

13 February 2014
EMA/CVMP/AWP/119489/2012-Rev.1¹
Committee for Medicinal Products for Veterinary Use

Reflection paper on use of pleuromutilins in food-producing animals in the European Union: development of resistance and impact on human and animal health

Draft Agreed by Scientific Advisory Group on Antimicrobials	29 November 2012
Adoption by Committee for Medicinal Products for Veterinary Use (CVMP) for release for consultation	13 December 2012
Start of public consultation	13 December 2012
End of consultation (deadline for comments)	30 June 2013
Agreed by Antimicrobials Working Party (AWP)	25 September 2013
Adopted by CVMP	7 November 2013
Further revision by AWP	23 January 2014
Further adoption by CVMP	13 February 2014

CVMP recommendations for action

Pleuromutilins (tiamulin and valnemulin) are used predominately in pigs but to some extent also in poultry and rabbits. It is an essential group of antimicrobial agents in veterinary medicine especially as it is the sole treatment option for enteritis in pigs caused by isolates of *Brachyspira hyodysenteriae* (swine dysentery) resistant to lincosamides and macrolides. The negative consequences in case such a pathogen were to become pleuromutilin resistant would thus be considerable both from an economic and an animal welfare perspective.

¹ Further revision to take into accounts from IFAH Europe and FVE. ESVAC data were updated and reanalysed.

There are several products containing pleuromutilins available on the EU market and most of the use is for group and herd/flock medication in feed or water. In addition to the originator products there are a number of different tiamulin containing generics available and the approved indications vary considerably. The overall use differs considerably between EU countries without known rationale. Partly the difference might reflect difference in prevalence for the major diseases where pleuromutilins are used but there is likely to be additional explanation that would merit further exploration.

To contain resistance, unnecessary use and also the preventive use of pleuromutilins without applying adequate control programmes should be avoided. For these reasons it is important that responsible use principles are outlined in the SPCs for approved products, e.g. as specified in section 4.9 of the SPC subsequent to the CVMP Tiamutin referral².

The CVMP notes that medicinal products containing pleuromutilins are being developed for treatment of various infections in humans, including methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA may be a zoonosis and thus the risk for spread of resistance from animals to humans is also to be considered. As detailed in the reflection paper on MRSA in food producing and companion animals in the EU (EMA/CVMP/SAGAM, 2009), the CVMP believes that animal associated MRSA is best addressed by promotion of responsible use of antimicrobials in general. Therefore all measures listed below would indirectly be effective in also reducing the risk for MRSA although they are primarily intended to reduce the risk for resistance in target animal pathogens.

For veterinary medicinal products containing pleuromutilins the CVMP concluded that the following recommendations are for consideration by Competent Authorities and applicants when considering new applications and post-authorisation procedures:

Pleuromutilins should only be used for treatment and metaphylaxis of disease³. The exception would be use in well-defined eradication programmes for swine dysentery and epizootic rabbit enterocolitis, that have been established by the veterinarian in conjunction with the farmer. Such eradication programmes should be restricted in time and include appropriate measures of effectiveness. This should be clearly stated in the SPCs for all products. Approved indications such as prevention of disease other than during eradication programmes should be withdrawn.

Indications should be worded to clearly express the intended use. General indications (e.g. those that do not include named target pathogens) should be avoided. Enhancement of production (e.g. increase of feed efficiency or growth promotion) is not regarded as an acceptable indication and should be withdrawn.

Duration of treatment should be limited to the time needed for cure of diseases. Summaries of Product Characteristics where the approved treatment duration is found unnecessarily long should be reviewed.

In July 2010, due to differences among the Summaries of Product Characteristics of nationally authorised for premixes containing tiamutin, the CVMP published an opinion on Tiamutin premix and associated names under article 34 of Directive 2001/82/EC recommending changes to SPCs in order to harmonise the indications for use, amount to be administered, administration route and withdrawal periods. Those recommendations should be applied to generic products.

² See

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/referrals/Tiamutin/vet_referral_000041.jsp&mid=WC0b01ac05805c5170

³ See revised the revision of the CVMP Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances

(http://www.ema.europa.eu/ema/doc_index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500143698&murl=menus/document_library/document_library.jsp&mid=0b01ac058009a3dc) for the definition of metaphylaxis

Additional comments

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.

Swine dysentery is a major disease in pig production in EU. Eradication programmes have successfully been applied in some countries in EU. CVMP would like to stress that such programmes are crucial to reduce the need for pleuromutilins. CVMP would therefore recommend responsible bodies to take further action against this disease. Noteworthy, control of spread of disease is crucial as resistant bacteria will spread with transport and trade of animals.

There is today no system in place to provide harmonised monitoring data for pleuromutilin resistance in *Brachyspira hyodysenteriae*. CVMP would recommend responsible bodies to create such a monitoring system, including sampling based on a standard protocol, for pleuromutilins and other relevant antimicrobials, first to allow baseline data to be collected and later to allow impact assessment of measures taken. Ideally, early warning systems should be created where treatment failures in case of swine dysentery is systematically monitored and related back to dose and duration of administration in the affected herd. Further exploration of differences in pleuromutilin use between EU countries and investigation of possible co-variability between use and resistance is recommended.

Table of contents

CVMP recommendations for action	1
1. Introduction	5
2. The use of pleuromutilins in veterinary medicine	5
3. Mechanisms and emergence of resistance in relevant bacteria	8
4. Problems of susceptibility testing.....	10
5. Occurrence of resistance in bacteria from food producing animals.....	11
6. Possible links between the use of pleuromutilins and other antimicrobials in animals and resistance in bacteria of animal origin.....	12
7. Impact on animal health and production	13
8. Potential impact on human health	13
9. Summary assessment.....	14
10. References	16

1. Introduction

Pleuromutilin is a natural antimicrobial substance produced by the fungus (basidiomycete) *Pleurotus mutilus* (Kavanagh et al., 1951) now called *Clitopilus scyphoides* (Singer, 1986). Tiamulin and valnemulin are semi-synthetic derivatives of pleuromutilin. Both tiamulin and valnemulin are used exclusively in veterinary medicine. Tiamulin was approved for use in veterinary medicine in 1979, followed by valnemulin in 1999 (Sader et al., 2012a). Retapamulin was the first pleuromutilin approved for topical use in humans in 2007 (Novak, 2011). BC-3781, a pleuromutilin for systemic use in humans, is currently under development (Sader et al., 2012a; Sader et al., 2012b).

Pleuromutilins are antibacterial agents that inhibit protein synthesis by binding to the 50S subunits of ribosomes of bacteria. Pleuromutilins are active against Gram-positive bacteria such as streptococci and staphylococci, anaerobic bacteria and *Mycoplasma* (Giguere, 2006; Islam et al., 2009; Jones et al., 2006). They have been used for decades in veterinary medicine for the control of respiratory and intestinal infections in different animal species, especially in pigs and to a lesser extent in poultry and rabbits (Giguere, 2006; Islam et al., 2009). The objective of this reflection paper is to summarize current knowledge on resistance development and the potential impact of this resistance on animal and human health as detailed in a concept paper

(http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500116978).

2. The use of pleuromutilins in veterinary medicine

In the Member States of the European Union tiamulin is authorised nationally and available in most of the EU Member States. Following a recent referral, the indications for tiamulin (EMA, 2010a) in pigs are treatment and prevention of swine dysentery (*Brachyspira hyodysenteriae*), treatment of colitis (*Brachyspira pilosicoli*), treatment of ileitis (*Lawsonia intracellularis*) and treatment of enzootic pneumonia (*Mycoplasma hyopneumoniae*). However as different products containing tiamulin are nationally approved other indications might still be listed. Tiamulin is also authorised for *Gallus gallus* (chickens) for the treatment and prevention of chronic respiratory disease (CRD) and airsacculitis caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and for turkeys for treatment and prevention of infectious sinusitis and airsacculitis caused by *Mycoplasma gallisepticum*, *Mycoplasma meleagridis* and *Mycoplasma synoviae* and for rabbits for treatment of epizootic rabbit enterocolitis. Valnemulin is authorised centrally for treatment and prevention of swine dysentery, spirocheatal diarrhoea and enzootic pneumonia and for treatment of clinical signs of porcine proliferative enteropathy⁴. Tiamulin is available as medicated feed premix, and oral solution and powder for medication in drinking water (Islam et al., 2009). In addition it is available as injectable for pigs. Valnemulin is available as premix for feed and oral powder⁵.

In some countries pleuromutilins are frequently used in the treatment of swine, especially in weaner pigs and finisher pigs (Jensen et al., 2012).

In pigs the dose of valnemulin varies between 1 and 12 mg/kg bw according to the indication and the duration of treatment can be related to clinical response and varies between 7 and 28 days (EMA, 2011). According to the authorisation for valnemulin, long term preventative use of valnemulin should

⁴ See

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/medicines/000042/vet_med_000116.jsp&mid=WC0b01ac058001fa1c

⁵ http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/veterinary/000042/WC500100721.pdf

be avoided by improving management practice and thorough cleansing and disinfection and consideration should be given to the eradication of infection from the farm.

In pigs the dose following the article 34 referral for the Tiamutin premix (EMA, 2010b) varies between 2 and 10 mg/kg bw according to the indication and the duration of treatment can be related to clinical response and varies between 7 and 28 days. According to the outcome of the referral (EMA, 2010b) preventive treatment with tiamulin should only be initiated after confirmed infection with *B. hyodysenteriae* and then as part of a programme including measures aiming to eradicate or control the infection in the herd. It is not known if such recommendation is included in the SPC for other products containing tiamulin.

There is limited data on the extent to which this advice on preventive use is followed; it is very important that such use is not undertaken without appropriate accompanying measures in order to minimise the emergence of resistance.

Data on trends in sales of antimicrobials 2005-2009 in nine countries were used to assess trends over time (EMA/ESVAC, 2011). Data for one country (Switzerland) was excluded as no data was available for 2005. The total sales of pleuromutilins expressed in tonnes active substance was divided by an estimate of the live weight of pigs expressed as mg per population correction unit (PCU). The PCU takes into account the estimated weight of livestock, slaughtered animals and transport of animals for fattening and slaughter in another Member State. It is probable that in most countries, most of the sales are for pigs but as pleuromutilins are also authorised for poultry, data are expressed both as mg/PCU pigs and as mg/PCU of pigs and poultry. The results shown in Figure 1 indicate an overall increasing trend in overall sales from 2005 to 2010 followed by a decrease in 2011.

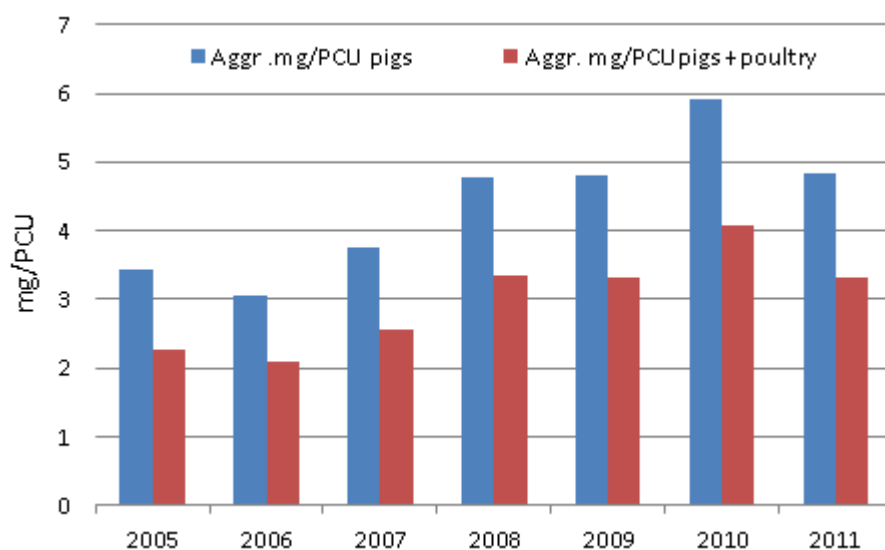


Figure 1. Total sales of pleuromutilins in eight countries expressed as mg/population correction unit (PCU) pigs and as mg/ PCU pig+poultry (EMA/ESVAC, 2011; EMA/ESVAC, 2013).

Data on sales of pleuromutilins in 25 countries in 2011 are shown in Figure 2 (based on data from (EMA/ESVAC, 2013)). As above, the sales in tonnes were calculated to mg/PCU pig and mg/PCU pig and poultry to represent an approximation of the exposure of the pig or pig and poultry population. Acknowledging that in some countries pleuromutilins will be used only for pigs while in others they are also used in poultry, the data still indicate that the population exposure varies widely between countries.

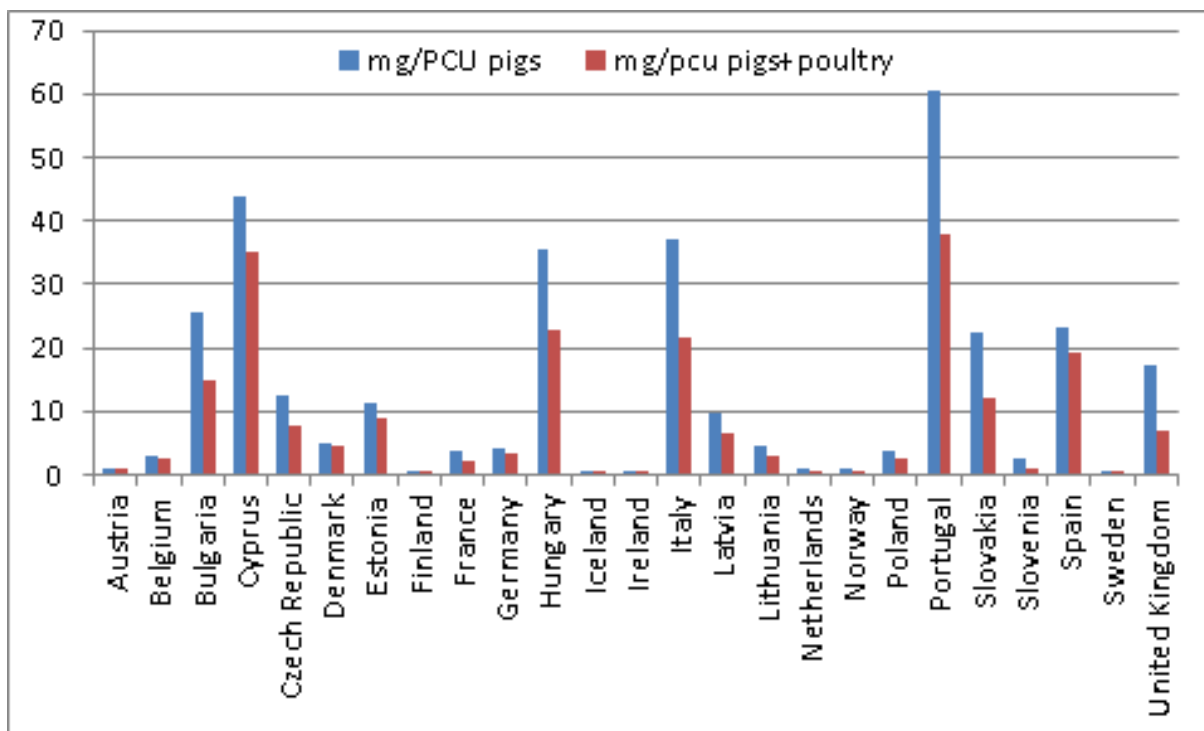


Figure 2. Sales of pleuromutilins expressed as mg/population correction unit (PCU) of pigs and of pigs and poultry (EMA/ESVAC, 2013).

Almost all of the sales are products formulated for in feed or in water medication (Figure 3), although the relative proportion between the different formulations varies between countries (Figure 4).

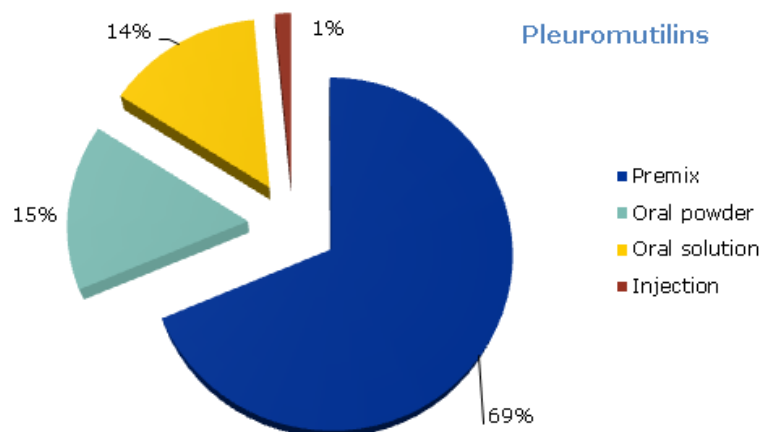


Figure 3. Sales of pleuromutilins in 2011 by pharmaceutical form in 25 countries expressed as percent of total sales in tonnes (EMA/ESVAC, 2013).

Figure 4. Distribution of sales by pharmaceutical form for pleuromutilins in 25 countries, in mg/PCU, for 2011 (EMA/ESVAC, 2013).

Sales of pleuromutilins in 25 countries, expressed as mg/PCU, were 2.5% of the total sales of antimicrobials to food producing animals (EMA/ESVAC, 2013).

3. Mechanisms and emergence of resistance in relevant bacteria

Pleuromutilins act by inhibiting protein synthesis by binding to the 50S subunit of the bacterial ribosome. They are strong inhibitors of peptidyl transferase. Resistance derives from chromosomal mutations in the 23S rRNA and *rpL3* genes. These chromosomal mutations emerge relatively slowly and in a stepwise fashion and are not transferred horizontally (Giguere, 2006). In addition, resistance genes can be located on plasmids or on transposons like the *vga* genes and the *cfr* gene (Long et al., 2006; Mendes et al., 2011; Novak, 2011; Schwendener and Perreten, 2011; Witte and Cuny, 2011). This type of resistance is transferable between bacteria and bacterial species. The mechanism of antimicrobial resistance varies according to the bacterial species investigated (Gentry et al., 2008; Long et al., 2006; Malbruny et al., 2011; Pringle et al., 2004; Wang et al., 2012a; Wang et al., 2012b; Wang et al., 2012c).

B. hyodysenteriae: The decreased susceptibility to tiamulin in *B. hyodysenteriae* clinical and in laboratory selected isolates has been associated to point mutations in the domain V of the 23S rRNA gene (positions 2032, 2055, 2447, 2499, 2504 and 2572 *Escherichia coli* numbering) and/or the ribosomal protein L3 gene (Hidalgo et al., 2011; Pringle et al., 2004). Hidalgo et al. (2011) has shown that one of the mutations, G2032A, that was present in the *B. hyodysenteriae* strain with the highest tiamulin MIC (>128 µg/ml) is associated with high tiamulin MICs in Spanish field isolates of *B. hyodysenteriae*, showing a connection between results from the *in vitro* study with clinical field isolates being exposed to tiamulin in the pig. Mutation in the nucleotide position 2032 seems to cause resistance to pleuromutilins as well as decreased susceptibility to lincosamides (Hidalgo et al., 2011). Tiamulin resistance in *B. hyodysenteriae* develops in a stepwise manner both *in vitro* and *in vivo* suggesting that multiple mutations are needed to achieve high level resistance (Karlsson et al., 2001; Karlsson et al., 2002). The MICs for valnemulin follow those for tiamulin in most cases but are generally a few dilution steps lower (Pringle et al., 2012). No resistance mechanism has yet been detected for *B. pilosicoli* (Pringle et al., 2012).

Staphylococcus species: Resistance in staphylococci can be due to point mutations in the domain V of 23S rRNA or in the *rpL3* gene encoding the ribosomal protein L3. Selected mutants of *S. aureus* resistant to linezolid exhibit cross-resistance to tiamulin (Gentry et al., 2007; Miller et al., 2008).

Transferable resistance in *S. aureus* and coagulase-negative staphylococci can be caused by *vga* genes, encoding ABC transporters, which export pleuromutilins, streptogramin A and lincosamides (Gentry et al., 2008; Hauschild et al., 2012). There are 7 known pleuromutilin-streptogramin resistance genes: *vga(A)*, *vga(A)v*, *vga(A)_{LC}*, *vga(B)*, *vga(C)*, *vga(D)* and *vga(E)* (Allignet and El Solh, 1997; Allignet et al., 1992; Jung et al., 2010; Kadlec and Schwarz, 2009; Schwendener and Perreten, 2011). Except for *vga(D)* which was found on a plasmid in *Enterococcus faecium* (Jung et al., 2010) all other genes were found on plasmids or transposons of staphylococci. Transferable resistance to pleuromutilins due to *vga* genes has been reported in methicillin-resistant *S. aureus* (MRSA) (Kadlec et al., 2010; Kadlec and Schwarz, 2009). Since 2005, a specific clone of MRSA, ST398 has emerged worldwide in livestock, especially swine (Catry et al., 2010; de Neeling et al., 2007). This clone is referred to as livestock-associated MRSA (LA-MRSA). Mendes, Smith et al. (2011) report the plasmid borne *vga (A)* gene in MRSA ST398 from a pig and a pig farmer in the United States. Kadlec and Schwarz (2009) identified a novel ABC transporter gene *vga(C)* which is located on the multidrug resistance plasmid pKKS825 in MRSA ST398. The *vga (A)v* gene has been detected in MRSA ST49 strains from pigs in Switzerland (Overesch et al., 2011). Porcine MRSA ST398 carrying small plasmids containing *vga(A)* or *vga(C)* genes have been identified in Portugal (Kadlec et al., 2010). Recently a new transposon Tn6133 containing *vga(E)* has been found in porcine MRSA ST398 isolates

(Schwendener and Perreten, 2011). The *vga(E)* gene, located on the same transposon, has also been detected in MRSA ST398 in clinical isolates from turkey and cattle as well as from chicken and turkey meat in Germany (Hauschild et al., 2012). This indicates that this resistance gene is disseminating in different countries and different animal species. Human *S. hominis* clinical isolates with low-level resistance to quinupristin/dalfopristin have also been shown to contain *vga(A)* (Petinaki et al., 2005).

Transferable resistance against five chemically distinct classes of antimicrobials (oxazolidinones, phenicols, streptogramin A, lincosamides and pleuromutilins) is mediated by the gene *cfr* encoding a rRNA methylase (Kehrenberg et al., 2007; Kehrenberg and Schwarz, 2006; Shore et al., 2010; Witte and Cuny, 2011). These antimicrobials bind to overlapping sites at the peptidyl transferase center. Each of these classes of antimicrobials contains important drugs that are used in human and veterinary medicine. This gene has been reported from several countries including Germany, Denmark, Spain, Ireland and China. It has been found in humans and animals, including pigs (Gopegui et al., 2012; Kehrenberg et al., 2009; Morales et al., 2010; Shore et al., 2010; Wang et al., 2012c). The gene was first detected on a plasmid originating from a bovine strain of the coagulase negative *Staphylococcus sciuri* (Schwarz et al., 2000) and has also been found in other coagulase-negative staphylococci (Kehrenberg et al., 2007; Kehrenberg and Schwarz, 2006). The *cfr* gene has also been found on a plasmid in porcine MRSA and MSSA of different clonal lineages (ST398 and ST9) (Kehrenberg et al., 2009). Recently the *cfr* gene has been detected in a Panton-Valentine-Leukocidin (PVL) positive MRSA of ST8 SCCmec type IV (USA300) (Shore et al., 2010). USA300 is a major community acquired MRSA causing skin and soft tissue infections in the United States of America and worldwide. A new multidrug resistance conjugative plasmid (pERGB) containing *cfr*, *tet(L)* (encoding tetracycline resistance), *ant(4)-Ia* (encoding tobramycin resistance) and *dfrK* (encoding trimethoprim resistance) was detected in a linezolid resistant MRSA strain with sequence type ST125. This MRSA strain was isolated from two patients with chronic obstructive pulmonary disease in Spain and both patients had been treated with linezolid (Gopegui et al., 2012). An outbreak of linezolid resistant *cfr*-positive MRSA has been reported in a Spanish hospital (Sanchez Garcia et al., 2010).

Recently, the enterococcal ABC transporter gene *lsa(E)* conferring resistance to pleuromutilins, lincosamides and streptogramin A has been detected in MSSA and MRSA (Wendlandt et al., 2012), suggesting exchange of this gene between *Enterococcus* spp. and *Staphylococcus aureus*.

E. coli: *Cfr*-mediated resistance has also been detected in *E. coli* (Long et al., 2006; Wang et al., 2012a). Analysis of 1230 *E. coli* isolates from pigs, ducks and chickens in China revealed one *cfr* positive isolate originating from a nasal swab of a pig. In addition to *cfr* these isolates also harboured the florfenicol resistance gene *floR* (Wang et al., 2012a).

Proteus vulgaris: The *cfr* gene has been reported in one *Proteus vulgaris*. This isolate was found when screening 557 nasal swabs of Chinese swine for florfenicol resistance (Wang et al., 2011). The isolate was also positive for the *floR* gene. To date, *cfr*-mediated resistance seems to be uncommon in *Enterobacteriaceae* such as *E. coli* and *Proteus vulgaris* (Wang et al., 2012a; Wang et al., 2011).

Enterococcus species: Liu et al. (2012a) and Liu et al. (2012b) reported the occurrence of the *cfr* gene in *Enterococcus faecalis* isolated from bovine and pig faeces in China. Insertion elements have been detected on a plasmid containing *cfr* and are thought to play an important role in the dissemination of resistance genes (Liu et al., 2012b). In *Enterococcus faecalis* resistance to pleuromutilins, streptogramin A and lincosamides is mediated by the *lsa(A)* gene (Wendlandt et al., 2012).

Bacillus species: The *cfr* gene located on a plasmid has been found in a *Bacillus* species isolated from a nasal swab of a pig in China (Wang et al., 2012b). The plasmid also carried a novel streptomycin resistance gene. Another *Bacillus* isolate, containing *cfr* and *erm(B)* conferring resistance to macrolides, lincosamides and streptogramin B located on a plasmid and *fexA* conferring resistance to

florfenicol located on the chromosomal DNA was found in an isolated from swine faeces in China (Dai et al., 2010).

Streptococcus agalactiae: Cross-resistance to pleuromutilins, lincosamides and streptogramin A has been found to be caused by a novel gene called *lsa(C)*. Expression of this gene in *S. agalactiae* led to increased minimal inhibitory concentrations (MICs) of lincomycin, clindamycin, dalbapristin, and tiamulin (Malbruny et al., 2011). The gene was found in 18 clinical isolates from humans in New Zealand (Malbruny et al., 2011).

Mycoplasma gallisepticum: Li et al. (2010a) studied the in vitro development of resistance to tiamulin and valnemulin in *Mycoplasma gallisepticum*. A single mutation of the 23S rRNA gene could cause elevated tiamulin and valnemulin MICs, but combinations of two or three mutations were necessary to produce high level resistance to these drugs. All mutants were cross-resistant to lincomycin, chloramphenicol and florfenicol and some mutants also to erythromycin, tilmicosin and tylosin (Li et al., 2010b).

Mycobacterium smegmatis: Long et al. (2010) found that single or double mutations at various locations in the 23S rRNA of *Mycobacterium smegmatis* resulted in unpredictable cross resistance between linezolid, chloramphenicol, clindamycin and valnemulin.

Data on resistance mechanisms of *Lawsonia intracellularis* are lacking.

4. Problems of susceptibility testing

Generally, accurate antimicrobial susceptibility testing of anaerobic, fastidious bacteria can be difficult to achieve. Different anaerobes require different supplements to the growth medium and this causes problems with standardisation of the methods. The fastidious nature of *B. hyodysenteriae* and *B. pilosicoli* has hampered standardisation of methods for antimicrobial susceptibility testing.

Antimicrobial susceptibility tests of *Brachyspira* spp. are not always performed on a routinely basis and there are no generally approved or recommended standards available. Different methods have been used such as broth dilution, microbroth dilution and agar dilution (Burch, 2005). Published susceptibility testing of *Brachyspira* spp. has been performed predominantly of *B. hyodysenteriae* isolates and by the agar dilution procedure. The most common medium used is trypticase soy agar (TSA) supplemented with 5% bovine or ovine blood. The MIC has been determined as the lowest concentration of the antimicrobial agent that prevents growth or hemolysis. A broth dilution method has been evaluated for monitoring of antimicrobial susceptibility in *Brachyspira* spp. (Karlsson et al., 2003). MIC quality control ranges for the type strain of *B. hyodysenteriae*, B78T (ATCC® 27164T), has been established in an inter-laboratory study for this method (Pringle et al., 2006a). For pleuromutilins, this method has been compared with agar dilution (Rohde et al., 2004). Both methods gave reproducible results, but the broth method on average gave one dilution lower MICs for both tiamulin and valnemulin.

Antimicrobial susceptibility testing of *Lawsonia intracellularis* is difficult as this obligate intracellular bacterium needs complicated cell culture systems to grow and published data on their in vitro susceptibility are scarce and include only a very limited number of isolates (McOrist et al., 1995; Wattanaphansak et al., 2009; Yeh et al., 2011).

Internationally accepted interpretative criteria are lacking except for tiamulin for *Actinobacillus* species (Clinical Laboratory Standards Institute (CLSI) 2012). To date, no tiamulin or valnemulin breakpoints have been established for *Brachyspira* species, but breakpoints of $\geq 2\mu\text{g/ml}$ (Clothier et al., 2011) have been used to classify isolates as resistant to tiamulin. According to Vyt and Hommez (2006) and

Karlsson et al. (2003) this breakpoint for tiamulin is too high to indicate decreased susceptibility. On the basis of a field survey on clinical efficacy it has been proposed that isolates with MICs $\geq 1\mu\text{g/ml}$ should be considered as not responding to therapy in vivo (Vyt and Hommez, 2006). Suggestion for interpretative criteria for tiamulin disk diffusion have been made for *Pasteurella multocida*, staphylococci, *Actinobacillus suis*, *Actinobacillus pleuropneumoniae* and *Erysipelothrix rhusiopathiae* (Jones et al., 2002). Burch (2005) suggested a breakpoint of $>0.125\mu\text{g/ml}$ for valnemulin (75 ppm in feed) using broth dilution and $>0.25\mu\text{g/ml}$ for the agar dilution method for *Brachyspira* species. For tiamulin (at a dose of 100 ppm) a breakpoint of $>0.5\mu\text{g/ml}$ and $>1.0\mu\text{g/ml}$ was suggested for broth dilution and agar dilution respectively (Burch, 2005). Pringle, Landen et al. (2012) suggest epidemiological cut-off values for monitoring antimicrobial susceptibility in *Brachyspira hyodysenteriae* of $>0.25\mu\text{g/ml}$ for tiamulin and $>0.125\mu\text{g/ml}$ for valnemulin.

5. Occurrence of resistance in bacteria from food producing animals

Brachyspira species: An increase in MIC's of tiamulin and valnemulin against *B. hyodysenteriae* has been reported in several countries. Reduced in vitro susceptibility of *B. hyodysenteriae* has been reported from Japan (Ohya and Sueyoshi, 2010), Spain (Hidalgo et al., 2011), The Netherlands (Duinhof et al., 2008), Germany (Rohde et al., 2004), Hungary (Molnar, 1996), the United Kingdom (Gresham et al., 1998) and Czech Republic (Lobova et al., 2004; Sperling et al., 2011). A study investigating 20 *Brachyspira intermedia* isolates from layers in Belgium and the Netherlands found that the MIC distribution was monomodal, but with tailing towards higher MIC values, possibly indicating low level acquired resistance in six isolates (Verlinden et al., 2011). Decreased susceptibility to tiamulin has also been found in *B. pilosicoli* isolates from Sweden (Pringle et al., 2006a) and in various *Brachyspira* spp. from the United States of America (Clothier et al., 2011). Seven out of 79 (4.7%) and 4/79 (3.2 %) *Brachyspira* isolates were resistant to tiamulin and valnemulin respectively applying a MIC $\geq 2\mu\text{g/ml}$ as breakpoint (Clothier et al., 2011). In Spain the susceptibility to tiamulin and valnemulin of *B. hyodysenteriae* decreased in 2008/2009 compared to previous years (Hidalgo et al., 2011). Resistance to pleuromutilins seems to be common in *B. hyodysenteriae* in Spain (Hidalgo et al., 2011). An increase in MICs was also seen in Japan where MICs for tiamulin and valnemulin were low and MIC distribution unimodal from 1985-2000, but higher MICs were recorded from 2001 onward and the distribution had a trend towards a bimodal distribution (Ohya and Sueyoshi, 2010). The MIC₉₀ of Czech *B. hyodysenteriae* isolates increased from $0.25\mu\text{g/ml}$ in 1997 to $4\mu\text{g/ml}$ in 2001 for tiamulin and from ≤ 0.031 in 1997 to 8 in 2001 for valnemulin (Lobova et al., 2004). In Germany MIC₉₀ for tiamulin increased from $0.125\mu\text{g/ml}$ (1989-1993) to 2-8 $\mu\text{g/ml}$ (2000-2002). For valnemulin the MIC₉₀ increased from $0.063\mu\text{g/ml}$ (1989-1993) to 2-4 $\mu\text{g/ml}$ (2000-2002) (Rohde et al., 2004).

Resistance to tiamulin has also been reported in *Haemophilus parasuis* and *Actinobacillus pleuropneumoniae* (Aarestrup et al., 2008). *S. aureus* and other staphylococci: A Canadian study found tiamulin MIC's to be significantly higher among MRSA ST398 than among human methicillin-susceptible *Staphylococcus aureus* and non-ST398 MRSA and porcine MSSA isolates (Rubin et al., 2011). Several studies have found *S. aureus* and MRSA isolates resistant to pleuromutilins (Gentry et al., 2008; Hauschild et al., 2012; Kadlec et al., 2009; Mendes et al., 2011). A high percentage of *S. aureus* isolates (40%) from slaughter pigs in Uruguay have been reported as resistant to tiamulin (Meyer et al., 2012). As described above, *cfr* and *vga* related transferable resistance in *S. aureus* including MRSA has been reported in different countries and different clonal lineages, including the livestock-associated MRSA ST398. In China, 149 staphylococcal isolates resistant to florfenicol were found when screening 557 pigs originating from 3 farms by taking nasal swabs. Of these isolates, 33 (22%) were found positive for *cfr* including *S. arlettae*, *S. saprophyticus*, *S. cohnii*, *S. sciuri* and *S. aureus*. Several

isolates contained the florfenicol resistance gene *fexA* in addition to *cfr*. Four different *cfr* carrying plasmids were identified and these plasmids sometimes also harboured other resistance genes such as *erm(C)* and *accA-aphD* (Wang et al., 2012c). Co-selection of *cfr* carrying isolates could therefore occur under selective pressure imposed by the use of florfenicol, aminoglycosides or macrolides.

Lawsonia intracellularis: In *Lawsonia intracellularis* no resistance to pleuromutilins has been reported, but only very few isolates have been investigated and accepted interpretative criteria for such susceptibility testing are lacking (McOrist et al., 1995; Wattanaphansak et al., 2009).

6. Possible links between the use of pleuromutilins and other antimicrobials in animals and resistance in bacteria of animal origin

Brachyspira species: The lack of authorised and effective drugs for the treatment of swine dysentery has increased the use of pleuromutilins, and this probably explains the emergence of resistant strains. In a Belgian study the MIC's of pleuromutilins for *B. hyodysenteriae* isolates from 17 farms were correlated with clinical efficacy of the drugs in the treatment of swine dysentery; 88% of the swine farms (n=15) that performed well were associated with susceptible isolates, whereas unfavourable clinical outcomes were associated with decreased susceptibility on two farrow-to-finish farms (Vyt and Hommez, 2006). In the Netherlands tiamulin resistant *B. hyodysenteriae* isolates were cultured from the faeces of pigs. The isolates were also resistant against lincomycin, tylosin, doxycycline, and tylvalosin. The repeated use of tiamulin on the affected farm was assumed to be the main cause of the development of resistance to the drug (Duinhof et al., 2008). Generally the use of pleuromutilins is high in Spain, Portugal and Czech Republic and relatively high percentages of *Brachyspira* isolates resistant to pleuromutilins have also been reported from Spain (Hidalgo et al., 2011) and Czech Republic (Lobova et al., 2004; Sperling et al., 2011). Multidrug-resistant and pleuromutilin-resistant *B. hyodysenteriae* isolates were associated with farms with endemic incidence of swine dysentery (Sperling et al., 2011). Increased consumption of pleuromutilins has been incriminated as cause for the increase in MICs of tiamulin and valnemulin (Lobova et al., 2004).

Staphylococci: It has been suggested that the use of pleuromutilins very likely selects for the emergence of *cfr* in animal isolates of staphylococci (Witte and Cuny, 2011). It must be noted that many isolates resistant to pleuromutilins are multidrug resistant. Mobile elements containing genes mediating resistance to pleuromutilins often contain resistance genes that confer resistance to other classes of antimicrobials. Therefore not only the use of pleuromutilins, but also the use of other antimicrobials can select for pleuromutilin resistance through co-selection. Plasmids carrying *vga(C)* genes have been found to contain the tetracycline resistance gene *tet(L)*, the kanamycin/neomycin resistance gene *aadD* and the trimethoprim resistance gene *dfpK* and therefore co-selection of *vga(C)* under selective pressure by the use of the other antimicrobials can potentially occur (Kadlec and Schwarz, 2009). Isolates harbouring the *vga(E)* gene were also resistant to beta-lactams, tetracyclines, trimethoprim, macrolides and lincosamides, spectinomycin and tiamulin and resistant or less susceptible to quinopristin/dalfopristin, which is used in human medicine to treat (severe) infections caused by MRSA and vancomycin resistant enterococci (Hauschild et al., 2012). Staphylococci carrying *cfr* were multidrug-resistant, resistance to erythromycin, tetracycline, spectinomycin, clindamycin and streptomycin being most common and three of six *cfr* positive isolates also carried the florfenicol resistance gene *fexA* (Kehrenberg and Schwarz, 2006).

Antibiotic usage records for Chinese pig farms indicate that multiple antimicrobial drugs, including florfenicol, lincomycin and tiamulin have been used on farms where *cfr* positive isolates have been found suggesting that selective pressure might have played a role (Wang et al., 2012b; Wang et al., 2011).

7. Impact on animal health and production

For most indications for which pleuromutilins are authorised there are alternative substances except for swine dysentery where high prevalence of resistance against alternative antimicrobials exists in many Member States. When resistance occurs to alternative antimicrobials, pleuromutilins are the only remaining treatment option for this indication. Thus impact of resistance to pleuromutilins on animal health and production is likely to be highest in the case of swine dysentery. In herds affected by this infection, the disease usually has a considerable impact on animal health as well as on production economy (Hampson et al., 2006; Wood and Lysons, 1988). Due to the lack of commercial vaccines, control and treatment of swine dysentery depends on the use of effective antimicrobial drugs. In most EU Member States there are no national programmes for control of swine dysentery.

Occurrence of resistance among *B. hyodysenteriae* to antimicrobial agents commonly used for treatment of swine dysentery such as macrolides (tylosin) and lincosamides is common (Hidalgo et al., 2011; Ohya and Sueyoshi, 2010; Sperling et al., 2011). Therefore the number of antimicrobials available for the treatment of swine dysentery is limited. Alternatives such as carbadox which is used in the United States of America, are not authorised in the EU. Thus, in many cases pleuromutilins are the only potentially effective choice among antimicrobials with swine dysentery as authorised indication. However, isolates with reduced susceptibility to pleuromutilins have emerged among *B. hyodysenteriae* in many countries (Duinhof et al., 2008; Hidalgo et al., 2011; Karlsson et al., 2004; Lobova et al., 2004; Ohya and Sueyoshi, 2010; Rohde et al., 2004; Sperling et al., 2011). Several of these reports document an increase in proportion of isolates with decreased susceptibility over time (Hidalgo et al., 2011; Ohya and Sueyoshi, 2010; Sperling et al., 2011), and in some cases therapy failure is described (Rohde et al., 2004; Sperling et al., 2011; Vyt and Hommez, 2006).

Lack of effective treatment options of swine dysentery would have considerable consequences for production economy due to mortality, impaired growth and secondary costs (Hampson et al., 2006). Depopulation of the farm and replacement of stock with non-infected animals may in such cases be the last resort measure (Hampson et al., 2006). To summarise, the loss of pleuromutilins as effective tools to treat swine dysentery because of a further increase in resistance or as a consequence of restrictions would present a considerable threat to the pig health, welfare and productivity.

8. Potential impact on human health

To date only one product containing pleuromutilins (retapamulin) is authorised for humans for topical use only. Concerns about lack of sufficient bioavailability, gastrointestinal side effects, hepatotoxicity, and the challenging side-chain chemistry of pleuromutilins labelled these drugs as difficult and hazardous to develop and several companies stopped their efforts in developing these drugs for human medicine (Novak, 2011). A new product, BC-3781, has been tested successfully during phase II trials for oral and intravenous administration to humans with serious multidrug-resistant skin infections and respiratory infections, including MRSA (Sader et al., 2012a; Sader et al., 2012b). Investigations exploring pleuromutilins for the treatment of *Mycobacterium tuberculosis* infections in humans are ongoing (Lotesta et al., 2011). Therefore, potential implications of the emergence of resistance to pleuromutilins in *S. aureus* and MRSA, including the livestock associated MRSA ST398 need to be considered. The emergence of successful epidemic clones of MRSA, like the PVL-positive ST8-IV/USA300 and ST125 carrying plasmids containing *cfr* is cause for concern and warrants close surveillance. PVL positive clones have not been reported in the EU pig population. Transfer of such plasmids between different bacteria and different hosts, including humans could potentially occur. The gene confers resistance to several important antimicrobials used in human medicine, such as oxazolidones and streptogramin A. Therefore, the emergence of this resistance gene in animals might

pose a threat to human medicine as they might compromise empirical treatment of human MRSA infections. To date linezolid resistance in *S. aureus* of human origin is still uncommon. Resistance to linezolid can be mediated by chromosomal mutations, but also through the acquisition of *cfr* by horizontal transfer (Stefani et al., 2010). An outbreak involving 12 patients with linezolid resistant MRSA has been reported in an intensive care unit in a Spanish hospital. Eleven of these patients had been treated with linezolid. In addition 3 patients in other wards were also infected with the linezolid resistant MRSA. All 15 isolates from the outbreak carried *cfr*. Six patients died and one death was directly attributed to the resistant MRSA (Morales et al., 2010; Sanchez Garcia et al., 2010). Contact with animals was not investigated. Retapamulin demonstrated excellent in vitro activity against MSSA and MRSA strains, but not against MRSA isolates harbouring the *cfr* gene (Candel et al., 2011).

Retapamulin MICs of ≥ 2 µg/ml were found in only 6 out of 5676 clinical *S. aureus* isolates. The ABC proteins Vga(Av) and Vga(A) were responsible for the reduced susceptibility to pleuromutilins in these six isolates (Gentry et al., 2008). Livestock-associated MRSA containing the plasmid-borne *vga(A)* gene has been reported from pigs and a pig farmer in the United States indicating that zoonotic transmission may occur (Mendes et al., 2011).

A special concern is the recent emergence of *cfr*-encoded plasmid-mediated linezolid resistance in *Enterococcus faecalis* and *Enterococcus faecium* in human clinical isolates in several countries, including Thailand (Diaz et al., 2012) and the United Kingdom (http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317135991530).

9. Summary assessment

Pleuromutilins are antimicrobial agents that are mainly used in veterinary medicine, especially in swine and to a lesser extent in poultry and rabbits. In pigs, tiamulin and valnemulin are used to treat swine dysentery, spirochaetal diarrhoea, porcine proliferative enteropathy, enzootic pneumonia and other infections where *Mycoplasma* is involved.

The vast majority of the sales of pleuromutilins comprise oral medication. Data on sales of pleuromutilins in different countries presented in figure 2 indicate that the amounts of pleuromutilins used vary markedly between countries. One possible explanation for this might be that in some countries, these substances are used more widely for treatment and prevention of not only swine dysentery but also porcine respiratory disease complex associated with *Mycoplasma spp.* and of infections with *Lawsonia intracellularis*. Other possible explanations might be differences in the prevalence of swine dysentery between the MSs and a high prevalence of resistance to alternative antimicrobials used to treat swine dysentery, e.g. the macrolides in countries with the highest use. A better understanding of the various factors explaining the observed differences would be valuable to support responsible use initiatives.

Decreased susceptibility of *B. hyodysenteriae* to pleuromutilins develops slowly and is caused by chromosomal mutations. The reported increase of the MICs of tiamulin and valnemulin against porcine *B. hyodysenteriae* isolates from different European countries is nevertheless alarming, as there is only a limited number of antimicrobials available for the treatment of swine dysentery and resistance to these antimicrobials is already very common. Considering that swine dysentery is a common and economically important disease a possible future loss of effective treatment could have considerable consequences for swine production.

Given the potential impact of resistance to pleuromutilins in *B. hyodysenteriae* on pig health, welfare and production, there is a need to include *B. hyodysenteriae* in national resistance monitoring programmes. Establishing approved standards for the methods used for susceptibility testing and

accepted criteria for the interpretation of the results could help to monitor the development of resistance.

Strategies to control or eradicate the infection from a herd or region could be implemented in order to reduce the continuous need for pleuromutilins on farms where swine dysentery is endemic. Such strategies rely on the supply of breeding animals that are certified free from *B. hyodysenteriae* and in most cases utilise strategic treatment with pleuromutilins for a limited period as part of the eradication protocol (Vyt et al., 2007; Vyt and Hommez, 2006). Successful programmes are in place in e.g. Sweden, Norway and Finland (Pringle et al., 2012). Another option to reduce the use of pleuromutilins would be to reserve this class of antimicrobials for the treatment of swine dysentery as alternative treatments for the other indications are available. Alternative strategies for the control of swine dysentery e.g. development of new antimicrobials, development of vaccines, increased hygiene and better management could be explored. Initiatives targeting responsible use of pleuromutilins could potentially reduce the risks associated with further emergence of resistance in *B. hyodysenteriae*.

To date the importance of pleuromutilins in human medicine is limited as only one product for topical treatment is authorised, but products for systemic use in humans with infections caused by multidrug-resistant bacteria are being developed. Therefore the importance of pleuromutilins for humans might increase in the future.

A special concern relating to human and veterinary medicine is the emergence of resistance to pleuromutilins in staphylococci (including MRSA) and enterococci, which can be located on mobile elements like plasmids and transposons and thus be horizontally transmitted (Kadlec et al., 2010; Kadlec and Schwarz, 2009; Witte and Cuny, 2011). A special concern are the *vga* genes conferring cross-resistance to pleuromutilins, streptogramin A and lincosamides and the *cfr* genes with an even broader spectrum conferring resistance to pleuromutilins, lincosamides, streptogramin A, phenicols and oxazolidinones.

Colonisation of animals with livestock-associated MRSA ST398 can lead to clinical infections in animals and zoonotic infections in humans and severe cases have been documented (Catry et al., 2010). The prevalence of MRSA in pigs is very high in many Member States (Catry et al., 2010; de Neeling et al., 2007) and in such situations there is a potential that the use of pleuromutilins for the treatment and prevention of other disease like swine dysentery further selects for pleuromutilin resistant staphylococci, including MRSA. The *vga* and *cfr* genes have been detected in isolates from humans and animals in many different countries (Gentry et al., 2008; Gopegui et al., 2012; Kadlec and Schwarz, 2009; Mendes et al., 2011; Shore et al., 2010; Witte and Cuny, 2011), and *cfr*-mediated resistance has been detected in several bacterial species, indicating inter-species and inter-genus transfer (Liu et al., 2012b; Long et al., 2006; Wang et al., 2012a; Wang et al., 2012b; Wang et al., 2011). Resistance selection and spread between animals and humans might jeopardise the efficacy of antimicrobial agents. The emergence of these resistance genes in animals poses a potential threat to human medicine as they might compromise empirical treatment of human MRSA infections. The use of linezolid or dalbavancin in humans may also select for resistant staphylococci and enterococci which might also be transmitted between humans, but also from humans to animals. As the pleuromutilin resistant isolates are often multidrug-resistant through the acquisition of genes *vga* and *cfr*, co-selection under selective pressure by numerous other antimicrobial agents in human and veterinary medicine may potentially occur. Nevertheless, resistance seems to be emerging and to further assess the situation, there is a need for the surveillance of bacteria especially staphylococci and enterococci from both animals and humans, for the presence of *vga* and/or *cfr* genes.

Co-selection for pleuromutilins with many different antimicrobials can potentially occur due to multidrug resistance genes and therefore prudent use of all antimicrobials in animals and humans is warranted.

10. References

- Aarestrup, F.M., C. Oliver Duran, and D.G. Burch. 2008. Antimicrobial resistance in swine production. *Animal health research reviews / Conference of Research Workers in Animal Diseases* 9:135-148.
- Allignet, J., and N. El Solh. 1997. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. *Gene* 202:133-138.
- Allignet, J., V. Loncle, and N. el Sohl. 1992. Sequence of a staphylococcal plasmid gene, *vga*, encoding a putative ATP-binding protein involved in resistance to virginiamycin A-like antibiotics. *Gene* 117:45-51.
- Burch, D.G.S. 2005. Pharmacokinetic, pharmacodynamic and clinical correlations relating to the therapy of colonic infections in the pig and breakpoint determinations. *The Pig Journal* 2005; 56: 8-24.
- Candel, F.J., G. Morales, and J.J. Picazo. 2011. In vitro activity of retapamulin against linezolid and methicillin-resistant *Staphylococcus aureus* isolates. *Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia* 24:127-130.
- Catry, B., E. Van Duijkeren, M.C. Pomba, C. Greko, M.A. Moreno, S. Pyorala, M. Ruzauskas, P. Sanders, E.J. Threlfall, F. Ungemach, K. Torneke, C. Munoz-Madero, and J. Torren-Edo. 2010. Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. *Epidemiol. Infect.* 138:626-644.
- Clothier, K.A., J.M. Kinyon, T.S. Frana, N. Naberhaus, L. Bower, E.L. Strait, and K. Schwartz. 2011. Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. *Journal of Veterinary Diagnostic Investigation* 23:1140-1145.
- Dai, L., C.M. Wu, M.G. Wang, Y. Wang, S.Y. Huang, L.N. Xia, B.B. Li, and J.Z. Shen. 2010. First report of the multidrug resistance gene *cfr* and the phenicol resistance gene *fexA* in a *Bacillus* strain from swine feces. *Antimicrob. Agents Chemother.* 54:3953-3955.
- de Neeling, A.J., M.J. van den Broek, E.C. Spalburg, M.G. van Santen-Verheuve, W.D. Dam-Deisz, H.C. Boshuizen, A.W. van de Giessen, E. van Duijkeren, and X.W. Huijsdens. 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* 122:366-372.
- Diaz, L., P. Kiratisin, R.E. Mendes, D. Panesso, K.V. Singh, and C.A. Arias. 2012. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* 56:3917-3922.
- Duinhof, T.F., C.M. Dierikx, M.G. Koene, M.A. van Bergen, D.J. Mevius, K.T. Veldman, H.M. van Beers-Schreurs, and R.T. de Winne. 2008. [Multiresistant *Brachyspira hyodysenteriae* in a Dutch sow herd]. *Tijdschrift voor diergeneeskunde* 133:604-608.
- EMA. 2010a. Opinion following an Article 34 referral for Tiamutin premix and associated names (EMA/118068/2010). In EMA web page.
- EMA. 2010b. Opinion following an Article 34(1) referral for Tiamutin premix and associated names. In.
- EMA. 2011. Econor. Valnemulin. Summary of product Characteristics. In.
- EMA/CVMP/SAGAM. 2009. Reflection paper on MRSA in food producing and companion animals in the European Union: epidemiology and control options for human and animal health (EMA/CVMP/SAGAM/68290/2009). In http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004311.pdf
- EMA/ESVAC. 2011. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. Trends in the sales of veterinary antimicrobial agents in nine European countries. Reporting period: 2005-2009. In European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf. 1-77.
- EMA/ESVAC. 2013. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011. Third ESVAC report. In European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Report/2013/10/WC500152311.pdf. 1-115.

- Gentry, D.R., L. McCloskey, M.N. Gwynn, S.F. Rittenhouse, N. Scangarella, R. Shawar, and D.J. Holmes. 2008. Genetic characterization of *Vga* ABC proteins conferring reduced susceptibility to pleuromutilins in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 52:4507-4509.
- Gentry, D.R., S.F. Rittenhouse, L. McCloskey, and D.J. Holmes. 2007. Stepwise exposure of *Staphylococcus aureus* to pleuromutilins is associated with stepwise acquisition of mutations in *rplC* and minimally affects susceptibility to retapamulin. *Antimicrob. Agents Chemother.* 51:2048-2052.
- Giguere, S. 2006. Lincosamides, pleuromutilins, and streptogramins. *Antimicrobial Therapy in Veterinary medicine*. Blackwell Publishing, Oxford.
- Gopegui, E.R., C. Juan, L. Zamorano, J.L. Perez, and A. Oliver. 2012. Transferable multidrug resistance plasmid carrying *cfr* associated with *tet(L)*, *ant(4')-Ia*, and *dfxK* genes from a clinical methicillin-resistant *Staphylococcus aureus* ST125 strain. *Antimicrob. Agents Chemother.* 56:2139-2142.
- Gresham, A.C., B.W. Hunt, and R.W. Dalziel. 1998. Treatment of swine dysentery--problems of antibiotic resistance and concurrent salmonellosis. *The Veterinary Record* 143:619.
- Hampson, D.J., C. Fellström, and J.R. Thomson. 2006. Swine Dysentery. Blackwell Publishing, Oxford. 785-805 pp.
- Hauschild, T., A.T. Fessler, K. Kadlec, C. Billerbeck, and S. Schwarz. 2012. Detection of the novel *vga(E)* gene in methicillin-resistant *Staphylococcus aureus* CC398 isolates from cattle and poultry. *J. Antimicrob. Chemother.* 67:503-504.
- Hidalgo, A., A. Carvajal, B. Vester, M. Pringle, G. Naharro, and P. Rubio. 2011. Trends towards lower antimicrobial susceptibility and characterization of acquired resistance among clinical isolates of *Brachyspira hyodysenteriae* in Spain. *Antimicrobial agents and chemotherapy* 55:3330-3337.
- Islam, K.M., U. Klein, and D.G. Burch. 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine--A review. *Poult. Sci.* 88:2353-2359.
- Jensen, V.F., H.D. Emborg, and F.M. Aarestrup. 2012. Indications and patterns of therapeutic use of antimicrobial agents in the Danish pig production from 2002 to 2008. *J. Vet. Pharmacol. Ther.* 35:33-46.
- Jones, R.N., T.R. Fritsche, H.S. Sader, and J.E. Ross. 2006. Activity of retapamulin (SB-275833), a novel pleuromutilin, against selected resistant gram-positive cocci. *Antimicrob. Agents Chemother.* 50:2583-2586.
- Jones, R.N., M.A. Pfaller, P.R. Rhomberg, and D.H. Walter. 2002. Tiamulin activity against fastidious and nonfastidious veterinary and human bacterial isolates: initial development of in vitro susceptibility test methods. *J. Clin. Microbiol.* 40:461-465.
- Jung, Y.H., E.S. Shin, O. Kim, J.S. Yoo, K.M. Lee, J.I. Yoo, G.T. Chung, and Y.S. Lee. 2010. Characterization of two newly identified genes, *vgaD* and *vatH*, [corrected] conferring resistance to streptogramin A in *Enterococcus faecium*. *Antimicrob. Agents Chemother.* 54:4744-4749.
- Kadlec, K., R. Ehrlich, S. Monecke, U. Steinacker, H. Kaspar, J. Mankertz, and S. Schwarz. 2009. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. *J. Antimicrob. Chemother.* 64:1156-1164.
- Kadlec, K., C.F. Pomba, N. Couto, and S. Schwarz. 2010. Small plasmids carrying *vga(A)* or *vga(C)* genes mediate resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-resistant *Staphylococcus aureus* ST398 from swine. *J. Antimicrob. Chemother.* 65:2692-2693.
- Kadlec, K., and S. Schwarz. 2009. Novel ABC transporter gene, *vga(C)*, located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrobial agents and chemotherapy* 53:3589-3591.
- Karlsson, M., C. Fellstrom, A. Gunnarsson, A. Landen, and A. Franklin. 2003. Antimicrobial susceptibility testing of porcine *Brachyspira* (*Serpulina*) species isolates. *J. Clin. Microbiol.* 41:2596-2604.
- Karlsson, M., C. Fellstrom, K.E. Johansson, and A. Franklin. 2004. Antimicrobial resistance in *Brachyspira pilosicoli* with special reference to point mutations in the 23S rRNA gene associated with macrolide and lincosamide resistance. *Microbial Drug Resistance* 10:204-208.
- Karlsson, M., A. Gunnarsson, and A. Franklin. 2001. Susceptibility to pleuromutilins in *Brachyspira* (*Serpulina*) *hyodysenteriae*. *Animal health research reviews / Conference of Research Workers in Animal Diseases* 2:59-65.

- Karlsson, M., J. Rohde, M. Kessler, and A. Franklin. 2002. Decreased susceptibility to tiamulin in German isolates of *Brachyspira hyodysenteriae*. *Proceedings of the 17th International Pig Veterinary Society Congress* Ames, IA, USA:189.
- Kavanagh, F., A. Hervey, and W.J. Robbins. 1951. Antibiotic Substances From Basidiomycetes: VIII. *Pleurotus Multilus* (Fr.) Sacc. and *Pleurotus Passeckerianus* Pilat. *Proceedings of the National Academy of Sciences of the United States of America* 37:570-574.
- Kehrenberg, C., F.M. Aarestrup, and S. Schwarz. 2007. IS21-558 insertion sequences are involved in the mobility of the multiresistance gene *cfr*. *Antimicrob. Agents Chemother.* 51:483-487.
- Kehrenberg, C., C. Cuny, B. Strommenger, S. Schwarz, and W. Witte. 2009. Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr*. *Antimicrob. Agents Chemother.* 53:779-781.
- Kehrenberg, C., and S. Schwarz. 2006. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob. Agents Chemother.* 50:1156-1163.
- Li, B.B., J.Z. Shen, X.Y. Cao, Y. Wang, L. Dai, S.Y. Huang, and C.M. Wu. 2010a. Mutations in 23S rRNA gene associated with decreased susceptibility to tiamulin and valnemulin in *Mycoplasma gallisepticum*. *FEMS Microbiol. Lett.* 308:144-149.
- Li, B.B., J.Z. Shen, X.Y. Cao, Y. Wang, L. Dai, S.Y. Huang, and C.M. Wu. 2010b. Mutations in 23S rRNA gene associated with decreased susceptibility to tiamulin and valnemulin in *Mycoplasma gallisepticum*. *FEMS Microbiol Lett* 308:144-149.
- Liu, Y., Y. Wang, S. Schwarz, Y. Li, Z. Shen, Q. Zhang, C. Wu, and J. Shen. 2012a. Transferable Multiresistance Plasmids Carrying *cfr* in *Enterococcus* spp. from Swine and Farm Environment. *Antimicrobial agents and chemotherapy*
- Liu, Y., Y. Wang, C. Wu, Z. Shen, S. Schwarz, X.D. Du, L. Dai, W. Zhang, Q. Zhang, and J. Shen. 2012b. First report of the multidrug resistance gene *cfr* in *Enterococcus faecalis* of animal origin. *Antimicrob. Agents Chemother.* 56:1650-1654.
- Lobova, D., J. Smola, and A. Cizek. 2004. Decreased susceptibility to tiamulin and valnemulin among Czech isolates of *Brachyspira hyodysenteriae*. *Journal of medical microbiology* 53:287-291.
- Long, K.S., C. Munck, T.M. Andersen, M.A. Schaub, S.N. Hobbie, E.C. Bottger, and B. Vester. 2010. Mutations in 23S rRNA at the peptidyl transferase center and their relationship to linezolid binding and cross-resistance. *Antimicrob. Agents Chemother.* 54:4705-4713.
- Long, K.S., J. Poehlsgaard, C. Kehrenberg, S. Schwarz, and B. Vester. 2006. The *Cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob. Agents Chemother.* 50:2500-2505.
- Lotesta, S.D., J. Liu, E.V. Yates, I. Krieger, J.C. Sacchettini, J.S. Freundlich, and E.J. Sorensen. 2011. Expanding the pleuromutilin class of antibiotics by de novo chemical synthesis. *Chem Sci* 2:1258-1261.
- Malbruny, B., A.M. Werno, D.R. Murdoch, R. Leclercq, and V. Cattoir. 2011. Cross-resistance to lincosamides, streptogramins A, and pleuromutilins due to the *Isa(C)* gene in *Streptococcus agalactiae* UCN70. *Antimicrob. Agents Chemother.* 55:1470-1474.
- McOrist, S., R.A. Mackie, and G.H.K. Lawson. 1995. Antimicrobial Susceptibility of *Iliont Symbiont Intracellularis* isolated from pigs with proliferative enteropathy. *J. Clin. Microbiol.* 1995 1314-1317.
- Mendes, R.E., T.C. Smith, L. Deshpande, D.J. Diekema, H.S. Sader, and R.N. Jones. 2011. Plasmid-borne *vga(A)*-encoding gene in methicillin-resistant *Staphylococcus aureus* ST398 recovered from swine and a swine farmer in the United States. *Diagn. Microbiol. Infect. Dis.* 71:177-180.
- Meyer, C., M. Fredriksson-Ahomaa, E. Stuber, S. Thiel, and E. Martlbauer. 2012. High frequency of multiresistant coagulase-positive *Staphylococcus aureus* found in slaughter pigs in Uruguay. *Foodborne pathogens and disease* 9:86-90.
- Miller, K., C.J. Dunsmore, C.W. Fishwick, and I. Chopra. 2008. Linezolid and tiamulin cross-resistance in *Staphylococcus aureus* mediated by point mutations in the peptidyl transferase center. *Antimicrob. Agents Chemother.* 52:1737-1742.
- Molnar, L. 1996. Sensitivity of strains of *Serpulina hyodysenteriae* isolated in Hungary to chemotherapeutic drugs. *Vet. Rec.* 138:158-160.
- Morales, G., J.J. Picazo, E. Baos, F.J. Candel, A. Arribi, B. Pelaez, R. Andrade, M.A. de la Torre, J. Fereres, and M. Sanchez-Garcia. 2010. Resistance to linezolid is mediated by the *cfr* gene in

- the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.* 50:821-825.
- Novak, R. 2011. Are pleuromutilin antibiotics finally fit for human use? *Ann. N. Y. Acad. Sci.* 1241:71-81.
- Ohya, T., and M. Sueyoshi. 2010. In vitro antimicrobial susceptibility of *Brachyspira hyodysenteriae* strains isolated in Japan from 1985 to 2009. *J. Vet. Med. Sci.* 72:1651-1653.
- Overesch, G., S. Buttner, A. Rossano, and V. Perreten. 2011. The increase of methicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type ST49 in slaughter pigs in Switzerland. *BMC veterinary research* 7:30.
- Petinaki, E., I. Spiliopoulou, M. Maniati, and A.N. Maniatis. 2005. Emergence of *Staphylococcus hominis* strains expressing low-level resistance to quinupristin/dalfopristin in Greece. *The Journal of antimicrobial chemotherapy* 55:811-812.
- Pringle, M., F.M. Aarestrup, B. Bergsjö, M. Fossi, E. Jouy, A. Landen, D. Mevius, K. Perry, C. Teale, J. Thomson, T. Skrzypczak, K. Veldman, and A. Franklin. 2006a. Quality-control ranges for antimicrobial susceptibility testing by broth dilution of the *Brachyspira hyodysenteriae* type strain (ATCC 27164T). *Microbial Drug Resistance* 12:219-221.
- Pringle, M., A. Landen, H.E. Unnerstad, B. Molander, and B. Bengtsson. 2012. Antimicrobial susceptibility of porcine *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* isolated in Sweden between 1990 and 2010. *Acta Veterinaria Scandinavica* 54:54.
- Pringle, M., J. Poehlsgaard, B. Vester, and K.S. Long. 2004. Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in *Brachyspira* spp. isolates. *Mol. Microbiol.* 54:1295-1306.
- Rohde, J., M. Kessler, C.G. Baums, and G. Amsberg. 2004. Comparison of methods for antimicrobial susceptibility testing and MIC values for pleuromutilin drugs for *Brachyspira hyodysenteriae* isolated in Germany. *Vet. Microbiol.* 102:25-32.
- Rubin, J.E., K.R. Ball, and M. Chirino-Trejo. 2011. Decreased susceptibility of MRSA ST398 to tiamulin. *Vet. Microbiol.* 151:422-423.
- Sader, H.S., D.J. Biedenbach, S. Paukner, Z. Ivezic-Schoenfeld, and R.N. Jones. 2012a. Antimicrobial activity of the investigational pleuromutilin compound BC-3781 tested against Gram-positive organisms commonly associated with acute bacterial skin and skin structure infections. *Antimicrob. Agents Chemother.* 56:1619-1623.
- Sader, H.S., S. Paukner, Z. Ivezic-Schoenfeld, D.J. Biedenbach, F.J. Schmitz, and R.N. Jones. 2012b. Antimicrobial activity of the novel pleuromutilin antibiotic BC-3781 against organisms responsible for community-acquired respiratory tract infections (CARTIs). *J. Antimicrob. Chemother.* 67:1170-1175.
- Sanchez Garcia, M., M.A. De la Torre, G. Morales, B. Pelaez, M.J. Tolon, S. Domingo, F.J. Candel, R. Andrade, A. Arribi, N. Garcia, F. Martinez Sagasti, J. Fereres, and J. Picazo. 2010. Clinical outbreak of linezolid-resistant *Staphylococcus aureus* in an intensive care unit. *JAMA : the journal of the American Medical Association* 303:2260-2264.
- Schwarz, S., C. Werckenthin, and C. Kehrenberg. 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrob. Agents Chemother.* 44:2530-2533.
- Schwendener, S., and V. Perreten. 2011. New transposon Tn6133 in methicillin-resistant *Staphylococcus aureus* ST398 contains *vga* (E), a novel streptogramin A, pleuromutilin, and lincosamide resistance gene. *Antimicrobial agents and chemotherapy* 55:4900-4904.
- Shore, A.C., O.M. Brennan, R. Ehricht, S. Monecke, S. Schwarz, P. Slickers, and D.C. Coleman. 2010. Identification and characterization of the multidrug resistance gene *cfr* in a Pantone-Valentine leukocidin-positive sequence type 8 methicillin-resistant *Staphylococcus aureus* IVa (USA300) isolate. *Antimicrob. Agents Chemother.* 54:4978-4984.
- Singer, R. 1986. The agaricales in Modern Taxonomy.
- Sperling, D., J. Smola, and A. Cizek. 2011. Characterisation of multiresistant *Brachyspira hyodysenteriae* isolates from Czech pig farms. *Vet. Rec.* 168:215.
- Stefani, S., D. Bongiorno, G. Mongelli, and F. Campanile. 2010. Linezolid resistance in staphylococci. *Pharmaceuticals* 3:1988-2006.
- Verlinden, M., F. Boyen, F. Pasmans, A. Garmyn, F. Haesebrouck, and A. Martel. 2011. Antimicrobial susceptibility pattern of *Brachyspira intermedia* isolates from European layers. *Microb Drug Resist* 17:485-488.

- Vyt, P., P. Heylen, M. Neven, and F. Castryck. 2007. A Practical approach to the elimination of swine dysentery (*Brachyspira hyodysenteriae*) from single-site, farrow-to-finish herds. . *Vlaams Diergeneek. Tijdschr.* 76:124-129.
- Vyt, P., and J. Hommez. 2006. Antimicrobial susceptibility of *Brachyspira hyodysenteriae* isolates compared with the clinical effect of treatment. *Vlaams Dier ge nees kun dig Tijd schrift*, 75:279-285.
- Wang, Y., T. He, S. Schwarz, D. Zhou, Z. Shen, C. Wu, L. Ma, Q. Zhang, and J. Shen. 2012a. Detection of the staphylococcal multiresistance gene *cfr* in *Escherichia coli* of domestic-animal origin. *J. Antimicrob. Chemother.* 67:1094-1098.
- Wang, Y., S. Schwarz, Z. Shen, W. Zhang, J. Qi, Y. Liu, T. He, J. Shen, and C. Wu. 2012b. Co-location of the multiresistance gene *cfr* and the novel streptomycin resistance gene *aadY* on a small plasmid in a porcine *Bacillus* strain. *J. Antimicrob. Chemother.* 67:1547-1549.
- Wang, Y., C.M. Wu, S. Schwarz, Z. Shen, W. Zhang, Q. Zhang, and J.Z. Shen. 2011. Detection of the staphylococcal multiresistance gene *cfr* in *Proteus vulgaris* of food animal origin. *J. Antimicrob. Chemother.* 66:2521-2526.
- Wang, Y., W. Zhang, J. Wang, C. Wu, Z. Shen, X. Fu, Y. Yan, Q. Zhang, S. Schwarz, and J. Shen. 2012c. Distribution of the multidrug resistance gene *cfr* in *Staphylococcus* species isolates from swine farms in China. *Antimicrob. Agents Chemother.* 56:1485-1490.
- Wattanaphansak, S., R.S. Singer, and C.J. Gebhart. 2009. In vitro antimicrobial activity against 10 North American and European *Lawsonia intracellularis* isolates. *Veterinary microbiology* 134:305-310.
- Wendlandt, S., C. Lozano, K. Kadlec, E. Gomez-Sanz, M. Zarazaga, C. Torres, and S. Schwarz. 2012. The enterococcal ABC transporter gene *Isa(E)* confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.*
- Witte, W., and C. Cuny. 2011. Emergence and spread of *cfr*-mediated multiresistance in staphylococci: an interdisciplinary challenge. *Future microbiology* 6:925-931.
- Wood, E.N., and R.J. Lysons. 1988. Financial benefit from the eradication of swine dysentery. *Vet. Rec.* 122:277-279.
- Yeh, J.Y., J.H. Lee, H.R. Yeh, A. Kim, J.Y. Lee, J.M. Hwang, B.K. Kang, J.M. Kim, I.S. Choi, and J.B. Lee. 2011. Antimicrobial susceptibility testing of two *Lawsonia intracellularis* isolates associated with proliferative hemorrhagic enteropathy and porcine intestinal adenomatosis in South Korea. *Antimicrob. Agents Chemother.* 55:4451-4453.