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Review

Macrolides and lincosamides in cattle and pigs: Use and development of antimicrobial resistance

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ABSTRACT

Macrolides and lincosamides are important antibacterials for the treatment of many common infections in cattle and pigs. Products for in-feed medication with these compounds in combination with other antimicrobials are commonly used in Europe. Most recently approved injectable macrolides have very long elimination half-lives in both pigs and cattle, which allows once-only dosing regimens. Both in-feed medication and use of long-acting injections result in low concentrations of the active substance for prolonged periods, which causes concerns related to development of antimicrobial resistance.

Acquired resistance to macrolides and lincosamides among food animal pathogens, including some zoonotic bacteria, has now emerged. A comparison of studies on the prevalence of resistance is difficult, since for many micro-organisms no agreed standards for susceptibility testing are available. With animal pathogens, the most dramatic increase in resistance has been seen in the genus *Brachyspira*. Resistance towards macrolides and lincosamides has also been detected in staphylococci isolated from pigs and streptococci from cattle. This article reviews the use of macrolides and lincosamides in cattle and pigs, as well as the development of resistance in target and some zoonotic pathogens. The focus of the review is on European conditions.

Introduction

Macrolides are classified according to the number of atoms which comprise the lactone ring, ranging from 12 to 16 members (Yao and Moellering, 2007) (Table 1). The first macrolide intended for food animal use was spiramycin, which was introduced in the early 1960s, followed by erythromycin and tylosin in the early 1970s (Prescott, 2008). The most recent macrolide to be approved in the EU was tildipirosin in 2011. Semi-synthetic, new generation macrolides, the azalides, were introduced into human medicine in the early 1990s (Ballow and Amsden, 1992; Bryskier and Butzler, 2003). The first azalide for animal use, gamithromycin, was approved for use within the European Union (EU) in 2008.

Lincomycin and its semi-synthetic derivatives clindamycin and pirlimycin, belong to the lincosamides. In addition, streptogramins (A and B) are classified along with macrolides and lincosamides (Edelstein, 2004). The only streptogramin used for animals is

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[1] See: http://www.eucast.org/expert_rules/.

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Table 1
Macrolides and related compounds and their approval for animal use in the EU (EMA/CVMP/SAGAM, 2011).

Macrolides			Lincosamides	Streptogramins (A,B)
14-Membered ring	15-Membered ring	16-Membered ring		
Clarithromycin	Azithromycin	Josamycin	Clindamycin[b]	Pristinamycin
Erythromycin[a]	Gamithromycin[a]	Mideacamycin	Lincomycin[a]	Quinupristin/dalfopristin
Oleandomycin	Tulathromycin[a]	Miocamycin	Pirlimycin[a]	Virginiamycin[c]
Roxithromycin		Rokitamycin		
Telithromycin[a]		Spiramycin[a]		
		Tildipirosin[a]		
		Tilmicosin[a]		
		Tylosin[a]		
		Tylvalosin[a]		

[a] Substances approved for veterinary use in one or more member states in the EU (having marketing authorisation [MA]).
[b] Only approved for small animal use.
[c] Not any longer approved in the EU.

virginiamycin, which, until 1998, was approved in the EU as a feed additive for production enhancement of food animals; it is still approved for this use in the US.

Macrolide antimicrobials inhibit bacterial protein synthesis via binding to the 50S subunit of the ribosome. They bind preferentially to the 23S rRNA of the 50S subunit, which overlaps with the binding site of lincosamides and streptogramin B, but differs from those of phenicols like chloramphenicol, and pleuromutilins. Due to this similar mechanism of action, resistance is also often linked, and macrolides, lincosamides and streptogramins B are often referred to as the MLS_B group. Macrolide and lincosamide (ML) antibacterials have generally a bacteriostatic action, which is mainly time-dependent (Giguère 2013a, 2013b). Some new generation macrolides can have bactericidal activity against some bacterial species, in laboratory conditions, although this effect is limited compared with other classes of antimicrobials (Seral et al., 2003).

ML antimicrobials are active against many Gram-positive bacterial genera such as *Streptococcus*, *Staphylococcus*, *Enterococcus* and *Trueperella* (*Arcanobacterium*), as well as against Gram-negative organisms, like *Actinobacillus*, *Haemophilus*, *Histophilus*, *Mannheimia*, *Pasteurella*, *Moraxella*, *Bordetella*, *Campylobacter* and *Lawsonia*. Anaerobes including *Fusobacterium*, *Clostridium* and *Bacteroides* spp. are usually susceptible. In addition, the spectrum covers spirochaetes (*Leptospira*, *Brachyspira*), and *Mycoplasma*. However, substantial differences exist between macrolides in their activity against different organisms (Hardy et al., 1988; Bryskier and Butzler, 2003). The spectrum of activity of lincosamides is similar but not identical than that of macrolides; for example, *Enterococcus faecalis* is intrinsically resistant to lincosamides (Roberts, 2008). Lincosamides have low activity against Pasteurellaceae (Giguère 2013b).

In vitro susceptibility testing for ML antimicrobials is problematic for many bacterial species, since guidelines for determination of minimal inhibitory concentrations (MIC) do not include all micro-organisms (Schwarz et al., 2010; CLSI, 2013). Comparison of resistance data is also difficult because different antimicrobials are often tested and criteria for interpretation may differ.

Macrolides penetrate well into tissues (Giguère 2013a; Rose et al., 2013). ML build up high intracellular concentrations and accumulate within phagocytes (Scorneaux and Shryock, 1999). The actual efficacy of bacterial killing within cells has not been unambiguously demonstrated (Madgwick et al., 1989; Barcia-Macay et al., 2006). After oral administration, macrolides are absorbed incompletely. Lincosamides are absorbed well when given orally to monogastric animals. ML antibiotics are eliminated mainly by the liver, with a variable part of the drug excreted in bile as the parent drug or metabolites. This leads to enterohepatic cycling and long terminal half-lives.

Semi-synthetic macrolides are very long-acting, due to their low clearance rates. For example, the elimination half-life of tulathromycin in cattle and swine is close to 4 days and that of gamithromycin in cattle is >2 days. The most recently authorised macrolide, tildipirosin, has the longest terminal half-life, approximately 9 days in cattle and >4 days in swine. Quantifiable concentrations of gamithromycin and tildipirosin are present for >2 weeks in plasma and 3–4 weeks in the lung (Giguère et al., 2011; Menge et al., 2012). Severe tissue irritation, causing pain and inflammation, is a common problem of all macrolides, particularly when administered parenterally (Giguère 2013a).

Use of ML antimicrobials in cattle and pigs

By 2013, eight macrolides and two lincosamides were authorised for use in food animals in some or all EU member states (Table 1). In the EU, ML are available for parenteral administration, including intramammary use, and for oral use including premix formulations. ML are used widely for the treatment of common infections in food-producing animals, and have been categorised as critically important in veterinary medicine by the World Organisation for Animal Health (OIE) (Collignon et al., 2009).

Use of macrolides as growth promoters began at the same time as therapeutic use, with spiramycin and tylosin being used within the EU until 1998 (Council Regulation EC2821/98 of 17 December 1998). The first injectable, long-acting macrolide with a one-dose only regimen for food animal use was tilmicosin. Other macrolides authorised with this regimen are tulathromycin, gamithromycin and tildipirosin. The total number of available ML products varies between EU member states from 5 to 183 products containing macrolides (Fig. 1) and from 1 to 32 products containing lincosamides. More than 60 combination products containing macrolides and other antimicrobials are available in the EU; in addition, numerous lincomycin products exist as combinations (EMA/CVMP/SAGAM, 2011). Most often macrolides are combined with colistin or aminoglycosides, but in some products also with sulfonamides, trimethoprim, oxytetracycline, or ampicillin. The approved duration of treatment for some in-feed products is long, up to 4–5 weeks in some cases.

In a recent report (EMA/ESVAC, 2013), data on the sales of antibacterials in 25 European countries were analysed in a harmonised manner using population correction unit (PCU) as an estimate of the eligible animal population. In Fig. 2, data on the sales of ML in different European countries in 2011 are presented. Usage of ML varies greatly between countries, as does proportion of total antimicrobial sales which are ML (ranging from 4% in Sweden to 14% in Denmark), with pigs being the main target species for ML use. The use of different pharmaceutical forms of ML also differs widely between countries (EMA/ESVAC, 2013). For macrolides, sales

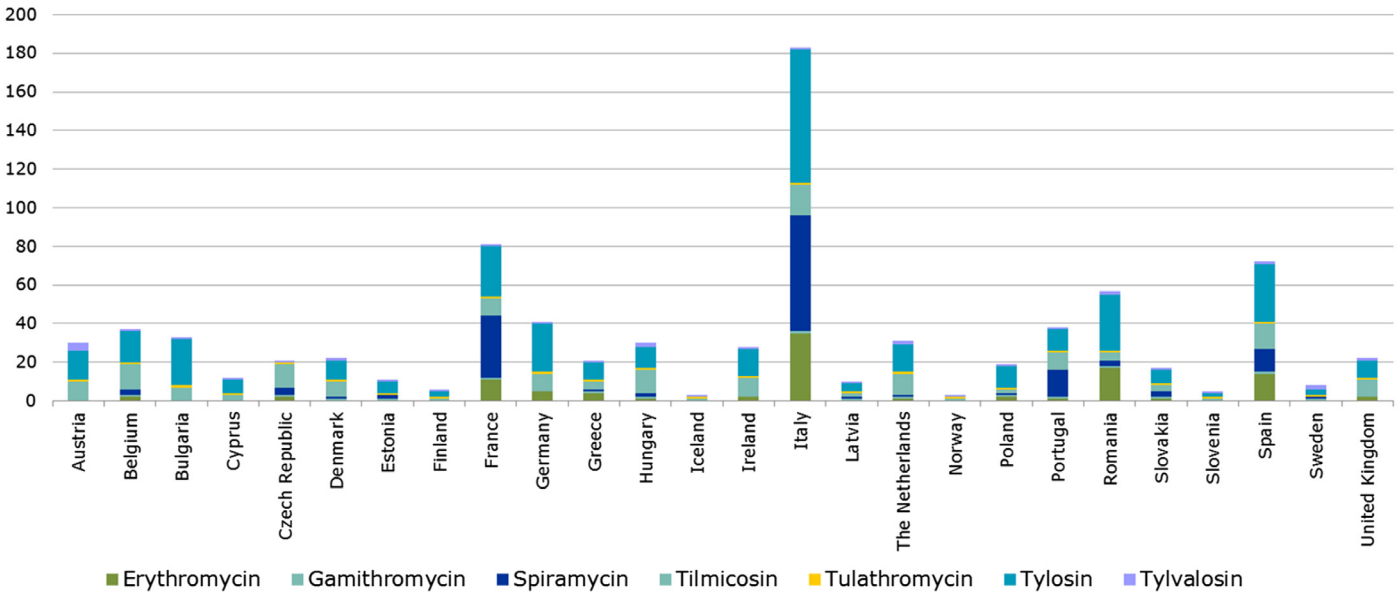

Fig. 1. Number of macrolide products authorised for food animal use per antimicrobial substance in Europe (25 countries, data from 2011). Data collected in the European Medicines Agency.

comprise premixes, oral powders, oral solutions and injectable products (Fig. 3), while for lincosamides almost all of the sales were products for in-feed medication (Fig. 4).

Older macrolide products have various indications and dose regimens. The main indications in cattle include all common infections such as respiratory and genital infections, foot lesions and mastitis, and, in pigs, pneumonia, enteritis and arthritis are label indications. More recently approved ML products list the target pathogens, with injectable macrolides indicated for the treatment and prevention of respiratory infections in non-lactating cattle caused by *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, or *Mycoplasma bovis*. Tulathromycin is also indicated for interdigital necrobacillosis associated with *Fusobacterium necrophorum* and *Porphyromonas levii*, and for treatment of infectious bovine keratoconjunctivitis associated with *Moraxella bovis*. In swine, injectable macrolides are indicated for the treatment and prevention of swine enzootic pneumonia caused by *Mycoplasma hyopneumoniae*, and respiratory infections caused by *Actinobacillus pleuropneumoniae*, *P. multocida*, and *Haemophilus parasuis* (EMA, 2010). Tylvalosin is authorised centrally in the EU for the oral treatment and prevention of porcine proliferative enteropathy caused by *Lawsonia intracellularis*, swine dysentery caused by *Brachyspira hyodysenteriae*, as well as swine enzootic pneumonia.

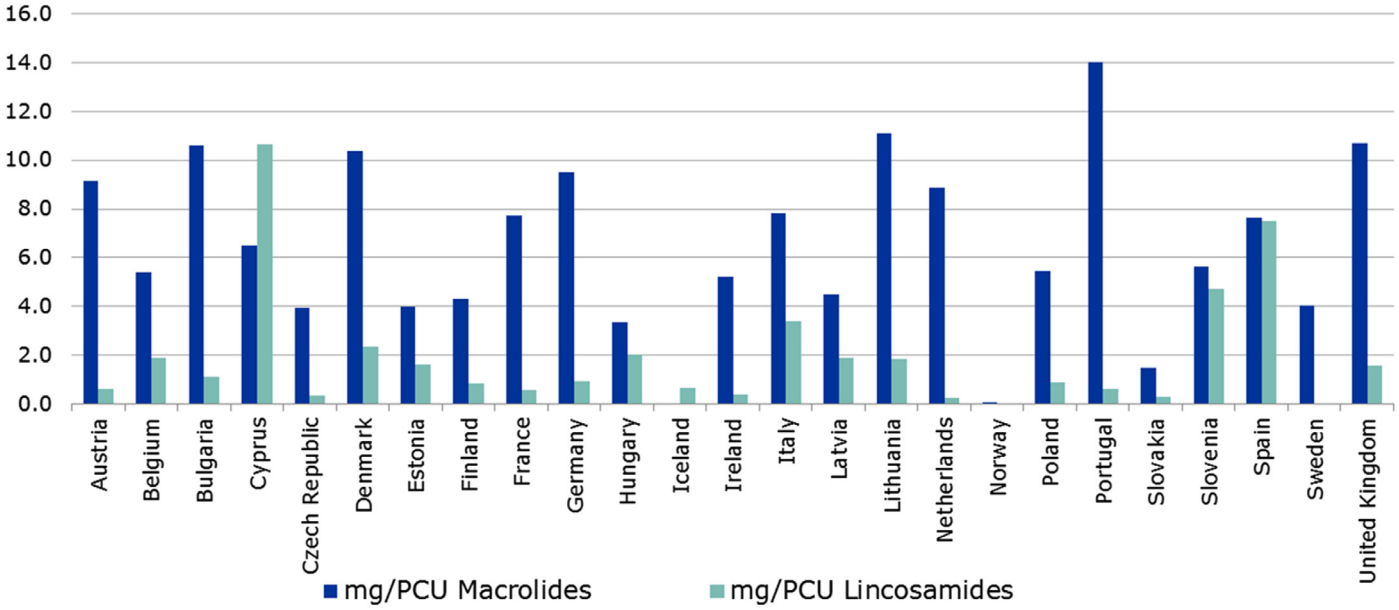

Fig. 2. Sales of macrolides and lincosamides in 25 European countries in 2011 expressed as mg of active ingredient per population correction unit (PCU) for all food-producing species, including horses. Data from ESVAC (EMA/ESVAC, 2013).

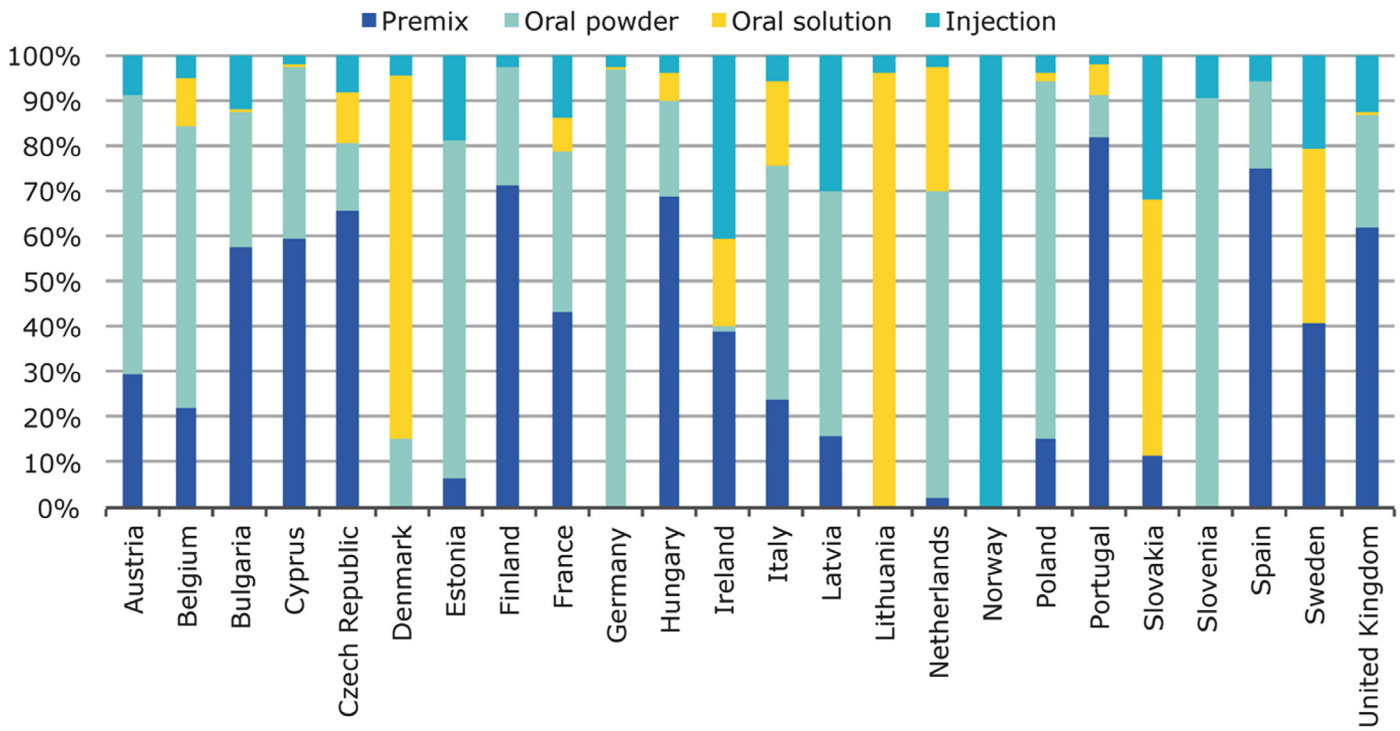

Fig. 3. Sales of macrolides in 24 European countries (no sales in Iceland or Norway) in 2011 by pharmaceutical form expressed as percentage of the sales in tonnes in each country. No sales in Iceland. In addition, negligible amounts were sold as intramammary and/or intrauterine preparations in some countries. Data from ESVAC (EMA/ESVAC, 2013).

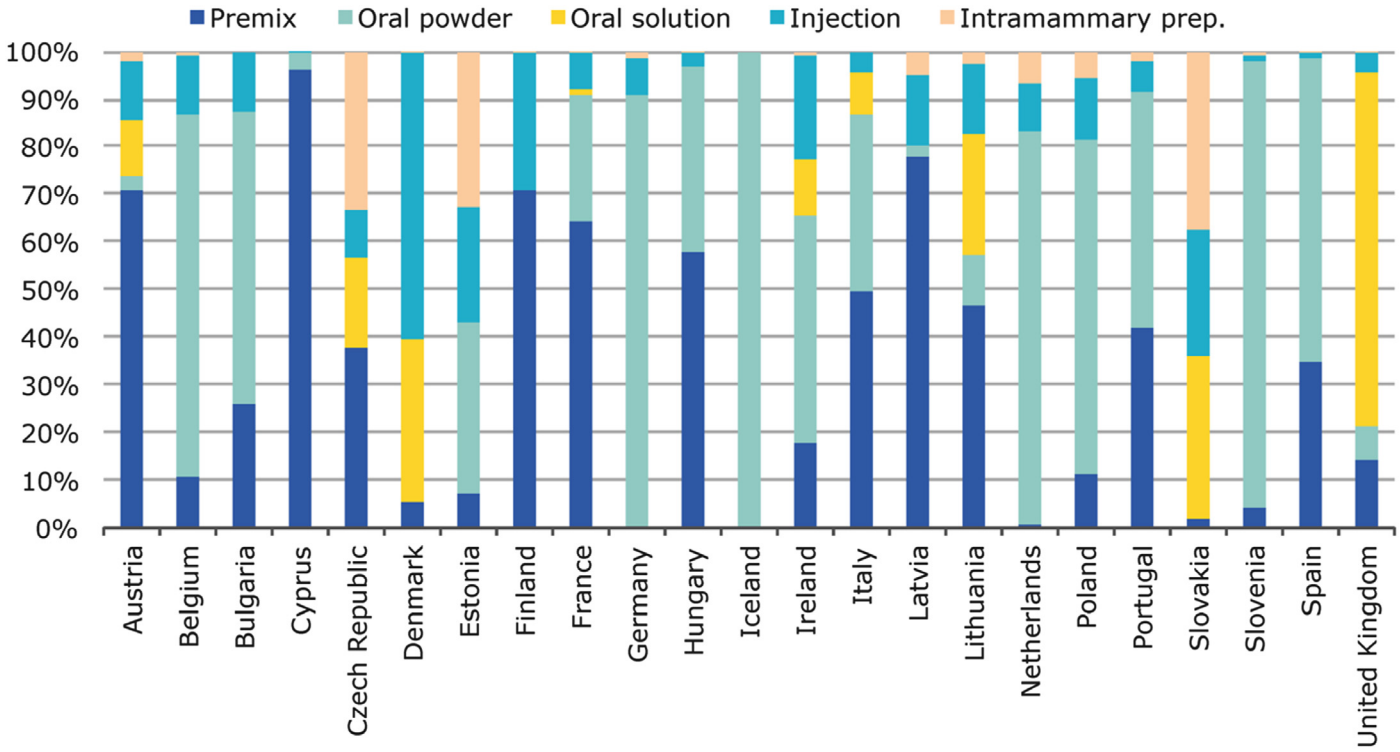

Fig. 4. Sales of lincosamides in 23 European countries in 2011 by pharmaceutical form expressed as percentage of the sales in tonnes in each country. No sales in Norway or Sweden. Data from ESVAC (EMA/ESVAC, 2013).

Resistance to ML antibiotics

Mechanisms of resistance

Macrolide-producing *Streptomycetes* are intrinsically ML-resistant as they have genes which provide a self-protective mechanism (Andini and Nash, 2006). Enterobacteriaceae such as *E. coli* and *Salmonella* spp. have a low susceptibility to macrolides, because of the poor permeability of these hydrophobic substances across their bacterial wall (Vaara, 1993). Gram-negative non-fermentative rods like *Pseudomonas aeruginosa* and *Acinetobacter* are inherently resistant to ML antimicrobials.[1] More than 70 genes encoding for acquired ML resistance are hosted by more than 60 different bacterial species (Roberts, 2011; van Hoek et al., 2011).

The most common resistance mechanism against ML antibiotics is a target site modification mediated by at least 36 different rRNA methylases (*erm* genes) (Leclercq and Courvalin, 1991; Diner and Hayes, 2009; Palmieri et al., 2013). These genes, the most prevalent being *erm*(A), *erm*(B), and *erm*(C), have been detected in numerous streptococci and staphylococci including *Strep. suis*, *Strep. uberis*, *Staph. hyicus*, and *Staph. aureus*, as well as many other food animal pathogens such as *Enterococcus*, *M. haemolytica*, *P. multocida*, *Trueperella pyogenes* and *Listeria monocytogenes* (Jensen et al., 1999; Boerlin et al., 2001; Martel et al., 2001, 2003; Jost et al., 2003; Culebras et al., 2005; Loch et al., 2005; Luthje and Schwarz, 2007; Luthje et al., 2007; Palmieri et al., 2007; Schmitt-Van de Leemput and Zadoks, 2007; Desmolaize et al., 2011; Haenni et al., 2011; Kadlec et al., 2011; Zastempowska and Lassa, 2012). *Erm* genes can be transferred horizontally because of the association with mobile genetic elements (Roberts, 2011).

A *Cfr* gene encoding for an unusual rRNA methylase and conferring a multi-drug resistance phenotype (including resistance to lincosamides, streptogramins A, phenicols, pleuromutilins, and oxazolidinones) has been detected in several bacterial species including staphylococci, enterococci and *E. coli* from food animals (Schwarz et al., 2002; Witte and Cuny, 2011; Liu et al., 2012; Zhang et al., 2014). This gene is of global concern as it is often located on plasmids and can be spread between bacterial species and genera (Shen et al., 2013).

The second most common ML resistance mechanism is the active expulsion of the antimicrobial from the bacteria mediated by efflux pump genes (*mef* and *msr*). *Mef* genes confer resistance to ML antibiotics and *msr* genes to both ML and streptogramin B. Many of the *mef* genes are associated with conjugative elements located in the chromosome, whereas *msr* genes are located mainly on plasmids. Chromosomal *msr*(E) was detected in *P. multocida* and *M. haemolytica* strains isolated from bovine respiratory disease (Kadlec et al., 2011). The *mef*(A) gene has been reported in streptococci, including *Strep. suis* (Martel et al., 2003). Other efflux genes are *vga* genes which have previously been detected in streptococcal and enterococcal species and code for transferable resistance to pleuromutilins, streptogramin A and lincosamides (De Graef et al., 2007). *Vga*(C) and *vga*(E) were found in porcine methicillin-resistant *Staph. aureus* (MRSA) isolates of type ST398 (Kadlec et al., 2009, 2011; Kadlec and Schwarz, 2009; Schwendener and Perreten, 2011) and *vga*(A) and *vga*(E) in bovine ST398 *Staph. aureus* isolates (Fessler et al., 2010; Hauschild et al., 2012). One more efflux gene *lsa*, which is intrinsic in *E. faecalis* and confers resistance to lincosamides and streptogramin B, has recently been detected in MRSA of swine origin (Li et al., 2013).

The third resistance mechanism is enzyme-catalysed inactivation of the drug mediated by phosphorylases (*mph* genes) conferring resistance only to macrolides, or transferases that render the bacteria resistant only to streptogramin A. Genes encoding for inactivating enzymes have been detected in food animal pathogens, including *mph*(C) in *Staph. aureus* and *lnu*/*lin* in *Staph. hyicus* and other coagulase-negative staphylococci (CNS) (Luthje and Schwarz, 2007; Luthje et al., 2007; Sampimon et al., 2011). *Strep. uberis* has been shown to express *mph*(B) or *lin*(B) (Schmitt-Van de Leemput and Zadoks, 2007; Achard et al., 2008; Haenni et al., 2011). A bovine *P. multocida* strain has been reported to carry the *mph*(E) gene in addition to *msr*(E) in its chromosome (Kadlec et al., 2011). Rare enzymes causing resistance are lyases (*vgb* genes) which confer resistance to streptogramin B and esterases (*ere* genes) conferring resistance to erythromycin (Morar et al., 2012).

Non-transferable resistance due to mutations in ribosomal RNA and ribosomal proteins can also lead to reduced macrolide susceptibility. Mutational events of the 23S rRNA confer MLS resistance (Vester and Douthwaite, 2001) and are the most prevalent or the only known resistance mechanism in certain food animal pathogens, including *B. hyodysenteriae*, *B. pilosicoli*, and *M. hyopneumoniae* (Karlsson et al., 1999, 2004b; Stakenborg et al., 2005; Hidalgo et al., 2011), as well as zoonotic organisms *Campylobacter jejuni* and *C. coli* (Gibreel and Taylor, 2006; Alfredson and Korolik, 2007; Caldwell et al., 2008).

Emergence of resistance to ML antibiotics in pathogens isolated from cattle

Macrolide resistance of *P. multocida* isolated from cattle is rare within the EU (Kehrenberg et al., 2006; Kaspar et al., 2007). In The Netherlands, in 2004–2005 0% and in 2006–2008 2.5% of isolates from cattle were reported to be resistant to tilmicosin, and no resistance to tulathromycin was found (MARAN, 2007–2008). In France in 2008, 7% of *P. multocida* but 35% of *M. haemolytica* isolated from cattle were resistant to tilmicosin (AFSSA, 2009). In Belgium, 13% of *P. multocida* isolates and 38% of *M. haemolytica* isolates from healthy cattle, including veal calves, were reported to be non-susceptible to tilmicosin (Catry et al., 2005). In The Netherlands, resistance to tilmicosin for *M. haemolytica* was reported to increase from zero to 6.5% (MARAN, 2007–2008), and the same figure was found for tulathromycin. Data from the US and Canada suggest that MICs for bovine *M. haemolytica* and *P. multocida* towards macrolides have increased. Portis et al. (2012) using the same breakpoints as used in European studies reported that in 2009, 25% of *M. haemolytica* isolates and 24% of *P. multocida* were not susceptible to tilmicosin; for tulathromycin the same figures were 9% and 4%, respectively.

Data on *H. somni* from cattle are scarce, but Portis et al. (2012) reported that the proportion of *H. somni* isolates from cattle which were non-susceptible to tilmicosin and tulathromycin increased from 3% and 2% in 2000 to 18% and 17% in 2009.

Macrolide resistance in *Staph. aureus* retrieved from bovine mastitis has generally been uncommon: 0–2% of the isolates have been reported to be resistant to erythromycin (Hendriksen et al., 2008). Higher figures were seen in Estonia where 5% of *Staph. aureus* mastitis isolates were resistant to erythromycin and as much as 18% to clindamycin (Kalmus et al., 2011). Resistance of *Staph. aureus* to clindamycin has not been recorded in the Nordic countries (Pitkala et al., 2004; NORM-VET, 2005–2008). For pirlimycin, 4% of the isolates were resistant in The Netherlands (MARAN, 2007–2008). Coagulase-negative staphylococci (CNS) have developed resistance to ML antimicrobials (Luthje and Schwarz, 2006; Sampimon et al., 2011), with 13–20% of CNS isolated from bovine mastitis in The Netherlands, Estonia and France resistant to lincosamides (MARAN, 2007–2008; AFSSA, 2009) and 14–15% to erythromycin (Botrel et al., 2010). This is less marked in the Nordic countries, where resistance to macrolides has been 4–6%, and no resistance to clindamycin has been found (Pitkala et al., 2004; NORM-VET, 2005–2008).

In some European countries, up to 22% of *Strep. uberis* and 17% of *Strep. dysgalactiae* isolates from bovine mastitis have been found to be resistant to erythromycin (Hendriksen et al., 2008); in a French study, 13–17% of *Strep. uberis* and 4–6% of *Strep. 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 *Strep. uberis* and 8% of *Strep. dysgalactiae* were resistant to clindamycin (MARAN, 2007–2008). In Sweden and Norway, no resistance towards erythromycin or clindamycin was reported for *Strep. uberis* and *Strep. dysgalactiae* (NORM-VET, 2005–2008; SVARM, 2002–2010). In Finland, 15% of *Strep. uberis* isolates were resistant to erythromycin but none to clindamycin; *Strep. dysgalactiae* isolates were fully susceptible for both (FINRES-Vet, 2005–2009).

In vitro susceptibility testing of *Mycoplasma* is challenging, due to their slow growth and need for specific media, and consequently little is known about ML resistance of *Mycoplasma* species isolated in food animals (Aarestrup and Kempf, 2006). Acquired resistance towards macrolides has been suspected in *M. bovis* isolated from cattle in some countries (Thomas et al., 2003; Gerchman et al., 2009).

Emergence of resistance to ML antibiotics in pathogens isolated from pigs

For *Brachyspira* isolated from swine, high levels of ML resistance have been reported for tylosin in most EU countries, with close to 100% of the isolates being resistant (FINRES-Vet, 1999; SVARM, 2002–2010; Vyt and Hommez, 2006; MARAN, 2007–2008; Hidalgo et al., 2009, 2011). Resistance to ML antimicrobials can develop rapidly in *B. hyodysenteriae* because only a single transversion mutation in one position of the 23S rRNA gene is required (Karlsson et al., 1999; Pringle et al., 2012). Data on susceptibility of *Brachyspira* to tylvalosin are limited, but isolates with high MIC values to tylosin have generally also had higher MICs to tylvalosin (Karlsson et al., 2004a; Hidalgo et al., 2011; Pringle et al., 2012). Recently, tentative wild-type cut-off values have been proposed for *B. hyodysenteriae* (Pringle et al., 2012). Resistance of *B. hyodysenteriae* towards lincomycin is close to that for tylosin (SVARM, 2002–2010; FINRES-Vet, 2005–2009), due to complete cross-resistance.

In a US study, MIC values for *Brachyspira* species isolated from pigs with clinical disease were reported to be consistently high to lincomycin (Clothier et al., 2011). In Europe, resistance in *B. pilosicoli* to tylosin has been reported to be 50–100%; also occasional high MICs for tylvalosin have been reported (SVARM, 2002–2010; Karlsson et al., 2004b; Pringle et al., 2006a; Pringle et al., 2012). Isolates of *B. hyodysenteriae* have been found with simultaneous resistance to lincomycin, tylosin, tylvalosin and tiamulin (Duinhof et al., 2008). For *L. intracellularis*, there are no standards for susceptibility testing and practically no data are available. The activity of tylosin and lincomycin was tested against 10 isolates of *L. intracellularis* (Wattanaphansak et al., 2009). The wide range of MIC distribution may indicate decreased susceptibility.

Results from in vitro susceptibility testing of *Mycoplasma* should be interpreted with caution as no agreed standards for testing are available. *M. hyopneumoniae* strains are intrinsically resistant to 14-membered macrolides. Development of resistance in *M. hyopneumoniae* towards 15- and 16-membered macrolides and lincosamides has been reported in Belgium (Vicca et al., 2004; Stakenborg et al., 2005). In a limited study from the US, field isolates of *M. hyopneumoniae* were reported to be fully susceptible to clindamycin and tylosin, but 25% were resistant to tulathromycin (Schultz et al., 2012). Resistance of *M. hyosynoviae* isolated from swine has been studied in Denmark; in 1968–1971 all isolates were susceptible to lincomycin and tylosin, but 20 years later 12% of isolates were resistant to tylosin (Aarestrup and Friis, 1998).

For *A. pleuropneumoniae* isolated from swine, data are limited. In France, close to 80% of *A. pleuropneumoniae* were resistant to spiramycin, but only 2% to tilmicosin (AFSSA, 2009). In Spain, MIC values of *A. pleuropneumoniae* for erythromycin increased compared with those reported two decades earlier (Gutierrez-Martin et al., 2006); however, trends such as these should be interpreted with caution since laboratory methods may not be the same. Macrolide resistance of *P. multocida* isolated from swine is rare within the EU (Kehrenberg et al., 2006; Kaspar et al., 2007). In France in 2008, no resistance to tilmicosin was found in porcine isolates of *P. multocida*, but 86% of the isolates were resistant to tylosin (AFSSA, 2009).

Staph. hyicus isolated from swine is more frequently resistant to macrolides (Werckenthin et al., 2001). The occurrence of macrolide resistance of *Staph. hyicus* isolated from swine in Denmark appears to correlate with the use of tylosin for growth promotion. The proportion of *Staph. hyicus* isolates resistant to erythromycin increased from 33% in 1996 to 62% in 1997, and decreased by 2001 to approximately 20%, thereafter stabilising at about 35% (Aarestrup and Schwarz, 2006; DANMAP, 2012). Tylosin is still used for treatment, which probably maintains the resistance at the present level. In Sweden, 12% of *Staph. hyicus* were resistant to erythromycin over a long time period (SVARM, 2002–2010).

Increasing resistance for macrolides in *Strep. suis* was found in Denmark during investigations 10 years apart (Aarestrup and Schwarz, 2006). In selected EU countries in 2002, resistance of *Strep. suis* to erythromycin was 19–65% (Hendriksen et al., 2008). In France, resistance was reported to be as high as 72–77% towards spiramycin and tylosin and 69% for lincomycin (AFSSA, 2009). In the German surveillance data from 2008, 30–45% of isolates were resistant to erythromycin (BVL, 2008).

Resistance among some zoonotic and indicator bacteria

There are clear differences in resistance of *Campylobacter* spp. originating from food animals across the EU (de Jong et al., 2009; EFSA/ECDC, 2013). *C. jejuni* from poultry is considered to be the main source of zoonotic *Campylobacter*; cattle and pigs are less important sources. However, macrolide resistance is substantially more common in *C. coli* than in *C. jejuni* (Payot et al., 2006; Belanger and Shryock, 2007). In the EU around 25% of porcine *C. coli* isolates were resistant to erythromycin, ranging from 0% in Sweden to 63 % in Spain (six countries reporting) (EFSA/ECDC, 2013). Data from The Netherlands showed that *C. jejuni* isolated from food animals has no erythromycin resistance but 15% of *C. coli* isolates did (MARAN, 2013).

In Denmark, approximately 80% of *E. faecium* isolated from pigs in the late 1990s were resistant to tylosin and 50–70% resistant to virginiamycin (Aarestrup et al., 2000). After the ban on tylosin, spiramycin and virginiamycin as feed additives within the EU in 1998, the proportions of macrolide-resistant enterococci decreased in countries with previously very high figures (DANMAP, 2012; MARAN, 2007–2008). At the same time, consumption of tylosin in the Danish pig industry decreased from almost 80 tons to about 20 tons (DANMAP, 2012). In The Netherlands in 2012, resistance to erythromycin of *E. faecalis* and *E. faecium* from cattle and pigs was 61% and 47%, respectively (MARAN, 2013), although since 2010, the proportion of resistant isolates *of E. faecium* has decreased. In Finland and Sweden, where use of ML antibiotics is limited compared with most other European countries (EMA/ESVAC, 2012), erythromycin resistance of enterococci isolated from cattle and pigs has ranged from 15% to 45% (FINRES-Vet, 2005–2009; SVARM, 2002–2010).

Clostridium spp. are normal microbiota but some are opportunistic pathogens which can cause enteric diseases in humans and animals. Only a few reports on the susceptibility of *C. perfringens* of porcine isolates are available, but ML resistance is common (Franklin et al., 2006). *C. difficile* is a leading cause of nosocomial diarrhoea in humans and also causes enteric infections in piglets. Ribotype 078 has recently been increasingly isolated in both

humans and pigs (Keessen et al., 2013). Isolates were mostly susceptible to clindamycin, but resistant to erythromycin, with human isolates being more resistant than porcine ones. The authors suggested that human and piglet type 078 isolates may have a common origin.

Discussion

A very high number of products containing ML antimicrobials are available. The indications and dosages of the old, nationally authorised macrolide products show a great variation, and their efficacy data are limited or non-existent. The approved duration of treatment for some old in-feed ML products is very long, up to 5 weeks, which raises concerns about possible use for growth promoting purposes. The most recently approved macrolides share once-only dosing regimen and the elimination of the substances is very slow. This regimen apparently meets the requirements for convenient treatment protocols of young cattle in the field conditions. Whether this is an optimal duration of the treatment (i.e. if the drug needs to be present in the tissues for 3–4 weeks as is maintained by these long-acting products) remains open to debate (Giguère et al., 2011; Menge et al., 2012).

In addition to the target pathogens, the exposure of the whole microbiome of the animal to the active substance is long, which could lead to the development of antimicrobial resistance. Even very low antibiotic concentrations have been shown to select for resistance (Gullberg et al., 2011). Studies comparing the efficacy of long- *versus* short-acting macrolides would be warranted. Another concern related to one-shot-only macrolide products is related to animal welfare: according to the summary of product characteristics of the products, pain and reactions at the injection site are commonly seen and they may last for weeks.

The indications for in-feed ML combination products can be particularly broad. The justification of the combinations over single components has not been proven, as the products were approved before specific requirements for demonstration of efficacy of fixed combination products were implemented (EMEA/CVMP, 2006a). To decrease the risk for resistance development it would be necessary to update and harmonise the dosing regimens of ML antibiotics in the EU, in particular those of the in-feed products used for long periods. Approvals of the combination products should be renewed and those with no justification should be withdrawn from the market.

The increase of macrolide resistance, in particular for *C. coli* from pigs after macrolide use as growth promoter and for treatment, has been documented in several studies (Aarestrup et al., 1997; Van Looveren et al., 2001). If the use is restricted, resistance among *Campylobacter* spp. can decrease markedly, as seen in Denmark after the ban of tylosin for growth promotion (DANMAP, 2012). The dynamics of antimicrobial resistance in *C. coli* was studied on a large pig farm in Finland (Juntunen et al., 2010), where tylosin treatment was selected for a high rate of resistance towards erythromycin, which significantly decreased when treatment was discontinued.

ML are important antimicrobials for the treatment of infections in cattle and pigs, though they are seldom the sole alternative for treatment. They share some advantageous pharmacokinetic characteristics such as good penetration into tissues and high intracellular concentrations, which makes them an attractive choice for several indications (Giguère 2013a, 2013b). Macrolides, like tilmicosin and tulathromycin, are recommended in national treatment guidelines and textbooks for the treatment of bovine respiratory disease in cattle, as alternatives to benzylpenicillin, oxytetracycline, or phenicols (Anonymous, 2003, 2012; Constable et al., 2008). The majority of respiratory pathogens of cattle have still remained susceptible to ML antibiotics, despite the decades-long use of these substances. Macrolides are better choices than critically important drugs such as fluoroquinolones or extended spectrum cephalosporins which also have bovine respiratory disease (BRD) as label indication, but should be used with caution (Anonymous, 2003; Constable et al., 2008). In the EU, the latter must have specific warning sentences in their summary of product characteristics, which means they should be second line treatments only (EMEA/CVMP, 2006b; EMA/CVMP/SAGAM, 2009).

ML have limited uses for the treatment of bovine mastitis caused by Gram-positive pathogens (Deluyker et al., 2005; Constable et al., 2008). Mastitis-causing streptococci have remained fully susceptible to benzylpenicillin (SVARM, 2002–2010; MARAN, 2007–2008; Hendriksen et al., 2008). Frequent use of ML for the treatment of mastitis in some countries may have contributed to the high prevalence of resistance mastitis-causing streptococci. Macrolides could be regarded as an alternative for treatment of mastitis caused by penicillin-resistant *Staph. aureus*. However, cure rates of mastitis caused by *Staph. aureus* with spiramycin or pirlimycin treatment have been reported to be low (Pyörälä and Pyörälä, 1998; Middleton et al., 2007). Culling seems to be a better option due to the poor prognosis and risk of spreading of the intramammary infections (Barkema et al., 2006).

For the treatment of swine dysentery caused by *B. hyodysenteriae*, macrolides have been the drugs-of-choice in addition to tiamulin and valnemulin (Giguère 2013a, 2013b). Due to widespread resistance, in many countries macrolides can only be used after susceptibility testing. Decreased susceptibility to tiamulin in *B. hyodysenteriae* isolates has also been reported (Gresham et al., 1998; Lobova et al., 2004), but it must be emphasised that no approved breakpoints for susceptibility testing are available. For diarrhoea caused by *B. pilosicoli*, pleuromutilins have been the first choice, but resistance to tiamulin has emerged and percentages of resistance from 5–16% to them have been reported (Fossi et al., 1999; Pringle et al., 2006b). For porcine proliferative enteropathy caused by *L. intracellularis*, pleuromutilins or tetracyclines are the first choices, and macrolides are the second choice (Burch et al., 2008). The therapeutic arsenal for major swine gastrointestinal infections is already limited, and strict prudent use principles should be adapted to slow down the development of antimicrobial resistance of the causing agents. Increases in macrolide, lincosamide and pleuromutilin resistance would considerably worsen the situation.

For swine enzootic pneumonia caused by *M. hyopneumoniae* as well as mycoplasmal arthritis, lincomycin and macrolides are important alternatives to pleuromutilins. Tylosin or lincomycin is used for neonatal diarrhoea in piglets caused by *C. perfringens*, as an alternative to penicillins. Swine pneumonia caused by *A. pleuropneumoniae* and *P. multocida* has remained mostly susceptible to penicillins, but macrolides are also used. Increasing resistance towards ML would not result in a situation of no treatment alternatives for pigs, but would seriously restrict alternatives available for treatment.

Macrolides are considered as critically important and lincosamides as highly important in human medicine (WHO, 2011). In humans, macrolides are used mostly for non-food-borne bacterial infections with the exception of *Campylobacter* and possibly *Salmonella* spp. (Gunell et al., 2010). Food-borne pathogens, but also bacteria infecting humans not linked directly to food of animal origin may acquire resistance determinants from bacteria originating from food-producing animals. Prudent use of all antimicrobials including ML antibiotics approved for food animals is of utmost importance. Antimicrobials should never be used to compensate for inadequate management of food animals, and all preventive measures such as consecutive production cycles and vaccinations should be applied.

Conclusions

Macrolides and lincosamides (ML) are important antimicrobials for the treatment of infections in cattle and pigs, although seldom the sole alternative. The most common indications for ML are the treatment of major swine gastrointestinal and respiratory infections and of bovine respiratory disease. The number of old products containing ML in the EU is substantial, and updating and harmonising the dosing regimens of these products are necessary. Acquired resistance to ML antimicrobials among food animal pathogens, including some zoonotic bacteria, has emerged, with the greatest increase in resistance in *Brachyspira*. In-feed medications and long-acting injections resulting in low concentrations of the active substance for long periods may particularly contribute to the development of antimicrobial resistance. Macrolides are considered as critically important and lincosamides as highly important in human medicine, but these substances are mostly used for non-food-borne infections. Prudent use of ML antimicrobials approved for food animals is of crucial importance to maintain the efficacy of these important therapeutic alternatives.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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