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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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CVMP recommendations for action

Macrolides and lincosamides are used for treatment of diseases that are common in food producing animals including medication of large groups of animals. 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 between animals and humans.

Macrolides have been used for group and herd/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 cure 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

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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 is questionable. The use of combinations in situations where products with a single active substance would be enough unnecessarily increases selection pressure for antibiotic resistance.

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 subsequently 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 including medication of large groups of animals. 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. In human medicine, macrolides and streptogramins are classified as critically important and lincosamides as important (AGISAR 2009). Prioritization of classes of antimicrobials to be addressed most urgently in terms of risk management strategies for non-human use of antimicrobials has resulted in the selection of three groups: quinolones, 3rd/4th generation cephalosporins, and macrolides (AGISAR 2009).

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 emergence of resistance against erythromycin has been 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 regions.

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) (Table 1). 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 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, 2003a). 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 (Bryskier and Butzler, 2003a; Xiong and Le, 2001). They have activity against macrolide-resistant streptococci (Pfister et al., 2004; Shain and Amsden, 2002). 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.

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Table 1. Macrolides and related compounds (Bryskier and Butzler, 2003a; Giguère, 2006a).

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

* 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

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, 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 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* (Jacobs et al., 2003; Munckhof et al., 2000) 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 (Bryskier and Butzler, 2003a; Hardy et al., 1988). 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 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 et al., 1988; Rise and Bonomo, 2007; Vaara, 1993). Azithromycin has moderate *in vitro* activity against *Salmonella* Typhi (Butler and Girard, 1993; Metchock, 1990); intracellular activity against non-typhoid Salmonella was also demonstrated (Chiu et al., 1999). Macrolides also have significant immunomodulatory effects independent of their antimicrobial activity (Chin et al., 2000; Tamaoki 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 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 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, 2003a), with an optimal activity at pH higher than 7. Macrolides and lincosamides produce high intracellular concentrations and are known to accumulate in phagocytic cells. Protein binding may reduce intra-bacterial uptake and interfere with the antibacterial activity as shown for clindamycin (Burian et al., 2011). The actual efficacy of bacterial killing within the cells however has not been documented (Barcia-Macay et al., 2006; Madgwick et al., 1989). 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 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 (Bryskier and Butzler, 2003b; Gilbert et al., 2009). Together with fluoroquinolones

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they are drugs of choice to treat human campylobacteriosis, in cases requiring antimicrobial therapy (Moss, 2003). In uncomplicated campylobacteriosis administration of antibiotics is not recommended (Moss, 2003; Ternhag et al., 2007). 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 (Gilbert et al., 2009; Greenwood, 2003).

Streptogramins (quinupristin/dalfopristin) are authorized for use in infections caused by *Enterococcus (E.) faecium*. Quinupristin/dalfopristin is one of the few potential substances for the treatment of infections due to multi-resistant *E. 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 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 2011, 8 macrolides and 2 lincosamides have been authorized for veterinary use in some or all Member States of the EU: erythromycin, tildipirosin, 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[4]
Macrolides			
Erythromycin	Injection, oral, intramammary[2]	National[1]	All food animals
Gamithromycin	Injection	Centralized (2008)	Bovine
Spiramycin	Injection, oral, intramammary[2]	National	Bovine, porcine and chicken
Tildipirosin	Injection	Centralized (2011)	Bovine and porcine.
Tilmicosin	Injection, oral	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
[4]Existence of an MRL does not imply the existence of a Marketing Authorization

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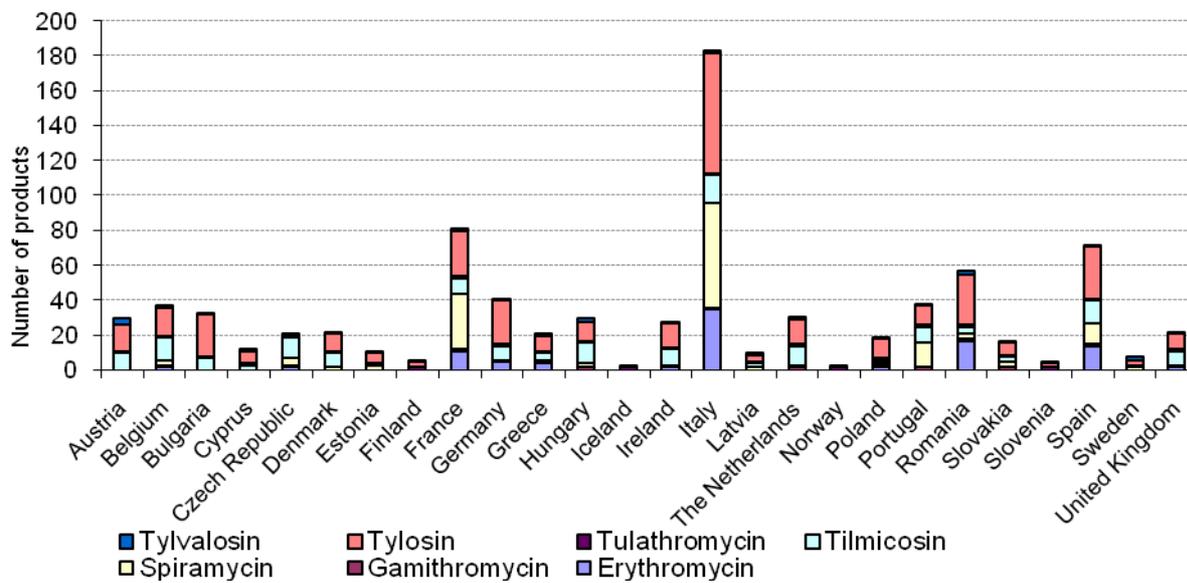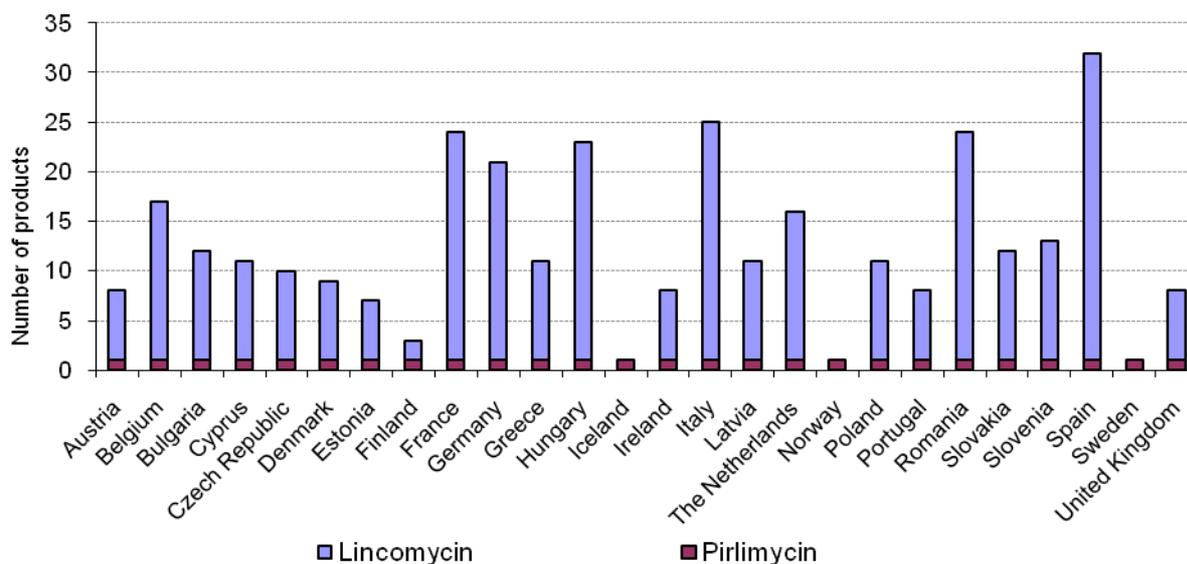

Figure 2. Number of lincosamides products formulated per antimicrobial substance and Member State (data from 2009).

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 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 or more) was already introduced for food animal therapy during late 1970'ies, when parenteral oxytetracycline products formulated in slow-release bases were brought into market. Later for macrolides, the prolonged effect (>48 hours activity) was achieved using molecules with a low clearance. The first macrolide introduced into veterinary medicine with one-dose only posology was tilmicosin in the early 1990ies. The next macrolide authorized with this regimen was tulathromycin in 2003, followed by gamithromycin in 2008 and tildipirosin in 2011. Some macrolides and lincosamides are also used by the intramammary route, erythromycin and lincomycin on national authorization and pirlimycin on centralized authorization. In this document, main attention is focused on the systemic use.

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

In a recent report from the European medicines Agency (ESVAC, 2011), data on sales during 2005-2009 from nine European countries were reanalysed in a harmonized manner and a measure for correction for population size was developed. Data on the sales of macrolides and lincosamides in 2009 have been retrieved from that report and are presented as mg antimicrobials /population correction unit in table 3. The unit used for population size correction reflects the total live weight of food producing animals including horses. In many countries, pigs are likely to be the main target species for medication with macrolides and therefore the proportion of pigs of the total PCU is also given in table 3. Even if that is taken into account, a large variation in amounts as well as of the proportion of macrolides and lincosamides of the total sales is observed. Examples of factors other than the relative importance of different species that may explain the observed differences are availability of veterinary antibacterial products per country, prices, risk-management measures implemented, the veterinarians' prescribing behaviour, animal production systems and the general situation with regard to infectious diseases.

Table 3. Overall national sales of macrolides and lincosamides expressed as mg/population corrected unit (mg/PCU) in nine European countries (ESVAC, 2011) - and proportion of pigs of the total population correction unit (PCU).

	Macrolides (in mg/PCU)	Lincosamides (in mg/PCU)	All antimicrobials (in mg/PCU)	Percent of total sales	Proportion pigs of total PCU
Czech Republic	6.1	0.3	106.4	6%	33%
Denmark	5.9	1.2	52.9	13%	75%
Finland	0.8	0.3	31.5	4%	37%
France	10.7	0.9	141.2	8%	27%
Norway	0.0	0.1	13.9	<1%	7%
Sweden	0.9	0.2	18.7	6%	28%
Switzerland	4.8	0.1	94.9	5%	31%
The Netherlands	14.9	0.3	165.4	9%	49%
United Kingdom	6.5	1.1	68.0	11%	12%

The nationally authorised macrolide products are mostly old, and their indications and posologies show a great variation. For the initial macrolide products, indications were not very specific, but the products were just aimed for treatment and prophylaxis of bacterial infections susceptible for these substances. The main indications in swine are pneumonia, enteritis and arthritis, in cattle all common infections such as respiratory and genital infections, foot lesions and mastitis, and in poultry respiratory infections and necrotic enteritis. Products for in-feed medication containing macrolides or lincosamides in combination with other antimicrobials are common. Most often macrolides are combined with colistin or aminoglycosides, but also with sulphonamides, trimethoprim, oxytetracycline, or ampicillin. More than 60 combination products containing macrolides with other antimicrobials are available in the EU; in addition, numerous lincomycin products in combinations exist. Some examples of combination products are presented in table 4. 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 (Catry et al., 2007; Samson et al., 2006; Timmerman et al., 2006).

Table 4. Examples of combination products with macrolides, authorized in Member States of the European Union in 2010.

Active substances	Target species	Indications (in brief) collected from different products	Comments
Erythromycin, ampicillin	All production animals except poultry	Treatment of gastro-intestinal infections	Potentially antagonistic combination (Pillai et al., 2005)
Tylosin, erythromycin, neomycin	Poultry	Mycoplasmosis, salmonellosis, colibacillosis, secondary infections	Contains 3 antibiotics. No rationale for two macrolides in the same product. No rationale to have components targeted

			towards respiratory and gastro-intestinal infections in the same product.
Tylosin, sulfonamide	Pig	Prevention of haemorrhagic enteritis and enzootic pneumonia	No rationale to have components targeted towards respiratory and gastro-intestinal infections in the same product.
Tylosin or spiramycin oxytetracycline	Pig, cattle	Treatment of intestinal and respiratory infections caused by micro-organisms sensitive to the active substances. Prevention of haemorrhagic enteritis	No rationale to have components targeted towards respiratory and gastro-intestinal infections in the same product.
Spiramycin or erythromycin, sulfonamide, trimethoprim	Pig, poultry	Treatment of coccidiosis, mycoplasmosis, respiratory and enteric infections incl. salmonellosis.	Contains 3 antibiotics – no rationale for the combination
Spiramycin (or tylosin), colistin	All production animals	Bacterial infections of gastrointestinal and respiratory tract.	No rationale to have components targeted towards respiratory and gastro-intestinal infections in the same product.

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 (M.) haemolytica*, *Pasteurella (P.) multocida* and *Histophilus (H.) somni*, treatment and prevention of bovine respiratory disease associated with *M 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 (A.) pleuropneumoniae*, *P. 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 (L.) intracellularis*, swine dysentery caused by *Brachyspira (B.) 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; Burch et al., 2008; Constable et al., 2008; Giguère, 2006a). 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 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 et al., 2006) that carry unique chromosomal *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* spp. 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* spp. (Capoor et al., 2007; Jones et al., 1988).

6.2. Acquired resistance

The first bacterial species with acquired resistance to macrolides described was a Staphylococcus showing resistance to erythromycin (Roberts, 2008; Zhanel et al., 2001). Later, over than 70 different genes, hosted by more than 60 different bacterial species, have been described in the context of MLS resistance (Table 5) (Roberts, 2011).

6.3. Horizontally transferable resistance

The most common resistance mechanism is a target site modification mediated by at least 34 different rRNA methylases (*erm* genes) described in 34 bacterial genera (Diner and Hayes, 2009; Leclercq and Courvalin, 1991) (table 5). 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 (Douthwaite et al., 2000; Roberts et al., 1999). This modification reduces the binding of the MLSB antimicrobials to the ribosomal target site.

The *erm* genes can be expressed constitutively or inducibly (Giguère, 2006a; Stepanovic et al., 2006). When the gene is constitutively expressed, the bacterial strain harbouring 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 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.

The *erm* genes have been identified in so far in 32 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, Tn917,5384,Tn2009, or Tn53982010), 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* has been detected e.g. in

streptococcal species such as *Streptococcus suis*, *S. uberis*, *S. dysgalactiae, S. agalactiae and Staphylococcus (S) pseudintermedius*, *S. hyicus*, *S. aureus,* enterococci, and *L. monocytogenes* (Boerlin et al., 2001; Culebras et al., 2005; Haenni et al., 2010; Jensen et al., 1999; Kadlec et al., 2011; Loch et al., 2005; Luthje and Schwarz, 2007; Luthje et al., 2007b; Martel et al., 2001; Martel et al., 2003; Palmieri et al., 2007; Schmitt-Van de Leemput and Zadoks, 2007). Different erm genes including *erm*T have been found in the emerging meticillin resistant *S. aureus* ST398 in livestock (Fessler et al., 2010) and *erm*C in coagulase-negative staphylococci (CNS) isolated in bovine mastitis (Sampimon et al., 2011).

In bacteria isolated in humans, inducible resistant strains (e.g. *Staphylococcus* species) predominated in the 1960s to 1970s (Roberts 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 plasmids (at least 13 of them including variants B to H, O, S to U, X, and Y) or transposons (variants A, B, F, G and X(Roberts, 2011). These genetic platforms usually harbour many different resistance genes; 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). Experimental *in vivo* and *in vitro* studies have demonstrated transfer of *erm*-genes within and between bacterial species. Transfer of different *erm* and *mef* genes carried on a plasmid or in a transposon together with other resistance determinants has been shown for instance between strains of *Haemophilus (H.) influenzae*, *E. faecalis,* or *Clostridium* species (Huycke et al., 1992; Mullany et al., 1995) and between *L. monocytogenes* and *E. faecalis* (Doucet-Populaire et al., 1991; Poyart-Salmeron et al., 1990) and *H. Influenzae* and *E. faecalis* (Roberts et al 2011) . *S. suis* isolates were shown to be capable of transmitting macrolide resistance to *E. faecalis* (Stuart et al., 1992; Wasteson et al., 1994), lactic acid bacteria to *Enterococci* and *Listeria* (McConnell et al., 1991; Toomey et al., 2009) and *E. faecalis* to *S. aureus* (Noble et al., 1992). These examples and others confirm that horizontal transfer of resistance determinants occurs, even between different genera, including transfer from Gram-negative to Gram-positive bacteria (Roberts et al. 2011). Knowledge on persistence of resistance in new reservoirs is limited; in one study intestinal carriage of resistant strains of *E. faecium* of animal origin in humans was found transient (Sørensen et al., 2001).

The second most common resistance mechanism is due to active expulsion of the antimicrobial from the bacteria mediated by efflux pumps. At least 18 different genes have been identified in relation to this mechanism (Table 5). 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. Chromosomal *msr*E was recently detected in a *P. multocida* strain isolated in bovine respiratory disease (Kadlec et al., 2011). Among animal pathogenic bacteria, *mef*(A) has been detected in S. suis (Martel et al., 2003). A novel macrolide efflux gene (mef(B)) has been detected in porcine isolates of *E. coli* (Liu 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 20 inactivating enzymes involved (table 5). At least two of the corresponding genes have linkage to integrons *ere*(A) (for erythromycin

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esterase), *lnu/lin*(F) (for lincomycin nucleotidyl transferase; (Roberts 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* and other CNS (Luthje and Schwarz, 2007; Luthje et al., 2007b; Sampimon et al., 2011). *Streptococcus uberis* has been shown to express several genes such as *mph*(B) or *lin*(B) to confer resistance to macrolides or lincosamides (Achard et al., 2008; Haenni et al., 2010; Schmitt-Van de Leemput and Zadoks, 2007).

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 5). 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 et al., 2002), and later also in other animal isolates like porcine *S. aureus* and bovine S. simulan*s* (Long et al., 2006). This gene has also been detected in human isolates of linezolid-resistant *S. aureus* (Arias 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 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 5). The bovine *P. multocida* strain reported to carry *msr*E had also *mph*E gene in its chromosome (Kadlec et al., 2011). 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 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 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* (Hidalgo et al., 2011; Karlsson et al., 1999; Karlsson et al., 2004b; Stakenborg et al., 2005), as well as in the zoonotic *C. jejuni* and *C. coli* (Alfredson and Korolik, 2007; Caldwell et al., 2008; Gibreel and Taylor, 2006). 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.

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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 rapidly dominate bacterial populations in which the individual cells possess only one or two rRNA operons (Vester and Douthwaite, 2001).

Table 5. Resistance genes and mechanisms of resistance for macrolides, lincosamides and streptogramins.

Resistance phenotype	Genes	Characteristics	HGT*
MLSB	erm (A to Z and 30 to 42)	rRNA methylases that confers resistance to macrolides, lincosamides and streptogramins B. Can be either inducible or constitutive	+
M(E)SB	msr (A, C, D and E)	Efflux pumps (ATB-binding transporter) that confers resistance to macrolides 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 E)	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 lincomycin	+
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 spiramycin	+
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 streptogramins	-

| 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 interpretation criteria for resistance differ (Schwarz 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, 2010a, 2011). For animal pathogens, comparable 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.

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; Hidalgo et al., 2009; Hidalgo et al., 2011; MARAN, 2008; SVARM, 2002-2009; Vyt and Hommez, 2006). 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 (Hidalgo et al., 2011; Karlsson et al., 2004a). Resistance of *B. hyodysenteriae* for lincomycin is close to that for tylosin (FINRES-Vet, 2007-2009; ITAVARM, 2003; SVARM, 2002-2009), 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 (Karlsson et al., 2004b; Pringle et al., 2006a; SVARM, 2002-2010) . Multiresistant isolates have also been found, with simultaneous resistance against lincomycin, tylosin, tylvalosin and tiamulin (Duinhof et al., 2008).In a field study on spontaneous infection of pigs caused by *B. 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 et al., 2006). However, in Belgium 34% of *C. perfringens* isolated in poultry were reported to be resistant to lincomycin (Martel et al., 2004). Some data are available for *Fusobacterium* spp. isolated in animals, indicating resistance against macrolides, but susceptibility to lincosamides (Jimenez et al., 2004; Jousimies-Somer et al., 1996). Recent data from

Sweden on susceptibility of *F. necrophorum* isolated in cows and sheep showed MICs for erythromycin from 2 to 8 mg/l (SVARM, 2002-2009). No accepted interpretation criteria for determining macrolide resistance of *F. necrophorum* exist.

6.6.3. Family Pasteurellaceae

In North America, resistance of *P. multocida* isolated in cattle and swine against macrolides has been frequently reported, but in the EU it has been rare (Kaspar et al., 2007; Kehrenberg et al., 2006). In the Netherlands, 0 % in 2004-2005 and 2.5 % of isolates from cattle in 2006-2007 were reported to be resistant to tilmicosin but none to tulathromycin (MARAN, 2008). In France in 2008, 7% of bovine *P. multocida* were reported to be 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 *M. haemolytica* isolates from healthy animals including veal calves were reported to be resistant to tilmicosin (Catry et al., 2005). As to *M. haemolytica* isolated in cattle in The Netherlands, resistance to tilmicosin was reported to increase from zero to 6.5 % (MARAN, 2008); the same figure was found for tulathromycin. In France in 2008, the proportion of *P. multocida* isolated in cattle resistant to tilmicosin was reported to be 7% but in *M. haemolytica* as high as 35% (AFFSA, 2009). 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, which recommended that diagnostic laboratories should not 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 reported for Danish isolates during early 2000 (Aarestrup 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 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 (AFFSA, 2009; Hendriksen et al., 2008). 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). CNS have developed resistance to MLS antimicrobials (Luthje and Schwarz, 2006; Sampimon et al., 2011).Resistance for macrolides has been 4-6%, and no resistance to clindamycin has been found in reports available (MARAN, 2007; NORM-VET, 2005; Pitkala et al., 2004). By contrast, 13-20% of CNS isolated from bovine mastitis in the Netherlands and France were resistant to lincosamides (AFFSA, 2009; MARAN, 2007) and up to 14% to erythromycin (Botrel et al., 2010).

Information available on meticillin-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 (Kehrenberg et al., 2009; Rich et al., 2005), but even higher figures were recently reported (Dewaele et al., 2011). As regards MRSA of

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type ST398 common in food animals, 40-50% of isolates from swine and bovine mastitis are also resistant for macrolides and lincosamides (Fessler et al., 2010; Kadlec et al., 2009). Recently, a novel mechanism mediating transferable resistance to lincosamides, streptogramin A antibiotics and pleuromutilins have been described in porcine and bovine ST398 isolates (Fessler et al., 2010; Kadlec and Schwarz, 2010).

Acquired macrolide resistance has emerged in *Streptococcus* species of animal origin. Available information indicates that the occurrence 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 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 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 (NORM-VET, 2008; SVARM, 2002-2010). 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, 2005-2006).

Resistance of *S. 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 reported to be as high as 72-77% to spiramycin and tylosin and 69% for lincomycin (AFFSA, 2009). Prevalence of *S. 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% (Aarestrup and Schwarz, 2006; DANMAP, 2004). In Sweden, 12 % of *S. hyicus* were resistant to erythromycin (SVARM, 2002-2010). Higher figures have been reported for some other EU countries (Aarestrup and Schwarz, 2006).

6.6.5. Other bacteria and Mycoplasma

For *L. intracellularis* there are no standards for susceptibility testing and practically no data are available. In one study, MIC_{90} values of *L. 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). Wattanaphansak (2009) tested activity of tylosin and lincomycin, among other antimicrobials, against 10 isolates of *L intracellularis*. The wide range of MIC distribution indicated occurrence of decreased susceptibility. The clinical relevance of these results is not known.

Reports on antimicrobial susceptibility of *Mycoplasma* species are scant. Furthermore, 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 for 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 et al., 2005). Resistance of *M. hyorhinis* to macrolides and lincosamides was reported in Japan (Kobayashi 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 et al., 2003). In one study using experimental

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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 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 in the reported prevalences between EU states (de Jong et al., 2009; EFSA, 2010a; EFSA/ECDC, 2011). According to the EFSA zoonosis reports (table 6) presenting data from 2004 to 2008, resistance to erythromycin among *C. coli* isolates from pigs was common: in 2008, 39% of a total of 662 isolates and in 2009 35% of a total of 551 were resistant (EFSA, 2010a, b). Among *C. jejuni* from poultry resistance to erythromycin had remained at a constantly low level. From a total of 1996 isolates from poultry in 2008, 3% were reported to be resistant and from a total of 577 isolates in 2009, 0.3%, respectively. A total of 6% of 423 isolates from broiler meat were resistant, but only 6 Member States were reporting (EFSA, 2010a), the respective figure from 2009 was 3% (EFSA/ECDC, 2011). Among *C. coli* isolates from poultry, 12% out of 997 studied isolates were resistant to erythromycin in 2008 and 14% out of 321 isolates in 2009. 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* (Belanger and Shryock, 2007; Payot et al., 2006). In *Campylobacter*, total cross-resistance between older macrolides (erythromycin) and new macrolides such as azithromycin has been shown (Harada et al., 2006). The EFSA Community Report (EFSA/ECDC, 2011) showed that in the EU in 2009, in average 1.6% of *C. jejuni* and 8.5% of *C. coli* isolated in humans were resistant to erythromycin. Based on data from EFSA/ECDC on human infections by *Campylobacter* in 2009, the prevalence of erythromycin resistance of *C. coli* ranged from 5% to 26% among six MS, and that of *C. jejuni* from to 0 to 6% among 10 MS (EFSA/ECDC, 2011). Human campylobacteriosis is mainly caused by *C. jejuni* (EFSA, 2011).

Table 6. Reported resistance to erythromycin in *Campylobacter* isolated in healthy animals in 2008. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2008 and 2009. Cut-off values used were 4 µg/ml for *C. jejuni* and 16µg/ml for *C. coli* (EFSA(b), 2010; EFSA/ECDC, 2011).

	Cattle		Pigs		Poultry	
Country	*C. jejuni* N	% R	*C. coli* N	% R	*C. jejuni* N	% R
Austria	125*	0	137*	13	125	0
Belgium					111*	8
Czech Republic					133*	0
Denmark	87	0	113	12	75	0
Finland	48	0			78	0
France			76	45	32	0
Germany	83	0	91**	27	93	1
Hungary			116	12	53	0
Ireland					110*	1
Italy	35	9	143**	60	55*	7
Latvia					57*	4
Malta						
Netherlands	31	3	79	18	61	2

Poland					105*	0
Portugal					89*	8
Romania					82*	4
Slovenia					97*	0
Spain	92	4	167	70	60	0
Sweden	68***	0	97****	0	38*	0
United Kingdom					130*	0
Norway**			67	0	99*	0
Switzerland*	10*	0	191	10	131	0

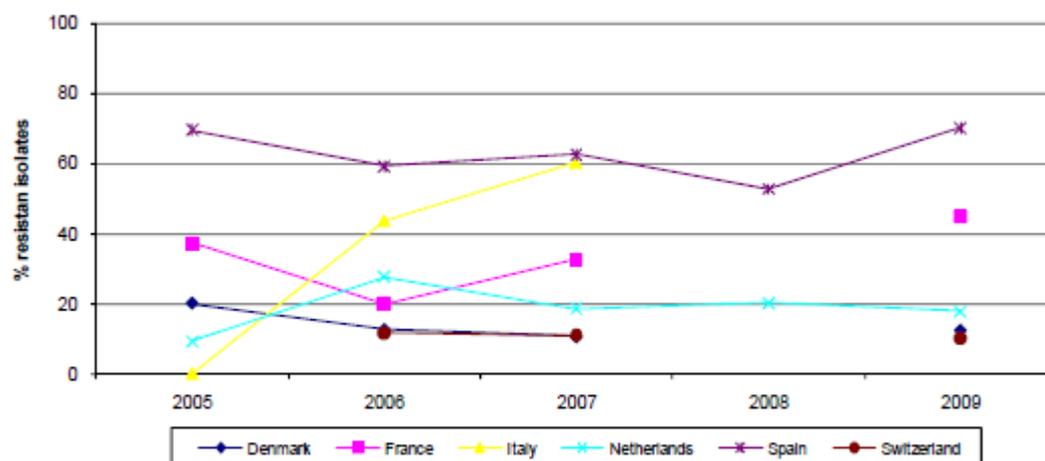*2008 **2007 ***2006 ****2005

Figure 3. Trends in erythromycin resistance in *Campylobacter coli* from pigs in the Member States (and non Member States) reporting these data ((EFSA, 2011).

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 et al., 2000). The prevalence of macrolide-resistant enterococci has since decreased (Figure 4); 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 national surveys in the EU show that proportion of erythromycin-resistant *E. faecalis* and *E. faecium* isolated from broiler meat was for example 11% and 23% in Denmark and 42% *vs* 34% in the Netherlands (EFSA, 2010a).
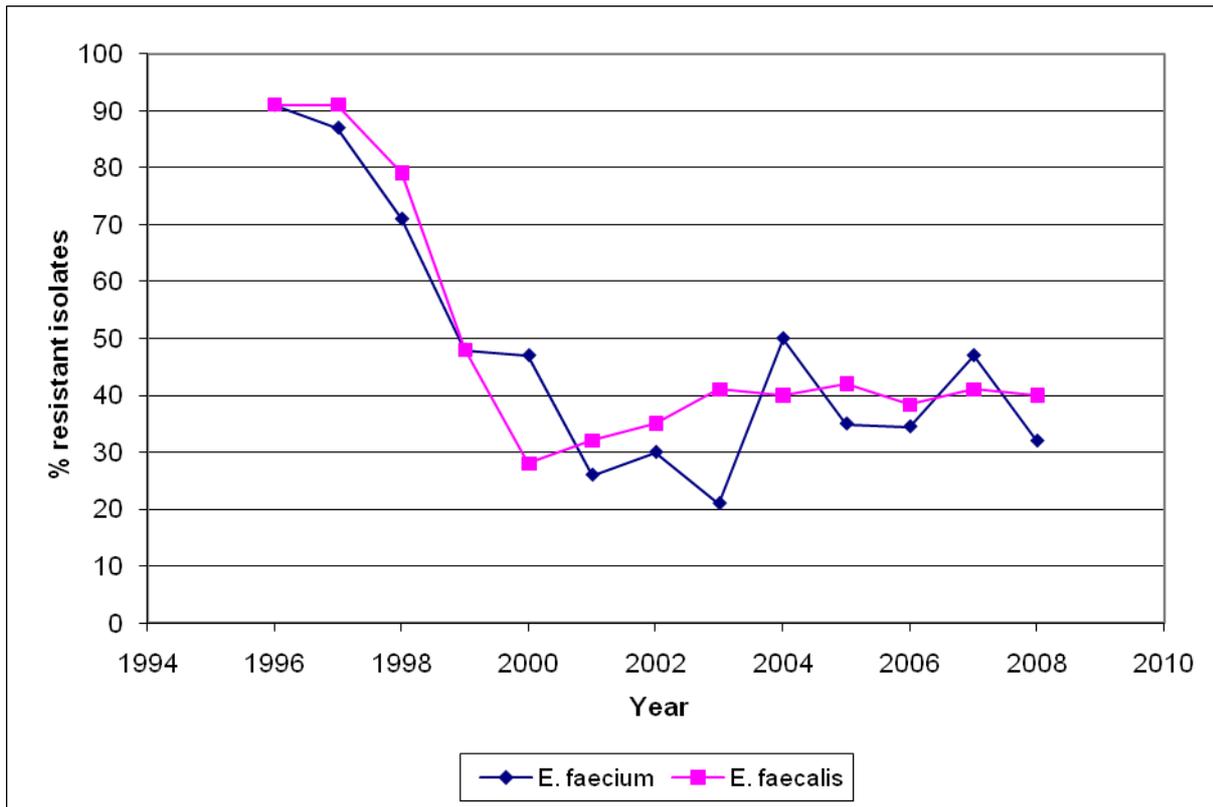
Figure 4. Occurrence of resistance (%) among Enterococcus faecium and Enterococcus faecalis from pigs in Denmark (DANMAP). Growth promoters were prohibited in the EU in 1998.

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 et al., 1983; Andremont et al., 1991; Pecquet 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 et al., 2002). Macrolides significantly increased the proportion of macrolide-resistant streptococci in the pharynx of human volunteers (Malhotra-Kumar 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 *S. pneumoniae* (Cizman, 2003). Several pharmaco-epidemiological studies have demonstrated a link between use of macrolides and resistance (Bergman et al., 2006; Karlowsky et al., 2009; Riedel et al., 2007). In a cross-national European study, an association between macrolide consumption and resistance was found (Goossens 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 et al., 2002).

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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 et al., 2007; Lin et al., 2007). Long-term exposure to low doses has resulted in significantly higher frequency of resistant isolates compared with therapeutic doses (Ladely et al., 2007). Therapeutic use of tylosin resulted in high-level resistance among *Campylobacter* in a large turkey flock (Logue et al., 2010). The authors concluded that once established in a production unit, macrolide-resistant *Campylobacter* have potential to persist and be transferred to the final product. 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 et al., 1997; Van Looveren 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, 2002-2010). Erythromycin-resistant *Campylobacter* have shown fitness burden, which may reduce their prevalence after removal of selection pressure (Caldwell et al., 2008; Logue et al., 2010; Luangtongkum et al., 2009). The dynamics of antimicrobial resistance in *C. coli* was studied on a large pig farm (Juntunen et al., 2010). Tylosin treatment selected for a high level of resistance to erythromycin. Resistance to nalidixic acid, ciprofloxacin and streptomycin also increased in *C. coli* isolates within a few days. Common resistance mechanisms for MLS antimicrobials and aminoglycosides are not known to exist. 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 (Aarestrup and Carstensen, 1998; Kaukas et al., 1988; Linton et al., 1985; Welton 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 et al., 1983). Virginiamycin is known to select for streptogramin resistance in *E. faecium* in food animals (Hammerum et al., 1998; Werner 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 (Aarestrup et al., 2008; Donabedian et al., 2003; Schwarz et al., 2006).

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, 2002-2010). 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 4).

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 *S. 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 (Botrel et al., 2010; Hendriksen et al., 2008). 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 MLST 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 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; Hidalgo et al., 2009; MARAN, 2008; SVARM, 2002-2009). 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 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 exceptions *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. This indirect risk from the use of macrolides in food animals should also be taken into account in determining risk profiles. Use of MLS antimicrobials in food animals may in general have an impact on human health.

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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 2005-2009 (EFSA, 2011). In food of animal origin, the highest proportion of *Campylobacter*-positive samples has been reported for fresh poultry meat where on average 31% (11%-90% of the reporting MS) of the samples in 2009 were positive (EFSA, 2011). Macrolide resistance of meat isolates reflects the situation among isolates originating from the respective species; for example in Denmark erythromycin resistance has been very low in domestic poultry meat (DANMAP, 2008, 2009). Public health aspects of macrolide resistance in *Campylobacter* are controversial. Most cases of campylobacteriosis in humans are sef-limiting, and invasive disease is in general rare (Pigrau et al., 1997). If antimicrobial treatment is necessary, macrolides are common alternatives for *Campylobacter* enteritis, because resistance to fluoroquinolones has increased (Blaser and Engberg, 2008; Guerrant et al., 2001). 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 might be increasing (Engberg et al., 2001). No published data on human treatment failures in infections caused by macrolide-resistant *Campylobacter* are available. Risk assessment studies to explore public health impacts from antibiotic use in food animals have been based on varying estimations and information available (Cox and Popken, 2006; Kelly et al., 2004). Infections with macrolide-resistant *Campylobacter* can be associated with an increased frequency of adverse events, invasive disease and death compared to infections caused by susceptible strains (Helms et al., 2005; Ternhag et al., 2007; Travers and Barza, 2002). 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 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 has been suggested to be even lower (Cox and Popken, 2006; Hurd and Malladi, 2008). Estimated benefits of using fluoroquinolones or macrolides in broiler production clearly overweighed calculated risks (Cox and Popken, 2006) . It is difficult to assess the implications of these studies for the EU conditions. A more 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 et al., 2008). 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 et al., 2008). Most published risk assessment studies estimate the risk of macrolide use in food animals for public health as very low (Alban et al., 2008; Cox and Popken, 2006; Hurd et al., 2004). On the other hand, these studies have been criticized for possibly underestimating the risks (Collignon, 2004; Tollefson et al., 2004). The methodology used, quality of the data and assumptions made are the critical points in risk assessment studies.

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 (Hakanen et al., 2006; Koningstein et al., 2010; Rise and Bonomo, 2007; Threlfall, 2002). 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 et al., 2007). Due to these resistance problems in *Salmonella*, azithromycin has been used off-label for treatment of salmonellosis, mainly for infections caused by *S.* Typhi with reduced susceptibility to fluoroquinolones (Capoor et al., 2007; Threlfall et al., 2008). Evidence on the clinical efficacy of azithromycin mainly in the treatment of typhoid fever is available (Chinh et al., 2000; Frenck et al., 2004; Frenck et al., 2000). 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 et al., 2010). Susceptibility testing of *Salmonella* strains is advisable before treatment, as resistance against azithromycin may develop (Capoor et al., 2007; Gunell et al., 2010). Influence of recently authorised macrolides approved for animal use on the development of resistance in *Salmonella* isolates of animal origin cannot be excluded.

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 et al., 2010; Hammerum 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 et al., 2005). Macrolide resistance is already a recognised problem among streptococci isolated in humans (Fines et al., 2001; Rantala 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, 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 et al., 1998; Lobova 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 et al., 1999; Pringle 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 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 or fluoroquinolones 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, oxytetracycline or spectinomycin. In situations where respiratory pathogens have developed resistance for these antimicrobials, macrolides or florfenicol are the recommended choices over reserve drugs

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fluoroquinolones or extended spectrum cephalosporins (Anonymous, 2003; Constable et al., 2008; WVAB, 2011)

Macrolides and lincosamides have a limited use for treatment of bovine mastitis caused by Gram-positive pathogens (Constable et al., 2008; Deluyker et al., 2005). Mastitis-causing streptococci isolated in the EU have remained fully susceptible to penicillin G (Hendriksen et al., 2008). Macrolides do not offer benefits 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 (Hendriksen et al., 2008; Loch et al., 2005). Macrolides can be regarded as an alternative for treatment of mastitis caused by penicillin-resistant *S. aureus*, but culling is mostly a better option in those cases, due to poor prognosis (Barkema 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 et al., 2008). Resistance in *M. gallisepticum* may already limit the use of macrolides to treat chronic respiratory disease in poultry (Migaki 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. 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. Streptogramins are no longer used in food producing animals in the European Union.

- 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.

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- 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*) 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. The majority of mastitis streptococci and respiratory pathogens remain susceptible to macrolides. 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 interpretation criteria for resistance differ. For many pathogens, no agreed standards and interpretation criteria for the *in vitro* susceptibility testing are available. The complexity of cross resistance mechanisms makes interpretation in a diagnostic laboratory challenging.

- 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. However, the majority of udder and respiratory pathogens still remain susceptible to macrolides. Among zoonotic bacteria, the highest prevalences of resistance are seen in *Enterococci* but also *Campylobacter* 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 strongly 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 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.

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- 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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