Pleuromutins: use in food-producing animals in the European Union, development of resistance and impact on human and animal health

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Introduction

Pleuromutilin is a natural antimicrobial substance produced by the fungus Pleurotus mutilus, now called Clitopilus scyphoides. Tiamulin and valnemulin are semi-synthetic derivatives of pleuromutilin and both drugs are used exclusively in veterinary medicine. Tiamulin was approved for use in veterinary medicine in 1979, followed by valnemulin in 1999. Retapamulin was the first pleuromutilin approved for topical use for humans, in 2007. A pleuromutilin for systemic use in humans, BC-3781, is currently under development. Pleuromutins are antibacterial agents that inhibit protein synthesis. They are active against Gram-positive bacteria such as streptococci and staphylococci, anaerobic bacteria and mycoplasmata; 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.

Use of pleuromutins in veterinary medicine

Tiamulin is authorized nationally in the member states of the European Union (EU) and is available in most EU member states. Following a recent referral, tiamulin is indicated in pigs for the 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). Other indications might still be listed, as different products containing tiamulin are nationally approved. Tiamulin is also authorized for chickens for the treatment and prevention of chronic respiratory disease and airsacculitis caused by Mycoplasma gallisepticum and Mycoplasma synoviae; for turkeys for the treatment and prevention of infectious sinusitis and airsacculitis caused by M. gallisepticum, Mycoplasma meleagridis and M. synoviae; and for rabbits for the treatment of epizootic rabbit enterocolitis. Valnemulin is...
authorized centrally for the treatment and prevention of swine dysentery, spirochaete-associated diarrhoea, enzootic pneumonia and for the treatment of clinical signs of porcine proliferative enteropathy.\textsuperscript{10} Valnemulin is also licensed for the treatment of epizootic rabbit enteropathy.\textsuperscript{11} Tiamulin is available as an oral solution, a powder for medication in drinking water, medicated feed premixes and as an injectable formulation for pigs and valnemulin is available as oral powder and premixes for feed.\textsuperscript{6,10,12}

In some countries pleuromutilins are used frequently in the treatment of swine, especially in weaner pigs and finisher pigs.\textsuperscript{13} For pigs, the dose of valnemulin varies between 1 and 12 mg/kg, depending on the indication and the duration of treatment, but can be related to clinical responses and varies between 7 and 28 days.\textsuperscript{11} According to the authorization for valnemulin, long-term preventative use should be avoided by improving management practice and thorough cleansing and disinfection; consideration should also be given to the eradication of infection from the farm.

For pigs, the dose recommendation following the EU article 34 referral for the Tiamulin premix varies between 2 and 10 mg/kg depending on indication and the duration of treatment, but can be related to clinical responses and varies between 7 and 28 days.\textsuperscript{14} According to the outcome of the referral, preventive treatment with tiamulin should only be initiated after confirmed infection with \textit{B. hyodysenteriae} and then as part of a programme including measures aiming to eradicate or control the infection within the herd.\textsuperscript{14} It is not known whether such recommendations are included in the Summary of Product Characteristics for all products containing tiamulin. There are limited data on the extent to which this advice on preventive use is followed; e.g. it is very important that such use is not undertaken without appropriate accompanying measures in order to minimize the emergence of resistance.

Initially, data on the trends in EU sales of antimicrobials during 2005–09 in nine countries were used to assess trends over time.\textsuperscript{9} Data for one country (Switzerland) were excluded as no data were available for 2005. In the second European Surveillance on Veterinary Antimicrobial Consumption (ESVAC) report,\textsuperscript{15} data for 2010 and 2011 were included for 19 countries. The total sales of pleuromutilins expressed in tonnes of active substance were divided by an estimate of the live weight of pigs expressed as milligrams 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 since pleuromutilins are also authorized for poultry, data are expressed both as mg/PCU of 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 slight decrease in 2011. Data on sales of pleuromutilins in 25 countries in 2011 are shown in Figure 2, based on data from ESVAC.\textsuperscript{15–17} As above, the sales in tonnes were converted into mg/PCU of pigs and mg/PCU of pigs and poultry to represent an approximation of the exposure to 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. Almost all of the sales are products formulated for in-feed or in-water medications, although the relative proportions of the different formulations vary between countries (Figure 3).

Mechanisms 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 \textit{rplC} genes. These chromosomal mutations emerge relatively slowly and in a stepwise fashion and are not transferred horizontally.\textsuperscript{7} In addition, resistance genes can be located on plasmids or transposons like the \textit{vga} genes and the \textit{cfr} gene.\textsuperscript{6,16–21} This type of resistance is transferable between bacteria and bacterial species. The mechanism of antimicrobial resistance varies according to the bacterial species investigated.\textsuperscript{18,22–27}

Target pathogens

\textbf{\textit{B. hyodysenteriae}}

The decreased susceptibility to tiamulin of clinical and laboratory-selected \textit{B. hyodysenteriae} isolates has been associated with point mutations in the \textit{V} domain of the 23S rRNA (positions 2032, 2055, 2447, 2499, 2504 and 2572 in \textit{Escherichia coli} numbering) and/or the ribosomal protein L3 gene.\textsuperscript{22,28} Hidalgo et al.\textsuperscript{28} reported that one of the mutations, G2032A, was present in Spanish field isolates of the \textit{B. hyodysenteriae} strain with the highest tiamulin MIC (>128 mg/L), showing an association of the results of the \textit{in vitro} study with exposure of clinical field isolates to tiamulin in pig herds. Mutation at nucleotide position 2032 appears to be related to pleuromutilin resistance as well as decreased susceptibility to lincosamides.\textsuperscript{28} Tiamulin resistance in \textit{B. hyodysenteriae} develops in a stepwise manner both \textit{in vitro} and \textit{in vivo}, suggesting that multiple mutations are needed to achieve high levels of resistance.\textsuperscript{28,30} In general, the MICs of valnemulin follow those of tiamulin in most cases but are generally a few dilution steps lower.\textsuperscript{31} No resistance mechanism has yet been detected for \textit{B. pilosicoli}.\textsuperscript{31}

\textbf{\textit{M. gallisepticum}}

Data on resistance mechanisms of mycoplasma are scarce. Li et al.\textsuperscript{32} studied the \textit{in vitro} development of resistance to tiamulin and valnemulin in \textit{M. 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 levels of resistance to these drugs. All mutants were cross-resistant to lincomycin, chloramphenicol and florfenicol and some mutants also to erythromycin, tilmicosin and tylosin.32

**L. intracellularis**

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

**Non-target bacteria**

*Staphylococcus spp.*

Resistance in staphylococci can be due to point mutations in the V domain of 23S rRNA or in the *rplC* gene, encoding the ribosomal protein L3. Selected mutants of *Staphylococcus aureus* that are resistant to linezolid also exhibit cross-resistance to tiamulin.33,34 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.27,35 Initially, *vga* genes were reported to confer resistance to streptogramin A only, but subsequent investigations described *vga*(A) variants that conferred reduced susceptibility or resistance to lincosamides and/or pleuromutilin agents.27 There are five known *vga* genes and three variants: *vga*(A) and its two variants *vga*(A)v (which is not a designation approved by the MLS nomenclature centre) and *vga*(A)LC, *vga*(B), *vga*(C), *vga*(D), *vga*(E) and its variant *vga*(E).21,36–40 Except for *vga*(D), which was found on a plasmid in *Enterococcus faecium*, all other genes were found on plasmids or transposons of staphylococci.39 Transferable resistance to pleuromutilins due to *vga* genes has been reported in methicillin-resistant *S. aureus* (MRSA)38,41 Since
2005, a specific clone of MRSA, ST398 (where ST stands for sequence type), has emerged worldwide in livestock, especially swine.\(^{42,43}\) This clone is referred to as livestock-associated MRSA (LA-MRSA). Mendes et al.\(^{19}\) reported the plasmid-borne vga(A) gene in MRSA ST398 from a pig and swine farmer in the USA. The vga(A) gene has also been identified in MRSA ST398 isolates from broilers in the Netherlands.\(^{44}\) Kadlec and Schwarz\(^ {18}\) identified a novel ABC transporter gene vga(C) that is located on the multidrug resistance plasmid pKKS825 in a clinical porcine MRSA ST398 isolated in Germany. Porcine MRSA ST398 carrying small plasmids containing vga(A) or vga(C) genes have been identified in Portugal.\(^ {41}\) This study has suggested the possible exchange of a vga(A)-carrying plasmid between humans and pigs in Portugal based on the close structural relatedness of the plasmids (pVGA and pCP321) of the human and porcine MRSA isolates.\(^ {51}\) The vga(A) gene has been detected in MRSA ST49 strains from pigs in Switzerland.\(^ {45}\) Recently a new transposon, Tn6133, containing vga(E) has been found in porcine MRSA ST398 isolates from Switzerland.\(^ {21}\) The vga(E) gene, located on the same transposon, has also been detected in MRSA ST398 in clinical isolates from turkeys and cattle as well as from chicken and turkey meat in Germany.\(^ {35}\) This indicates that this resistance gene is disseminating in different countries and different animal species. A novel vga(E)-variant conferring resistance to pleuromutilins, lincomamides and streptogramin B has been detected in two porcine coagulase-negative staphylococcal isolates, a Staphylococcus simulans and a Staphylococcus cohnii isolate, from China.\(^ {40}\)

Recently, the enterococcal ABC transporter gene isa(E), conferring resistance to pleuromutilins, lincomamides and streptogramin A, has been detected in methicillin-susceptible Staphylococcus aureus (MSSA) and MRSA, suggesting exchange of this gene between Enterococcus spp. and S. aureus.\(^ {46}\) Transferable resistance to five chemically distinct classes of antimicrobials (phenicols, lincomamides, oxazolidinones, pleuromutilins, streptogramin A; the PhLOPS\(_\text{A}\) phenotype) is mediated by the gene cfr, encoding an rRNA methylase.\(^ {47,49,51}\) These antimicrobials bind to overlapping sites at the peptidyl transferase centre. 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, and has been found in humans and animals, including pigs.\(^ {4,17,50,53}\) The gene was first detected on a plasmid originating from a bovine strain of the coagulase-negative Staphylococcus sciuri and has also been found in other coagulase-negative staphylococci.\(^ {14,42,54}\) Most of the cfr-positive animal isolates originate from swine, but cfr has also been detected in isolates of bovine, equine and poultry origin.\(^ {53}\) The cfr gene has been found on a plasmid in porcine MRSA and MSSA of different clonal lineages (ST398 and ST9).\(^ {50}\) Recently, the cfr gene has been detected in a Panton–Valentine leucocidin (PVL)-positive human clinical MRSA isolate of ST8 SCCmec type IV (USA300).\(^ {37}\) USA300 is a major community-acquired MRSA causing skin and soft tissue infections in the USA and worldwide. A new multidrug resistance conjugative plasmid (pPERGB) containing cfr, tet(L) (encoding tetracycline resistance), ant(4′)-Ia (encoding tobramycin resistance) and dfrK (encoding trimethoprim resistance) was detected in a linezolid-resistant human MRSA strain with sequence type ST125. This MRSA strain was isolated from two patients with chronic obstructive pulmonary disease in Spain, both of whom had been treated with linezolid.\(^ {51}\) An outbreak of linezolid-resistant cfr-positive MRSA has been reported in a Spanish hospital.\(^ {55}\) A recent review has summarized current knowledge of the genetic environment of cfr.\(^ {53}\)

**Pleuromutilin resistance in other bacteria**

Cfr-mediated resistance has also been detected in *E. coli*, *Proteus vulgaris*, *Enterococcus* spp., *Bacillus* spp., *Jeotgalicoccus* spp. and *Micrococcus* spp.\(^ {18,24,53}\) To date, cfr-mediated resistance seems to be uncommon in Enterobacteriaceae such as *E. coli* and *P. vulgaris*.\(^ {24,56}\) 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*.\(^ {24}\) A cfr-positive *P. vulgaris* isolate was found when screening 557 nasal swabs from Chinese swine for florfenicol resistance.\(^ {56}\) Liu et al.\(^ {57,58}\) 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.\(^ {58}\) The cfr gene located on a plasmid has been found in a *Bacillus* species isolated from a nasal swab of a pig in China.\(^ {25}\) The plasmid also carried a novel streptomycin resistance gene, aadY. Another *Bacillus* isolate, containing cfr and erm(B) conferring resistance to macrolides, lincomamides, and streptogramin B and located on a plasmid, and fexA, conferring resistance to florfenicol and located on chromosomal DNA, was cultured from swine faeces in China.\(^ {59}\) Cross-resistance to pleuromutilins, lincomamides and streptogramin A in *Streptococcus aggalactiae* has been found to be caused by a novel gene, called *isa(C)*. Expression of this gene in *S. agalactiae* led to increased MICs of lincomycin, clindamycin, dalfopristin and tiamulin.\(^ {60}\) The gene was found in 18 clinical isolates from humans in New Zealand.\(^ {61}\) Long et al.\(^ {60}\) found that single or double mutations at various locations in the 23S rRNA gene of *Mycobacterium smegmatis* resulted in unpredictable cross-resistance between linezolid, chloramphenicol, clindamycin and valnemulin.

*E. faecalis* is intrinsically resistant to pleuromutilins, streptogramins A and lincomamides by the production of the ABC transporter Lsa(A).\(^ {66}\) In *E. faecium*, acquired resistance to pleuromutilins, streptogramins A and lincomamides is mediated by the ABC transporter gene eat(A).\(^ {61}\)

**Problems of susceptibility testing**

Accurate antimicrobial susceptibility testing of anaerobic, fastidious bacteria can be difficult to achieve as different anaerobes require different supplements in the growth medium. This causes problems with standardization of the methods. The fastidious nature of *B. hyodysenteriae* and *B. pilosicoli* has hampered standardization of methods for antimicrobial susceptibility testing. Antimicrobial susceptibility tests for *Brachyspira* spp. are not performed on a routine basis and there are no generally approved or recommended standards available. Different methods have been used, such as broth dilution and agar dilution.\(^ {62}\) Published susceptibility testing for *Brachyspira* spp. has been performed predominantly in *B. hyodysenteriae* isolates and by the agar dilution...
procedure. The most common medium used is trypticase soy agar supplemented with 5% bovine or ovine blood. The MIC has been determined as the lowest concentration of the antimicrobial agent that prevents growth or haemolysis. A broth dilution method has been evaluated for monitoring of antimicrobial susceptibility in Brachyspira spp.63 MIC quality control ranges for the type strain of B. hyodysenteriae, B78T (ATCC® 27164T), have been established in an inter-laboratory study of this method.64 For pleuromutilins, this method has been compared with agar dilution.65 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 L. intracellularis is difficult as this obligate intracellular bacterium needs complicated cell culture systems to grow and published data on in vitro susceptibility are scarce and include only a very limited number of isolates.66–68

Internationally accepted interpretative criteria are lacking except for tiamulin for Actinobacillus species (CLSI 2012). To date, no tiamulin or valnemulin breakpoints have been established for Brachyspira spp., but breakpoints of ≥2 mg/L have been used to classify isolates as resistant to tiamulin.59 According to Vyt and Hommez70 and Karlsson et al.,63 this breakpoint for tiamulin is too high to indicate decreased susceptibility. On the basis of a field survey of clinical efficacy, it has been proposed that isolates with MICs ≥1 mg/L should be considered as not responding to therapy in vivo.70 Suggestions for interpretative criteria for tiamulin disc diffusion have been made for Pasteurella multocida, staphylococci, Actinobacillus suis, Actinobacillus pleuropneumoniae and Erysipelothrix rhusiopathiae.61 Burch suggested a breakpoint of >0.125 mg/L for valnemulin (75 ppm in feed) using broth dilution and >0.25 mg/L for the agar dilution method for Brachyspira spp.62 For tiamulin (at a dose of 100 ppm) a breakpoint of >0.5 mg/L was suggested for broth dilution and agar dilution, respectively.62 Pringle et al.61 suggest epidemiological cut-off values for monitoring antimicrobial susceptibility in B. hyodysenteriae of ≤0.125 mg/L for valnemulin and ≤0.25 mg/L for tiamulin.

Occurrence of resistance in bacteria from food-producing animals

Brachyspira spp.

An increase in MICs of tiamulin and valnemulin for B. hyodysenteriae in Spain has been reported in several countries. Reduced in vitro susceptibility of B. hyodysenteriae has been reported from Japan,28 Spain,28 the Netherlands,28 Germany,65 Hungary,74 the UK75 and the Czech Republic.67 A study investigating 20 Brachyspira intermedia isolates from layers in Belgium and the Netherlands found that the MIC distribution was monomodal, but tailed towards higher MIC values, possibly indicating low-level acquired resistance in six isolates.78 Decreased susceptibility to tiamulin has also been found in B. pilosicoli isolates from Sweden and in various Brachyspira spp. from the USA.65,66 Four out of 79 (5.1%) and 6/79 (7.6%) Brachyspira isolates were resistant to tiamulin and valnemulin, respectively, applying MIC >2 mg/L as the breakpoint.69 In Spain the susceptibility of B. hyodysenteriae to tiamulin and valnemulin decreased in 2008/09 compared with previous years.28 Resistance to pleuromutilins appears to be common in B. hyodysenteriae in Spain.28 An increase in MICs of tiamulin and valnemulin was also observed in Japan, changing from a low and unimodal MIC distribution observed from 1985 to 2000 to higher MICs recorded from 2001 onwards, with a trend to a bimodal distribution.72 The MIC90 of Czech B. hyodysenteriae isolates increased from 0.25 mg/L in 1997 to 4 mg/L in 2001 for tiamulin and from ≤0.031 mg/L in 1997 to 8 mg/L in 2001 for valnemulin.77 In Germany the MIC90 of tiamulin increased from 0.125 mg/L (1989–93) to 2–8 mg/L (2000–02). For valnemulin the MIC90 increased from 0.063 mg/L (1989–93) to 2–4 mg/L (2000–02).65

Other bacterial species

Resistance to tiamulin has also been reported in Haemophilus parasuis, A. pleuropneumoniae and S. aureus, as well as other staphylococci.79 A Canadian study found tiamulin MICs to be significantly higher among human and porcine MRSA ST398 compared with human MSSA and non-ST398 MRSA, as well as porcine MSSA isolates.80 However, it is worth mentioning that the authors investigated only 17 MRSA ST398 isolates compared with 48 MSSA from humans, 15 MSSA from pigs and 50 non-ST398 MRSA from humans. Several studies have found S. aureus and MRSA isolates resistant to pleuromutilins.19,27,35,81 A high percentage of MSSA isolates (40%) from slaughter pigs originating from one farm in Uruguay were reported as resistant (MIC >16 mg/L) to tiamulin; the rate was 100% when using a different breakpoint (MIC >2 mg/L).82 A monitoring study of MRSA isolates from the nasal cavities of pigs at slaughterhouses in Switzerland found that 90% of the MRSA isolates were resistant to tiamulin and one-third of these tiamulin-resistant MRSA (MIC >2 mg/L) isolates harboured vga(A) genes.53 Detection of this kind of resistance is hampered by the fact that staphylococci are not target pathogens for pleuromutilins and as a consequence pleuromutilins are usually not included in susceptibility testing of staphylococci. In addition there are no approved clinical breakpoints. 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 LA-MRSA ST398. In China, 149 staphylococcal isolates resistant to florfenicol were found when screening nasal swabs from 557 pigs originating from three farms. Of these isolates, 33 (22%) were found to be positive for cfr; they included Staphylococcus arlettae, Staphylococcus 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; these plasmids sometimes also harboured other resistance genes, such as erm(C) and accA-aphD.23 Co-selection of cfr-carrying isolates could therefore occur under selective pressure imposed by the use of florfenicol, aminoglycosides or macrolides. In L. intracellularis no resistance to pleuromutilins has been reported to date, but very few isolates have been investigated and accepted interpretative criteria for such susceptibility testing are lacking.66,67 Antimicrobial susceptibilities of M. hyopneumoniae field isolates have been rarely determined and a limited number of isolates have been investigated in these studies. In a study investigating 21 Belgian field isolates, no resistance against tiamulin was found.51 Data on antimicrobial susceptibility of other mycoplasmae are scarce, with a limited number of isolates and/or from countries outside of the EU.
Possible links between the use of pleuromutilins and other antimicrobials in animals and resistance in bacteria of animal origin

The lack of authorized 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 MICs 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. 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. Generally, the use of pleuromutilins is high in Spain and the Czech Republic and relatively high percentages of *Brachyspira* isolates resistant to pleuromutilins have also been reported from these two countries. Multidrug-resistant and pleuromutilin-resistant *B. hyodysenteriae* isolates were associated with farms where there were endemic problems with swine dysentery. Increased consumption of pleuromutilins has been incriminated as a cause of the increase in MICs of tiamulin and valnemulin.

It has been suggested that the use of pleuromutilins likely selects for the emergence of *cfr* in animal isolates of staphylococci. It must be noted that many isolates resistant to pleuromutilins are multidrug resistant. Mobile elements containing genes mediating resistance to pleuromutilins often also 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 *dfrK*, and therefore co-selection of *vga(C)* under selective pressure by the use of other antimicrobials can potentially occur. The *vga(E)* gene was located on transposon Tn6133 carrying the resistance genes *spc* and *erm(A)* and therefore isolates were not only resistant to tiamulin, lincomamines and streptogramin A, but also to macrolides, streptogramin B and spectinomycin. In addition, the MRSA isolates carried other resistance genes conferring resistance not only to β-lactams, but also to tetracyclines and trimethoprim. Due to the combined presence of *vga(E)* and *erm(A)*, the isolates were resistant or less susceptible to quinupristin/dalfopristin, which is used in human medicine to treat (severe) infections caused by MRSA and vancomycin-resistant enterococci. Staphylococci carrying *cfr* were multidrug-resistant, with 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*. Antibiotic usage records for Chinese pig farms indicate that multiple antimicrobial drugs, including florfenicol, lincomycin and tiamulin, have been used on farms with *cfr*-positive isolates and that selective pressure might have played a role.

Impact on animal health and production

For most indications for which pleuromutilins are authorized there are alternative substances, except for swine dysentery, where high prevalences of resistance against alternative antimicrobials exists in many EU member states. When there is resistance to alternative antimicrobials, pleuromutilins are the only remaining treatment option for this indication. Thus, the impact of resistance to pleuromutilins on animal health and production is likely to be highest in cases 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. Due to the lack of commercial vaccines, the 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 the control of swine dysentery.

Resistance in *B. hyodysenteriae* commonly concerns antimicrobial agents used for treatment of swine dysentery, such as macrolides (tylosin) and lincosamides. Therefore, the number of antimicrobials available for the treatment of swine dysentery is limited. Alternatives such as carbadox, which is used in the USA, are not authorized in the EU. Thus, in many cases pleuromutilins are the only potentially effective choice for swine dysentery as an authorized indication. However, isolates with reduced susceptibility to pleuromutilins have emerged among *Brachyspira* spp. in many countries. Several of these reports document an increase in the proportion of isolates with decreased susceptibility over time, and in some cases therapy failure has been described. Lack of effective treatment options for swine dysentery would have considerable consequences for production economy due to mortality, impaired growth and secondary costs. Depopulation of the farm and replacement of animals with non-infected animals may, in such cases, be the measure of last resort.

Potential impact on human health

To date only one product containing pleuromutilins (retapamulin) is authorized for humans, for topical use only. Concerns about lack of sufficient bioavailability, gastrointestinal side effects, hepatotoxicity and the challenging side-chain chemistry of pleuromutilins have labelled these drugs as difficult and hazardous to develop, and several companies stopped their efforts to develop these drugs for human medicine. 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. Investigations exploring pleuromutilins for the treatment of *Mycobacterium tuberculosis* infections in humans are ongoing. Therefore, potential implications of the emergence of resistance to pleuromutilins in *S. aureus* and MRSA, including LA-MRSA ST398, need to be considered.

The emergence of successful epidemic clones of MRSA, such as the PVL-positive ST8-IV/USA300, and ST125-carrying plasmids containing *cfr* is major cause for concern and warrants close surveillance. PV-L-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 oxazolidinones and streptogramin A. Linezolid is an important antimicrobial agent for the treatment of vancomycin-resistant enterococci, methicillin-resistant staphylococci and penicillin-resistant pneumococci. Therefore, the emergence of this resistance gene in animals might pose a threat to human medicine as it might compromise the 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. 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, three patients in other wards were also infected with linezolid-resistant MRSA. All 15 isolates from the outbreak carried cfr. Six patients died and one death was directly attributed to the resistant MRSA. Contact with animals was not investigated.

The simultaneous presence of cfr and erm genes results in resistance to streptogramin A/streptogramin B combinations, such as quinupristin/dalfopristin, which are used to treat human infections with staphylococci and vancomycin-resistant E. faecium.

Retapamulin MICs of $\geq 2$ mg/L 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. LA-MRSA containing the plasmid-borne vga(A) gene has been reported from pigs and a pig farmer in the USA, indicating that zoonotic transmission may occur.

A special concern is the recent emergence of cfr-encoded plasmid-mediated linezolid resistance in E. faecalis and E. faecium in human clinical isolates in several countries, including Thailand and the UK.

Discussion

To date, pleuromutilins are used mainly in veterinary medicine. The vast majority of the sales of pleuromutilins are accounted for by oral medications. Data on sales of pleuromutilins in different countries 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 the treatment and prevention of not only swine dysentery but also porcine respiratory disease complex associated with Mycoplasma spp. and of infections with L. intracellularis. Other possible explanations might be differences in the prevalence of swine dysentery between countries and a high prevalence of resistance to alternative antimicrobials used to treat swine dysentery, e.g. 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. Nevertheless, the reported increases in MICs of tiamulin and valnemulin against porcine B. hyodysenteriae isolates from different European countries are alarming, as there are only a limited number of antimicrobials left available for the treatment of swine dysentery due to high levels of resistance. Considering that swine dysentery is a common and economically important disease, a possible future loss of effective treatment options 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 real 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 in monitoring 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 utilize strategic treatment with pleuromutilins for a limited period as part of the eradication protocol. Successful programmes are in place, for example Sweden, Norway and Finland. Another option for reducing 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 the responsible use of pleuromutilins could potentially reduce the risks associated with further emergence of resistance in B. hyodysenteriae.

Currently, the importance of pleuromutilins in human medicine is limited to one product authorized for topical treatment, 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 transmitted horizontally. A special concern is the vga genes, conferring cross-resistance to pleuromutilins, streptogramin A and lincosamides, and the cfr genes, with an even broader spectrum conferring resistance to phenicols, lincosamides, oxazolidiones, pleuromutilins and streptogramin A.

Colonization of animals with LA-MRSA ST398 can lead to clinical infections in animals and zoonotic infections in humans, and severe cases have been reported. The prevalence of MRSA in pigs is very high in many EU member states, and in such situations there is the potential that the use of pleuromutilins for the treatment and prevention of other diseases, such as 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, and cfr-mediated resistance has been detected in several bacterial species, indicating inter-species and inter-genus transfer. Resistance selection and spread between animals and humans might jeopardize the efficacy of antimicrobial agents. The emergence of these resistance genes in animals poses a potential threat to human medicine as they might compromise the empirical treatment of human MRSA infections. The use of linezolid or dalfopristin 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 because of the acquisition of vga and cfr and other resistance genes, co-selection may potentially occur under selective pressure by numerous other antimicrobial agents in human and veterinary medicine. 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 pleuromutilin resistance with many different antimicrobials can potentially occur due to multidrug resistance genes and the co-localization of these resistance genes with other resistance genes. The prudent use of all antimicrobials in animals and humans is therefore warranted.

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Transparency declarations
Conflicts of interest: none to declare.
A reflection paper on the same subject has been published on the website of the European Medicines Agency. This reflection paper is similar, but not identical to this review.

Disclaimer
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