for lincomycin) only partially predicted the clinical effect of treatment (Vyt and Hommez 2006).

### 6.6.2. Anaerobic bacteria other than Brachyspira

Data on resistance of anaerobic bacteria including *Clostridium* to macrolides and lincosamides are limited. Percentages of macrolide-lincosamide resistance among *C. perfringens* isolated from animals have been generally low in the EU (Franklin, Pringle et al. 2006). However, in Belgium 34% of *C. perfringens* isolated in poultry were resistant to lincomycin (Martel, Devriese et al. 2004). Some data are available for *Fusobacterium* spp. isolated in animals, indicating resistance against macrolides, but susceptibility to lincosamides (Jousimies-Somer, Pyorala et al. 1996; Jimenez, Piriz et al. 2004). Recent data from Sweden on susceptibility of *F. necrophorum* ssp. *necrophorum* isolated in cows and sheep showed MICs for erythromycin from 2 to 8 mg/l (SVARM 2002-2009). No accepted cut-off values for determining macrolide resistance of *F. necrophorum* exist.

### 6.6.3. Family Pasteurellaceae

In North America, resistance of *Pasteurella multocida* isolated in cattle and swine against macrolides has been frequently reported, but in the EU it has been rare (Kehrenberg, Walker et al. 2006). In the Netherlands, 0 % in 2004-2005 and 2.5 % of isolates from cattle in 2006-2007 were resistant to tilmicosin but none to tulathromycin. In France in 2008, 7% of bovine *P. multocida* were resistant to tilmicosin; among porcine isolates no resistance to tilmicosin was found but 86% of the isolates were resistant to tylosin (AFFSA 2009). In Belgium, 13% of *P. multocida* isolates and 38% of *haemolytica* isolates from healthy animals including veal calves showed resistance to tilmicosin (Catry, Haesebrouck et al. 2005). As to *Mannheimia haemolytica* isolated in cattle in The Netherlands, resistance to tilmicosin has increased from zero to close to 5 % (MARAN 2008); no resistance to tulathromycin has been found. In France in 2008, the proportion of *M. haemolytica* isolated in cattle resistant to tilmicosin was as high as 35%. In many national monitoring systems, susceptibility of *Pasteurellaceae* for macrolides has not been tested. Furthermore, if the cut-off breaks through the population, analysis of the distribution of inhibition zone diameters or MIC values may be problematic. This was for instance underlined by a French organization (Comité de l'antibiogramme - Société Française de Microbiologie), which recommended for diagnostic laboratories not to establish an interpretation for macrolides and *Pasteurellaceae* (Vet 2009).

Data on *Haemophilus parasuis* in pigs or *Histophilus somni* in cattle are scarce; no resistance for tilmicosin was found in Danish isolates during early 2000 (Aarestrup, Seyfarth et al. 2004). For A. pleuropneumoniae isolated in swine data are also very limited; in France already close to 80% of A. pleuropneumoniae were resistant to spiramycin, but only 2% to tilmicosin (AFFSA 2009). In Spain, minimal inhibitory concentrations (MIC) values of *A. pleuropneumoniae* for erythromycin had increased compared with those reported two decades earlier (Gutierrez-Martin, del Blanco et al. 2006), but changes like this should be interpreted with caution as methods may not be the same.

### 6.6.4. Staphylococcal and streptococcal species

Resistance of staphylococci (*S. aureus*) isolated in bovine mastitis against macrolides is rare in most EU member states where data are available: 0-2 % of the isolates were resistant against erythromycin. In *some* countries, higher figures have been reported; e.g. in France up to 7% of *S. aureus* isolates were resistant to macrolides and lincosamides (Hendriksen, Mevius et al. 2008; AFFSA 2009). Resistance of *S. aureus* for clindamycin was not reported in Finland, Sweden and Norway, and was 1-4% in the Netherlands. For pirlimycin, resistance in *S. aureus* has emerged in the Netherlands and was 4% in 2007 (MARAN 2007). Coagulase-negative staphylococci (CNS) have

developed resistance to MLS antimicrobials (Luthje and Schwarz 2006).Resistance for macrolides has been 4-6%, and no resistance to clindamycin has been found in reports available (Pitkala, Haveri et al. 2004; NORM-VET 2005; MARAN 2007). By contrast, 13-20% of CNS isolated from bovine mastitis in the Netherlands and France were resistant to lincosamides (MARAN 2007; AFFSA 2009) and up to 14% to erythromycin (Botrel, Haenni et al. 2010).

Information available on methicillin-resistant *S. aureus* (MRSA) isolated from animals shows that MRSA is often resistant also to MLS antimicrobials. Generally, close to 50% of the MRSA isolates from animals have been resistant to macrolides and lincosamides (Rich, Deighton et al. 2005; Kehrenberg, Cuny et al. 2009). As regards MRSA of type ST398 common in food animals, 40-50% of isolates from swine and bovine mastitis are also resistant for macrolides and lincosamides (Kadlec, Ehricht et al. 2009; Fessler, Scott et al. 2010). Recently, a novel mechanism mediating transferable resistance to lincosamides, streptogramin A antibiotics and pleuromutilins have been described in porcine and bovine ST398 isolates (Fessler, Scott et al. 2010; Kadlec and Schwarz 2010).

Acquired macrolide resistance has emerged in *Streptococcus* species of animal origin. Available information indicates that the ocurrence of resistant isolates varies between countries. In a limited study in some European countries, 0-22% of *S. uberis* and 0-17% of *S. dysgalactiae* isolates from bovine mastitis were found resistant to erythromycin (Hendriksen, Mevius et al. 2008); in a recent French study 13-17% of *S. uberis* and 4-6% of *S. dysgalactiae* isolates from clinical and subclinical mastitis were resistant to erythromycin, spiramycin and lincomycin (Botrel, Haenni et al. 2010). Data from the Netherlands revealed that 43% of *S. uberis* and 8% of *S. dysgalactiae* were resistant to clindamycin (MARAN 2007). In Sweden and Norway, no resistance for erythromycin or clindamycin was reported for *S. uberis* and *S. dysgalactiae* isolated in bovine mastitis (SVARM 2002-2009; NORM-VET 2008). In Finland, 15% of *S. uberis* isolates were resistant to erythromycin but none to clindamycin; *S. dysgalactiae* isolates were fully susceptible for both (FINRES-Vet 2007).

Resistance of *Streptococcus suis* isolated in pigs towards macrolides has varied between EU countries. Increasing resistance for macrolides among *S. suis* was found in Denmark during investigations ten years apart (Aarestrup and Schwarz 2006). In selected EU countries in 2002, resistance of *S. suis* to erythromycin was 19-65% (ARBAO-II). In France, resistance of *S. suis* was recently reported to be as high as 72-77% to spiramycin and tylosin and 69% for lincomycin (AFFSA 2009). Prevalence of *Staphylococcus hyicus* resistant to macrolides has been monitored in Denmark, where resistance for erythromycin increased from 33% in 1996 to 62% in 1997, and decreased from 2001 to approximately 20%, being at present about 35% (DANMAP 2004; Aarestrup and Schwarz 2006). In Sweden, 12 % of *S. hyicus* were resistant to erythromycin (SVARM 2002-2009). Higher figures have been reported for some other EU countries (Aarestrup and Schwarz 2006).

### 6.6.5. Other bacteria and Mycoplasma

For *Lawsonia intracellularis* there are no standards for susceptibility testing and practically no data are available. In one study, MIC90 values of *Lawsonia intracellularis* were higher for tylosin (64 µg/ml) as compared to those for tilmicosin (2 µg/ml) or erythromycin (0.5 µg/ml), but the clinical relevance of this remains unknown (Giguère 2006a).

Reports on antimicrobial susceptibility of *Mycoplasma* species are scant. Futhermore, results from *in vitro* susceptibility testing of *Mycoplasma* should be considered with caution as no agreed standards for testing are available. *M. hyopneumoniae* is intrinsically resistant to 14-membered macrolides. In reports published two decades ago, isolates from pigs were fully susceptible to 16-membered macrolides such as tylosin (Aarestrup and Kempf 2006). More recently, acquired resistance to macrolides and lincosamides was reported in Belgium (Stakenborg, Vicca et al. 2005). Resistance of *M. hyosynoviae* for macrolides and lincosamides was reported in Japan (Kobayashi, Nakajima et al. 2005).

Resistance of *M. hyosynoviae* isolated in swine was examined in Denmark; in 1968-1971 all isolates were susceptible to lincomycin and tylosin but twenty years later 12% of the isolates were resistant to tylosin (Aarestrup and Friis 1998). Many field isolates of *M. bovis* isolated from cattle in Belgium during early 2000 showed in vitro resistance to macrolides (Thomas, Nicolas et al. 2003). In one study using experimental *M. bovis* infection model, clinical efficacy of tulathromycin was not associated with the *in vitro* susceptibility of the challenge strain to that macrolide drug (Godinho, Rae et al. 2005). Clinical efficacy of tulathromycin did not correlate with the *in vitro* susceptibility in experimental infection caused by *Mycoplasma bovis* in calves (Godinho, Rae et al. 2005).

## *6.7. Emergence of resistance among zoonotic and commensal bacteria*

### *6.7.1. Campylobacter spp*

Resistance to macrolides has emerged in zoonotic pathogens such as *Campylobacter spp* isolated in food animals, with clear differences the reported prevalences between EU states (de Jong, Bywater et al. 2009; EFSA 2010). According to the recent EFSA zoonosis report (table 5) presenting data from 2004 to 2007, resistance to erythromycin among *C. coli* isolates from pigs was common: in 2007, 39% of a total of 662 isolates were resistant, with an increasing trend. Among *C. jejuni* from poultry resistance to erythromycin had remained at a constantly low level. From a total of 534 isolates from poultry, 4% were resistant, with no significant differences between isolates from poultry and broiler meat. Resistance among *C. jejuni* from cattle was very low and remained close to 0. Acquired macrolide resistance is substantially more common in *C. coli* than in *C. jejuni* (Payot, Bolla et al. 2006; Belanger and Shryock 2007). In *Campylobacter*, total cross-resistance between older macrolides (erythromycin) and new macrolides such as azithromycin has been shown (Harada, Asai et al. 2006). The EFSA Community Report (EFSA 2007) showed that in the EU in 2006, 2.3% of *C. jejuni* and 10% of *C. coli* isolated in humans were resistant to erythromycin. Based on data from ECDC on human infections by *Campylobacter* in 2006, the prevalence of erythromycin resistance ranged from 0% to 14% among eight MS (ECDC 2010).

**Table 5.** Reported resistance to erythromycin in *Campylobacter* isolated in healthy animals in 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2007. (EFSA 2007)

| | **Cattle** | | **Pigs** | | **Poultry** | |
|---|---|---|---|---|---|---|
| Country | *C. jejuni* n | % R | *C. coli* N | % R | *C. jejuni* N | % R |
| Austria | 202 | 0 | 219* | 18 | 26 | 0 |
| Czech Republic | | | | | 53 | 6 |
| Denmark | 84 | 1 | 104 | 11 | 94 | 1 |
| Finland | | | | | 94 | 0 |
| France | | | 77 | 32 | 56 | 0 |
| Germany | | | 91 | 27 | 100 | 13 |
| Italy | 54* | 0 | 143 | 60 | 48 | 4 |
| Netherlands | 71 | 0 | 103 | 18 | 45 | 2 |
| Norway | | | | | 99 | 0 |
| Slovenia | | | | | 71*** | 2.8 |
| Spain | 55 | 0 | 144 | 63 | 19 | 5 |
| Sweden | 68*** | 0 | 97* | 0 | 94** | 0 |
| Switzerland | | | 46 | 11 | 122 | 3 |

*2005 **2004 ***2006

*Figure CA16. Trends in erythromycin resistance in* Campylobacter coli *from pigs in reporting MSs, 2004-2007, quantitative data*

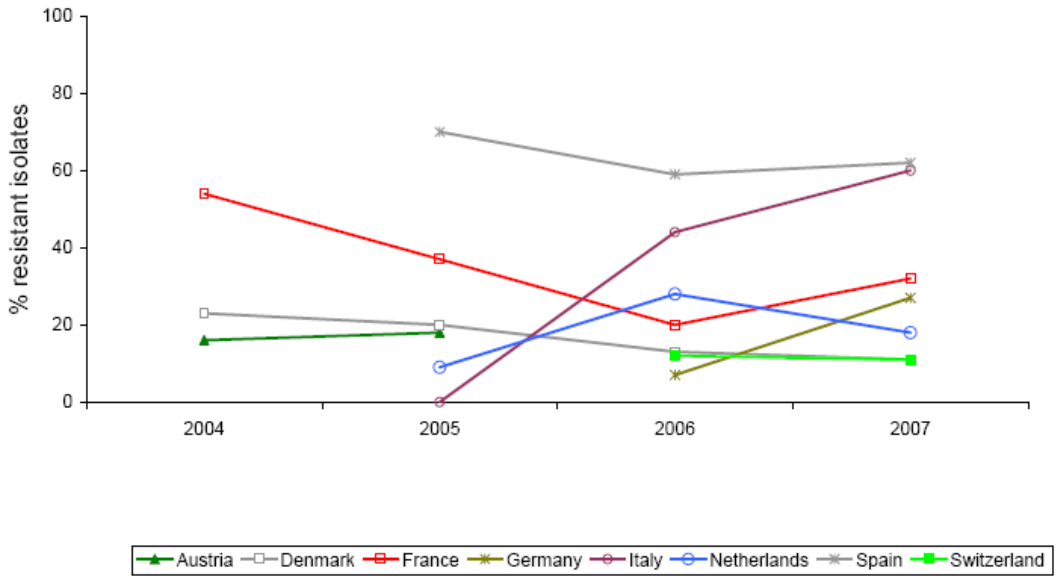



Figure 3. Trends in resistance in *Campylobacter coli* from pigs in the Member States of the EU reporting these data. Source: European Food Safety Authority; The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2004-2007. EFSA Journal 2010; 8(4):1309. (EFSA 2010)

### *6.7.2. Enterococcus spp*

Transferable resistance genes have emerged in *Enterococcus spp* of animal origin, and resistance against macrolides is at high levels. Proportions of resistant isolates vary between different EU member states. In Denmark, approximately 80% of *E. faecium* isolated from broilers and pigs in the late 1990ies were resistant to tylosin and 50-70% resistant to virginiamycin; at the same time respective figures were about 15% and 17% vs 2% in Finland and 7% and 0% (broilers) in Norway (Aarestrup, Kruse et al. 2000). The prevalence of macrolide-resistant enterococci has since decreased (Figure 3); in 2008 16% and 32% of E. faecium and 10% and 40% of *E. faecalis* isolated in broilers and pigs, respectively, were resistant to erythromycin in Denmark and the Netherlands (DANMAP 2008; MARAN 2008). The recent national surveys in the EU show that proportion of erythromycin-resistant *E. faecalis* and *E. faecium* isolated from broiler meat is for example 11% and 21% in Denmark and 42% *vs* 34% in the Netherlands (EFSA 2010).

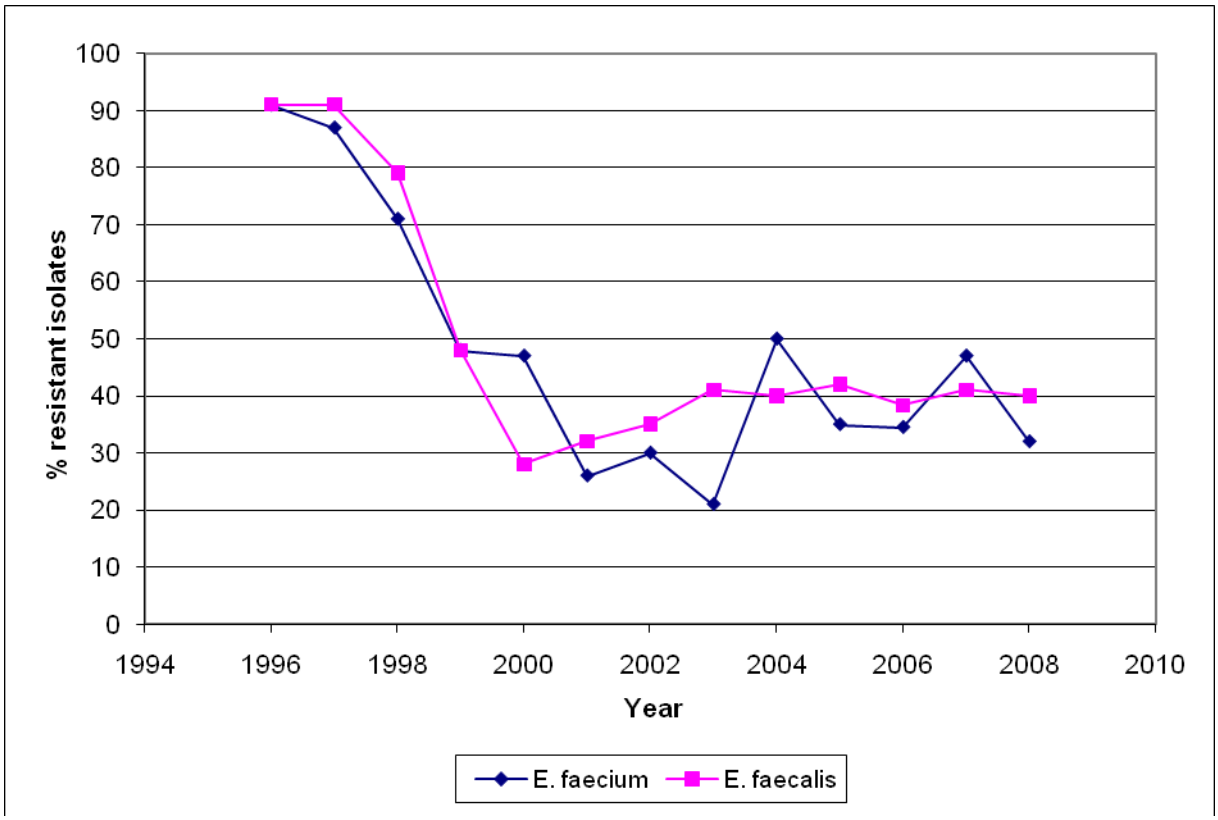



*Figure 4. Occurrence of resistance (%) among Enterococcus faecium and Enterococcus faecalis from pigs in Denmark (DANMAP). Growth promoters were prohibited in the EU in 1998.*

## 6.8. Influence of use of macrolides, lincosamides and streptogramins in human medicine on resistance

A strong association between use of macrolides and resistance of commensal or pathogenic bacteria has been noted in humans. In early exposure studies, impact of several macrolides was studied experimentally in human healthy volunteers (Andremont, Raibaud et al. 1983; Andremont, Trancrede et al. 1991; Pecquet, Chachaty et al. 1991). Faecal concentrations of highly resistant bacteria of the gastro-intestinal tract were found to increase during and after macrolide treatment. More recent studies using macrolides or streptogramins have confirmed these findings (Scanvic-Hameg, Chachaty et al. 2002). Macrolides significantly increased the proportion of macrolide-resistant streptococci in the pharynx of human volunteers (Malhotra-Kumar, Lammens et al. 2007).

Increased consumption of macrolides, especially the long-acting products, has significantly correlated with the level of macrolide resistance of group A streptococci and *Streptococcus pneumoniae* (Cizman 2003). Several pharmaco-epidemiological studies have demonstrated a link between use of macrolides and resistance (Bergman, Huikko et al. 2006; Riedel, Beekmann et al. 2007; Karlowsky, Lagace-Wiens et al. 2009). In a cross-national European study, an association between macrolide consumption and resistance was found (Goossens, Ferech et al. 2005). Use of macrolides may also select for resistance against other antimicrobials; they were shown to be stronger selectors for penicillin-resistant *S. pneumoniae* than beta-lactams, possibly because of linked resistance and great mucosal penetration of macrolides (Garcia-Rey, Aguilar et al. 2002).

## 6.9. Influence of macrolide use in food animals on occurrence of macrolide resistant Campylobacter

Oral administration of therapeutic or sub-therapeutic doses of macrolides has been shown to decrease susceptibility of *Campylobacter* species, mainly *C. jejuni*, to macrolides in chicken (Ladely, Harrison et al. 2007; Lin, Yan et al. 2007). Long-term exposure to low doses has resulted in significantly higher frequency of resistant isolates compared with therapeutic doses (Ladely, Harrison et al. 2007). The increase of macrolide resistance in *C. coli* in pigs after use of macrolides as antimicrobial growth promoters and for treatment has been documented in several studies (Aarestrup, Nielsen et al. 1997; Van Looveren, Daube et al. 2001). On the other hand, an example on the positive effect of restricting the use of antimicrobials on resistance comes from Denmark, where resistance among *C. coli* from pigs dramatically decreased after the ban of the use of tylosin for growth promotion (DANMAP 2006). In Sweden where the use of growth promoting antimicrobials was prohibited already in 1986, the occurrence of macrolide-resistant isolates of *C. coli* from pigs has stabilized at or below 1% since 1999 (SVARM 2002-2009). The dynamics of antimicrobial resistance in *C. coli* was recently studied at a large pig farm (Juntunen, Heiska et al. 2010). Tylosin treatment selected for a high level of resistance to erythromycin and resistance to ciprofloxacin, nalidixic acid and streptomycin also increased in *C. coli* isolates within a few days. Resistances significantly decreased when tylosin treatment was discontinued.

## 6.10. Influence of use of macrolides in food animals on occurrence of macrolide resistant enterococci

Several experimental studies have shown that use of in-feed tylosin or virginiamycin to pigs or poultry is associated with an increased proportion of intestinal enterococci with resistance to MLS antimicrobials (Linton, Hinton et al. 1985; Kaukas, Hinton et al. 1988; Aarestrup and Carstensen 1998; Welton, Thal et al. 1998). Similar results were obtained for *Enteroccocus* or *Staphylococcus* species isolated from the nares or skin of pigs fed with tylosin-containing feed (Christie, Davidson et al. 1983). Virginiamycin is known to select for streptogramin resistance in *E. faecium* in food animals (Hammerum, Jensen et al. 1998; Werner, Klare et al. 2000). Use of virginiamycin as a feed additive resulted in selection of resistance among enterococci in food animals, with cross-resistance against quinupristin/dalfopristin (Donabedian, Thal et al. 2003; Schwarz, Cloeckaert et al. 2006; Aarestrup, Wegener et al. 2008).

Tylosin has been widely used for growth promotion in swine and poultry production in the EU. The prevalence of resistance has been very high in many countries. In Finland and Sweden, the use of macrolides in animal production has been much more restricted and use as feed additive was finished earlier than elsewhere. In these countries the susceptibility of enterococci isolated in food animals has remained at a lower level: erythromycin resistance of *E. faecium* and *E. faecalis* isolated in pigs and poultry has been 10-30% and resistance to virginiamycin from 0 to 12% (Anonymous 1997; SVARM 2002-2009; NORM-VET 2008). After the ban of tylosin, spiramycin and virginiamycin as feed additives in the EU in 1998, the prevalence of macrolide-resistant enterococci decreased in countries with previously very high figures. In Denmark, proportion of erythromycin resistant *E. faecalis* and *E. faecium* isolated in pigs decreased from 80-90% to less than 40%; at the same time consumption of tylosin in pig industry decreased from almost 80 tons to about 20 tons (DANMAP 2008) (Figure 3).

## 6.11. Influence of macrolide use in food animals on resistance among Gram-positive cocci other than enterococci

*Staphylococcus hyicus* isolated swine is more frequently resistant against macrolides compared with e.g. *S. aureus* isolated in cattle. The possible reason for this situation can be the more widespread use

of macrolides in swine production. Macrolide resistance has been monitored for decades in Denmark. The occurrence of macrolide resistance of *S. hyicus* isolated from swine in Denmark seems to correlate with the use of tylosin for growth promotion: macrolide resistance of *S. hyicus* increased in Denmark from 33% in 1996 to over 60% in 1997, followed by a decrease to 21% in 2003 (DANMAP 2004). Tylosin was the most common antimicrobial used as a feed additive for pigs in Denmark. It is still used for treatment, which probably maintains the resistance at the present level.

For *Staphylococcus aureus* it has been shown in vitro that the non-inducers 16-member macrolides and lincosamides are able to select for constitutively expressed *erm*(C) (Luthje and Schwarz 2007a). Significant differences in occurrence of constitutive and induced *erm*(C) genes were demonstrated in staphylococcal isolates from reservoirs of swine, cattle and humans with different use of tylosin; constitutive genes were much more common in animal isolates (Jensen and Aarestrup 2005). Mastitis causing *streptococci* have developed resistance against macrolides, and the prevalences vary between countries (Hendriksen, Mevius et al. 2008; Botrel, Haenni et al. 2010). The effect of abundant use of macrolides and lincosamides for treatment of mastitis in some Member states on this phenomenon cannot be excluded.

MRSA of type ST398 has emerged in food animals and is a concern also related to antimicrobial use. MRSA strains can carry resistant genes against macrolides, and use of any substance in that group may provide selective pressure (Catry, Van Duijkeren et al. 2010). The potential influence of the use of products with long half-lives deserves special attention, as the time when concentrations close to the MIC of intestinal and skin microbiota can be long.

### *6.12. Influence of macrolides use in food animals on resistance among other bacterial species*

Regarding *Brachyspira* isolated in swine, high levels of resistance have been reported for tylosin in most EU countries, and close to 100 % of the isolates are resistant (FINRES-Vet 1999; SVARM 2002-2009; MARAN 2008; Hidalgo, Carvajal et al. 2009). The selective pressure exerted on spirochetes from the widespread use of tylosin as a growth promoting agent and for therapy is a probable reason for the present situation. Resistance for tylosin can develop rapidly, because it is caused by a single point mutation, and can develop within two weeks *in vitro* (Karlsson, Fellstrom et al. 1999).

# 7. Impact of MLS resistance on human and animal health

## 7.1.1. Impact on human health

In humans, macrolides are mostly used for infections caused by bacteria which are not transmitted via food, with exemptions *Campylobacter* and possibly *Salmonella*. However, even bacteria causing human infections not directly linked to food of animal origin may acquire resistance determinants from animal bacteria. Use of MLS antimicrobials in food animals may in general have an impact also on human health.

#### *7.1.1.1. Campylobacter*

Food of animal origin can transmit drug resistant *Campylobacter* from animals to humans. In the EU, *Campylobacter*-associated enteritis has been the most commonly reported gastrointestinal zoonotic disease during 2004-2007 (EFSA 2010). The proportion of *Campylobacter* positive samples has been highest for fresh poultry meat, where on average 26% of samples have been positive (EFSA 2010). In general, human cases of campylobacteriosis are self-limiting. If antimicrobial treatment is necessary, macrolides are common alternatives for *Campylobacter* enteritis, because resistance to

fluoroquinolones has increased (Guerrant, Van Gilder et al. 2001; Blaser and Engberg 2008). In young children who not always can be treated with fluoroquinolones, macrolides are the drugs of choice. Approximately 90% of human campylobacteriosis is caused by *C. jejuni* (Belanger and Shryock 2007). It has been suggested that the absolute number of serious *Campylobacter* infection cases is increasing (Engberg, Aarestrup et al. 2001). Infections with macrolide-resistant *Campylobacter* have been associated with an increased frequency of adverse events, invasive disease and death compared to infections caused by susceptible strains (Travers and Barza 2002; Helms, Simonsen et al. 2005). Contrary to this, risk analysis studies have suggested that the risk for an impaired human treatment in cases of infection with macrolide-resistant *C. coli* of porcine origin is very low (Hurd, Doores et al. 2004; Hurd and Malladi 2008). The risk for suboptimal treatment for infections due to macrolide-resistant *C. jejuni* of broiler or bovine origin was even lower (Hurd and Malladi 2008). In an US study (Cox and Popken 2006), benefits of using fluoroquinolones or macrolides in broiler production clearly overweighed calculated risks. It is difficult to assess the implications of this study for the EU conditions. A recent human health risk assessment study from Denmark concluded that it is questionable whether any excess risk exists related to infection with macrolide-resistant *Campylobacter* compared to macrolide-susceptible *Campylobacter* (Alban, Nielsen et al. 2008). It was concluded that the risk associated with the veterinary use of macrolides in Danish pigs for human health in Denmark was low, but according to the used exposure model, which included origin of meat as well as consumption patterns, most human cases of macrolide-resistant campylobacteriosis (157 out of 186) were ascribed to imported meat. Only seven cases could be explained by the veterinary usage of macrolides in Danish pig production (Alban, Nielsen et al. 2008). On the other hand, the published risk assessment studies have been criticized for underestimating the risks (Collignon 2004; Kelly, Smith et al. 2004).

#### *7.1.1.2. Other indications*

Resistance to fluoroquinolones among *Salmonella* has increased, and the use of fluoroquinolones as the first-line treatment is not always possible (Threlfall 2002; Hakanen, Kotilainen et al. 2006; Rise and Bonomo 2007). Severe clinical infections caused by *Salmonella* are treated by 3rd generation cephalosporins like ceftriaxone. Resistance to these extended-spectrum cephalosporins has been detected in *S. Typhimurium* isolates, together with resistance to ciprofloxacin (Threlfall 2002; Whichard, Gay et al. 2007). Due to these resistance problems in *Salmonella*, azithromycin has been introduced for treatment of salmonellosis, mainly for infections caused by *S.* Typhi with reduced susceptibility to fluoroquinolones (Capoor, Rawat et al. 2007; Threlfall, de Pinna et al. 2008). Evidence on the clinical efficacy of azithromycin mainly in the treatment of typhoid fever is available (Chinh, Parry et al. 2000; Frenck, Nakhla et al. 2000; Frenck, Mansour et al. 2004). Azithromycin has shown a good in vitro activity against nontyphoidal *S. enterica* against isolates with reduced susceptibility to fluoroquinolones, and could thus be a candidate for treatment of clinical nontyphoidal salmonellosis (Gunell, Kotilainen et al. 2010). Susceptibility testing of *Salmonella* strains is advisable before treatment, as resistance against azithromycin can develop (Capoor, Rawat et al. 2007; Gunell, Kotilainen et al. 2010). Gamithromycin, the first azalide approved for animal use, may have an influence on the development of resistance in Salmonella isolates of animal origin.

Quinupristin-dalfopristin belongs to the few available therapies for the treatment of infections due to multiresistant *E. faecium*, keeping also the emergence of strains resistant to linezolid in mind. Another limited indication for streptogramins is treatment of infections caused by multiresistant *S. aureus*. For both bacterial species, animal origin is a possibility and resistance can be linked with use of MLS substances in animals (Catry, Van Duijkeren et al. 2010; Hammerum, Lester et al. 2010). Systemic use of macrolides for food animals can select for MLS resistance among staphylococci residing on animal skin. Acquired macrolide resistance has also emerged in streptococcal species (Leclercq 2002; Leclercq and Courvalin 2002). Some species such as *S. suis* and *S. agalactiae* have zoonotic potential,

but transfer of resistance determinants between species is also a possibility (Martel, Decostere et al. 2005). Macrolide resistance is already a recognised problem among streptococci isolated in humans (Fines, Gueudin et al. 2001; Rantala, Haanpera-Heikkinen et al. 2006).

### 7.1.2. Impact on animal health

Macrolides, in addition to pleuromutilins tiamulin and valnemulin, have been the drugs of choice for treatment of swine dysentery caused by *B. hyodysenteriae* (Giguère 2006a; Giguère 2006b). Due to wide-spread resistance, macrolides are in most countries no more an alternative for this indication, and could only be used based on susceptibility testing. Decreased susceptibility for tiamulin among *B. hyodysenteriae* has been reported (Gresham, Hunt et al. 1998; Lobova, Smola et al. 2004). This is alarming, as the therapeutic arsenal for swine dysentery is very limited. In swine diarrhoea caused by *B. pilosicoli*, pleuromutilins have been the first choice, but resistance to tiamulin has emerged and percentages of resistance from 5 to 16% have been reported (Fossi, Saranpaa et al. 1999; Pringle, Landen et al. 2006b). Alternatively, macrolides or lincosamides can be used after susceptibility testing. For porcine proliferative enteropathy caused by *L. intracellularis*, pleuromutilins or tetracyclines are the first choices and macrolides the second choice (Burch, Duran et al. 2008).

For swine enzootic pneumonia caused by *M. hyopneumoniae* and in mycoplasmal arthritis, lincomycin and macrolides are important alternatives to pleuromutilins. Tylosin or lincomycin are used for neonatal diarrhoea in pigs caused by *Clostridium perfringens*, as an alternative to penicillins. *A. pleuropneumoniae* and *P. multocida* causing swine pneumonia have mostly remained susceptible for penicillins, but macrolides are also used. Resistance to macrolides and lincosamides would thus not result in situation with no treatment at all for these infections in pigs, but would seriously restrict the alternatives available for treatment.

Macrolides like tilmicosin and tulathromycin are recommended in national treatment guidelines and textbooks for treatment of bovine respiratory disease in cattle, as alternatives for penicillin G, oxytetracylin or spectinomycin. In situations where respiratory pathogens have developed resistance for these antimicrobials, macrolides or florfenicol are the recommended choices over reserve drugs fluoroquinolones or extended spectrum cephalosporins.

Macrolides and lincosamides have a limited use for treatment of bovine mastitis caused by Gram-positive pathogens (Deluyker, Van Oye et al. 2005; Constable, Pyörälä et al. 2008). Mastitis-causing streptococci isolated in the EU have remained fully susceptible to penicillin G (Hendriksen, Mevius et al. 2008). Macrolides do not offer any benefit over beta-lactams for treatment of streptococcal mastitis. On the contrary, resistance towards macrolides has emerged among them, which may risk the efficacy of treatment (Loch, Glenn et al. 2005; Hendriksen, Mevius et al. 2008). Macrolides can be regarded as an alternative for treatment of mastitis caused by penicillin-resistant *Staphylococcus aureus*, but culling is mostly a better option in those cases, due to poor prognosis (Barkema, Schukken et al. 2006).

In poultry, macrolides and lincosamides are alternatives for treatment of many indications. They are used e.g. as alternatives of penicillin G for treatment of necrotic enteritis, staphylococcal and streptococcal infections, and as alternatives to pleuromutilins or fluoroquinolones for Mycoplasma infections (Löhren, Ricci et al. 2008). Resistance in *Mycoplasma gallisepticum* may already limit the use of macrolides to treat chronic respiratory disease in poultry (Migaki, Avakian et al. 1993). The substances with authorization for poultry include macrolides and lincosamides; development of resistance to these substances would restrict the panel of the authorized substances for these species.

As conclusion, macrolides and lincosamides are very important antimicrobials for treatment of animal infections, though they are seldom the sole alternative. They share some advantageous

pharmacokinetic characteristics such as high lipid solubility, large volume of distribution and high intracellular concentrations, making them good alternatives for many infections. Specific studies on the negative impact of macrolide resistance on food animal health and welfare are not available. It can be estimated that it would result in delay of clinical recovery, higher mortality, increased animal suffering, and economical losses to the industry. The effects could be substantial as macrolides and lincosamides are commonly used drugs and susceptibility testing before treatment of food animals is not routinely carried out. Resistance for the present alternative drugs may also emerge, increasing the therapeutic importance of macrolides and lincosamides. Development of resistance against macrolides and lincosamides would have a serious negative impact on animal health.

# 8. Summary assessment

- In humans, macrolides are used primarily to treat respiratory infections, skin infections, or infections of the genital tract. Macrolides belong to the few available substances for treatment of serious *Campylobacter* infections. Macrolides (azalides) have also limited use in the treatment of Legionella and multi-resistant *Salmonella* infections. Streptogramins are reserve drugs indicated for certain infections caused by multi-resistant bacteria.
- Macrolides are relatively old substances in animal use as they have been on the market since the early 1960ies. Use of macrolides for growth promotion as feed additives began at the same time as the therapeutic use, until withdrawn in the EU in 1998.
- At present, macrolides and lincosamides are used for treatment and prevention of a variety of common infectious diseases in food animals in the EU. A very high number of products containing these substances are available. Nationally authorised macrolide products are mostly old, and their indications and posologies show a great variation. Products for in-feed medication with macrolides or lincosamides in combination with other antimicrobials are common. The indications for combination products can be particularly broad. The approved duration of treatment for some products is long, even from 4 to 5 weeks.
- The indications for the recently approved macrolide products are more restricted. The main indications in cattle are common infections such as respiratory and genital infections, foot lesions and mastitis, in swine pneumonia, enteritis and arthritis, and in poultry respiratory infections and necrotic enteritis.
- Acquired resistance mechanisms against MLS group antimicrobials are common and complex. A high number of genes coding for resistance have been detected in many bacterial genera, and new genes appear. The most significant genes which are transferred horizontally are rRNA methylases (erm genes) and the efflux genes (mef). Resistance mechanisms due to mutations have also been detected in increasing numbers in many bacterial species. Bacteria isolated in animals and humans share the same resistance determinants which can be transferred between bacterial strains, species and genera and between different hosts.
- Resistance against MLS among animal pathogens as well as zoonotic bacteria has emerged, and is now common in different bacterial species. It is apparent that situations in different EU member states greatly differ, regarding the susceptibility of animal pathogens for antimicrobials of the MLS group.
- It is difficult to compare prevalence data of resistance between different time periods and geographical sites, because origin of isolates, panels of antimicrobials used, methods used for susceptibility testing and cut-off values for resistance differ. For many pathogens, no agreed standards for the in vitro susceptibility testing are available.

- Resistance against macrolides and lincosamides has emerged among animal pathogens as well as in zoonotic bacteria, and is common in some species. In animal pathogens the most dramatic increase of resistance has been seen in the genera of Brachyspira where nearly all isolates at present are resistant. Significant resistance for macrolides and lincosamides has also appeared among staphylococci isolated in pigs and streptococci isolated in cattle. Among zoonotic bacteria, the highest prevalences of resistance are seen in *Enterococci* but also Campylobacteria need attention in this respect.
- A strong association between use of macrolides and resistance of both commensal and pathogenic bacteria has been noted in humans.
- Several studies have demonstrated the role of the use of macrolides on macrolide (erythromycin) resistance among *Campylobacter* in food animals. These studies unequivocally suggest that long-term, in particular low-dose use of macrolides selects for emergence of erythromycin resistant *Campylobacter* in animal reservoirs. Increase of macrolide resistance in *C. coli* in pigs after use of macrolides as antimicrobial growth promoters and for treatment is well documented. Resistance among *C. coli* from pigs dramatically decreased after the ban of the use of tylosin for growth promotion.
- The use of macrolides and lincosamides in food animals has apparently resulted in increased resistance among certain animal pathogens e.g. *Brachyspira* where today practically all isolates are resistant. Another example is *S. hyicus* where data from Denmark showed a strong correlation with the use of tylosin for growth promotion and emergence of resistance.
- Results from risk assessments on the impact of macrolide-resistant *Campylobacter* on public health are equivocal. The possible consequences on human health greatly depend on conditions which vary between continents and countries.
- In humans, MLS antimicrobials are mostly used for infections caused by bacteria which are not transmitted via food, except for campylobacteriosis and sometimes for salmonellosis. However, even if the bacteria causing human infections are not directly linked to food of animal origin they may acquire resistance determinants from animal bacteria.
- Macrolides and lincosamides are important substances for treatment of many common infections in food animals, though seldom the sole alternative.

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