EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 July 2013
EMA/755938/2012

Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

An agency of the European Union

Table of contents

1. Abstract

Soon after its introduction in the 1950s, the use of colistin in human medicine was restricted to predominantly topical administrations due to its toxicity if given systemically. Severe nosocomial infections due to multidrug-resistant (MDR) Gram-negative bacteria now account for high morbidity and mortality (Schorr, 2009). Colistin is therefore nowadays a last resort drug in human medicine in the context of treatment of infections caused by MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*), for which mortality can be extremely high.

Colistin has been used regularly in veterinary medicine for decades, both as curative treatment and for prevention of disease. The antibiotic has been predominantly administered as group treatment for Gram-negative gastrointestinal infections in conditions of densely populated livestock by oral administration, the route by which the compound demonstrates poor systemic absorption. The use in veterinary medicine is being questioned given the ever growing need for antimicrobials for treatment of MDR infections in humans.

This document reviews the current knowledge with respect to the impact of and need for colistin use for human and animal health. The document provides a description of toxicity issues, colistin resistance mechanisms, susceptibility testing problems, and risk management options. Among other recommendations, we here conclude that antimicrobial use in both human and veterinary medicine must be rationalised. For colistin use in particular, detailed monitoring of colistin resistant bacteria is required to confirm horizontal gene transfer is not involved and overall prevalence remains low. As soon as colistin resistance determinants are found on mobile genetic elements in the bacteria of concern as well as from human or animal origin, or a clonal explosion of virulent bacteria takes place, a new risk assessment would be required.

2. Introduction

The global emergence and steady increase in bacteria that are resistant to multiple antimicrobials has become a global public health threat (Carlet et al., 2012). Human infections with resistant bacteria are associated with higher patient morbidity and mortality, higher costs and longer length of hospital stay (Cosgrove, 2006). In the current state of increasing resistance coupled with a worrisome decrease in the availability of new antibiotics, there is a need to explore all options that would allow as far as possible the preservation of the current armamentarium (ECDC/EMEA, 2009).

Colistin (polymyxin E) is a cationic, multicomponent lipopeptide antibacterial agent discovered soon after the end of the Second World War (1949). An antibiotic originally named "colimycin" was first isolated by Koyama et al, from the broth of *Paenibacillus (Bacillus) polymyxa* var. *colistinus* in 1950 (Koyama et al., 1950). Colistin has been used for decades in veterinary medicine, especially in swine and veal calves. Based on national consumption data, Gram-negative infections of the intestinal tract, due to *E. coli* and *Salmonella* are the primary indications. Most of the colistin applications in animals are for oral group treatments.

In human medicine, colistin was initially restricted to topical use due to its systemic toxicity (Nord and Hoeprich, 1964). Recently hospital outbreaks with carbapenemase-producing Enterobacteriaceae (*E. coli, Klebsiella*), and multidrug-resistant *Pseudomonas* and *Acinetobacter* species (non-fermenters), have forced clinicians to re-introduce systemic colistin treatment, in the form of its inactive pro-drug, colistin methanesulphonate (CMS), as a last resort drug for nosocomial infections in which these

organisms are involved. Colistin is therefore playing nowadays a key role for public health, despite all the limitations deriving from its safety profile and uncertainties around the best way of using it (Nation and Li, 2009). Also, CMS is used by inhalation for the treatment of *Pseudomonas aeruginosa* lung infections in patients with cystic fibrosis.

Due to the critical importance of colistin for use in human medicine, there is a need to focus on the possible consequences of veterinary use of colistin for human public health. Simultaneously, the impact of current or future use of colistin products in veterinary medicine for animal health and welfare needs to be re-explored.

3. The use of colistin in human and veterinary medicine

3.1. Human medicine

In human medicine, the two salt forms of polymyxin E (colistin) have been widely commercially available, namely colistin sulphate and colistimethate sodium (syn colistin methanesulphate, colistin sulphonyl methate, pentasodium colistimethanesulphate). Colistimethate sodium (CMS) is microbiologically inactive (Bergen et al., 2006) and less toxic than colistin sulphate (Li et al., 2006). It is administered predominantly as parenteral formulations and via nebulisation (Falagas and Kasiakou, 2005). After administration, CMS is hydrolysed to colistin, which is the base component that is responsible for its antibacterial activity (Lim et al., 2010). Besides polymyxin E (colistin), polymyxin B is also widely used in human medicine. Although parenteral formulations exist and are used in various part of the world, polymyxin B is used only for topical administration in Europe for humans. Polymyxin B has been reported to be associated with a similar or even worse toxicity pattern when administered systemically (Ledson et al., 1998; Nord and Hoeprich, 1964).

Colistin sulphate is available in tablets and syrup for selective digestive tract decontamination (SDD) and as topical preparations for skin infections. CMS is available for administration intravenously, intramuscularly as well as topically via aerosol (nebulisation) or intraventricular administration. Polymyxin B is available in parenteral formulations and can be administered intravenously, intramuscularly, or intrathecal.

Until recently, due to the major concerns for neuro- and nephrotoxicity (Koch-Weser et al., 1970; Ryan et al., 1969), the use of polymyxins has been limited to ophthalmic and topical use (Falagas and Kasiakou, 2005; Koch-Weser et al., 1970). Cystic fibrosis patients have been an exception to this practice, and such patients have received systemic or nebulised colistin to control lower airway bacterial infections and complications (Beringer, 2001).

Healthcare-associated infections caused by MDR Gram-negative organisms are being increasingly reported, especially in patients in intensive care units and haematology/oncology units (Zarb et al., 2012). Colistin has re-emerged as a last-resort therapeutic option to treat infections due to multi-resistant, lactose-fermenting and non-fermenting Gram-negative bacilli, including *P. aeruginosa* and *Acinetobacter baumannii,* for which there is a growing unmet medical need. In particular, clinicians nowadays have to resort to colistin to treat nosocomial infections in critically ill patients, such as bacteraemia and VAP (ventilator-associated pneumonia), due to carbapenem-resistant Gram-negative bacteria (Daikos et al., 2012; Petrosillo et al., 2013). In most cases these carbapenem-resistant organisms produce a serine-based carbapenemase (e.g. the KPC or OXA enzymes) (Canton et al., 2012) or a metalloenzyme (e.g. the New Delhi Metalo-β-Lactamase 1, NMD-1) (Bogaerts et al., 2010; Cornaglia et al., 2011; Kumarasamy et al., 2010). These bacterial strains appear to be spreading within the EU and have become a major problem in some centres/countries (Huang et al., 2011).

Colistin in combination with other antibiotics such as tygecycline or carbapenems is currently used in some countries as preferred approach particularly for carbapenemase producing *Klebsiella pneumoniae* based on current evidence (Daikos et al., 2012; Qureshi et al., 2012; Tumbarello et al., 2012). A recent randomised trial failed to establish a clinical benefit for the combination of colistin with rifampicin for the treatment of serious infections due to extreme drug resistant (XDR) *Acinetobacter baumannii*, despite synergism was shown *in vitro* (Durante-Mangoni et al., 2013). Studies are ongoing to determine the efficacy of colistin combination with carbapenems for severe infections due to MDR Gram negative pathogens.

The use of colistin by inhalation as adjunctive therapy or as monotherapy for VAP treatment has also been explored (Lu et al., 2012; Michalopoulos and Falagas, 2008; Rattanaumpawan et al., 2010), however larger randomised trials are needed in order to conclude on the utility of this approach.

Available evidence, mainly from old case series, suggests that systemic colistin is an effective and acceptably safe option for the treatment of children without cystic fibrosis who have multidrug-resistant Gram-negative infections (Falagas et al., 2009).

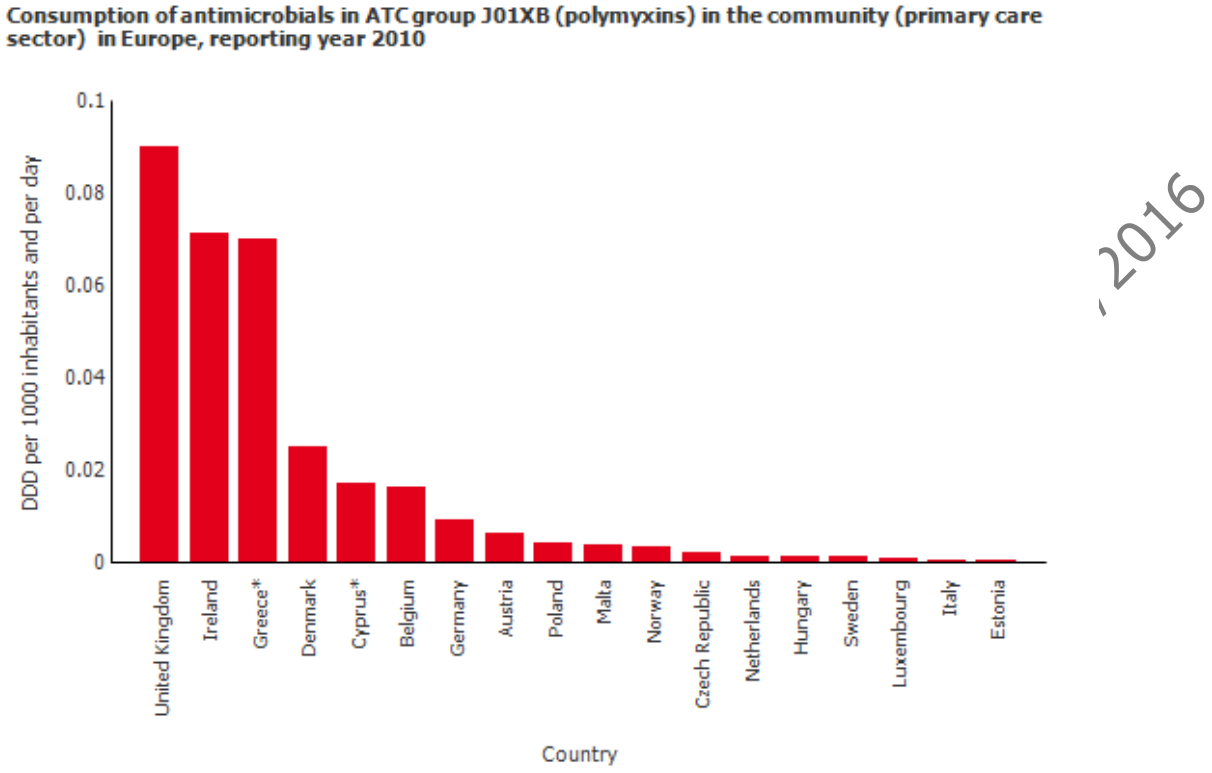
Major adverse effects of the systemic use of colistin in humans are nephrotoxicity (acute tubular necrosis), and neurotoxicity such as paraesthesia, dizziness/vertigo, weakness, visual disturbances, confusion, ataxia, and neuromuscular blockade, which can lead to respiratory failure or apnoea (Falagas and Kasiakou, 2005). Older studies show a much higher frequency of neuro - and occasionally irreversible – nephrotoxicity (approximately 7%), compared to more recent studies. The exception is cystic fibrosis patients in which up to 29% adverse (neurological) effects have been found (Bosso et al., 1991; Reed et al., 2001). The need of higher doses of CMS in order to achieve adequate colistin concentrations for therapeutic effect, as shown in recent studies (Garonzik et al., 2011; Plachouras et al., 2009), raises the concern around the consequent further increase in nephrotoxicity (Pogue et al., 2011). To contain toxic side effects following systemic use, close monitoring of renal function and avoidance of co-administration with other nephrotoxic agents (e.g. aminoglycosides) are recommended (Falagas and Kasiakou, 2005). New derivatives of polymyxins, with a more favourable toxicity profile are under evaluation (Vaara and Vaara, 2013).

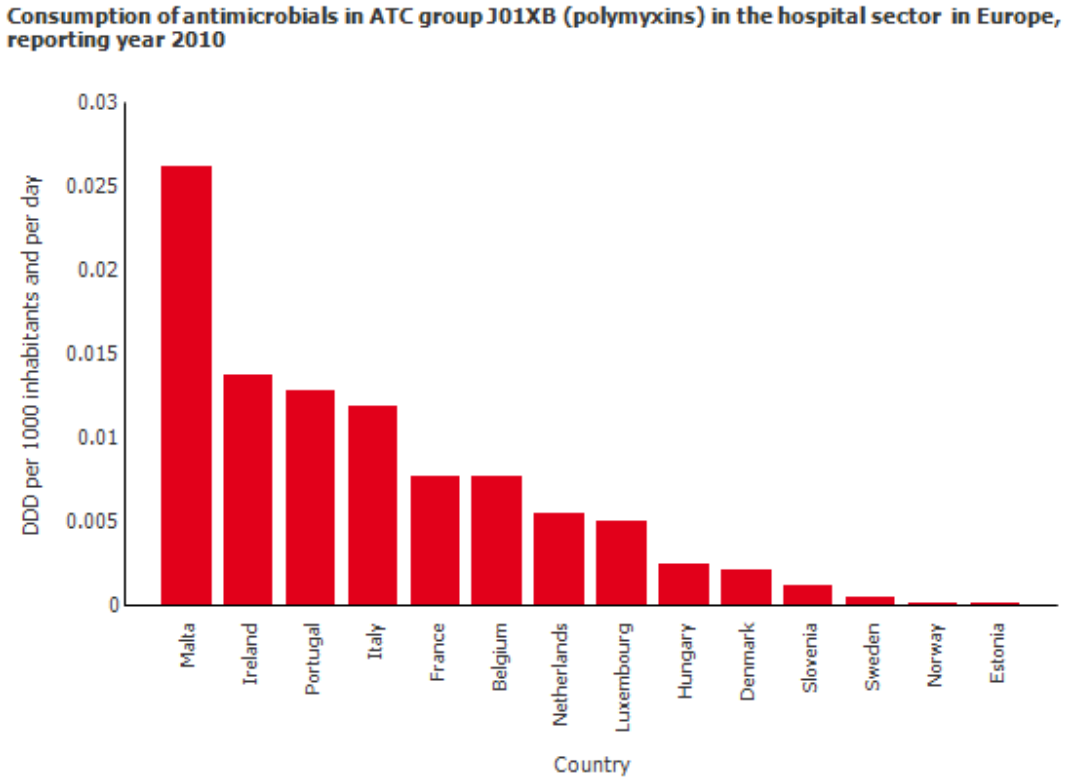
The use of parenteral colistin to treat serious human infections is somewhat hampered by continuing uncertainty regarding the optimum dose regimen, by the use of different ways to describe and express the dose (in grams colistin base and as International Units) as well as the uncertainty regarding what is actually delivered as active substance to the patient. There does, however, seem to be a consensus on a need to use higher doses, adjusted according to creatinine clearance, and a loading dose (Garonzik et al., 2011; Mohamed et al., 2012; Vicari et al., 2013).

A recent study with four different brands of CMS (He et al., 2013) showed that the pharmacokinetics (PK) in rats was markedly different, with differences in the amount of colistin formed from the various products. This is worrisome, considering the narrow therapeutic index of colistin and the risk of failure/resistance associated with lower exposure. All recent PK studies measured colistin A and B with chemical bioassay but not the other minor components (Couet et al., 2011; European Pharmacopoeia, 2013; Mohamed et al., 2012). The Ph. Eur. monograph for CMS is not particularly detailed and specifications are quite broad (European Pharmacopoeia, 2013). Also a minimum titre for CMS is specified in IU (potency test is based on turbidimetric and/or agar diffusion techniques) which would need some re-evaluation considering CMS is an inactive prodrug (Bergen et al., 2006).

Colistin is used both in the community and hospital sectors. Total consumption (reflecting topical, inhalational and systemic routes of administration combined) varies widely between countries. In Figure 1, variation in systemic administered colistin between different countries is shown.

Figure 1. Human colistin consumption as monitored in the European Surveillance of Consumption of Antimicrobials (ESAC-net) for community (top) and Figure 2, hospital sector (bottom).





Total care includes data both from the hospital sector and community (primary care sector) and overestimates the figures when used for reporting for the community sector. Some countries report reimbursement data that do not

*include consumption of antimicrobials obtained without prescription and other non-reimbursed sources. *Country provided only total care data. See ESAC for full details and methodology (ECDC, 2012).*

Colistin resistance has been emerging rapidly following its reintroduction as shown in different reports with an associated increased mortality (Capone et al., 2013; Kontopoulou et al., 2010; Zarkotou et al., 2010). In a hospital in Greece, resistance rates rose from 0% in 2007 to 8.13% in 2008 and to 24.3% in 2009 (Meletis et al., 2011). The use of colistin for selective digestive tract decontamination (SDD) in intensive care units has resulted in an increase of colistin resistance including cases of bacteraemia associated with colistin resistant strains, prompting the recommendation to stop the use of colistin for SDD (Halaby et al., 2013).

As outlined under the section 4.1 on resistance mechanisms, the most recent evolution consists of virulent clones of *K. pneumoniae* or other difficult to treat Gram-negative bacteria becoming resistant during therapy and associated with hospital outbreaks within Europe and world-wide (Balm et al., 2013; Brink et al., 2013; Comandatore et al., 2013; Del Bono, 2013; Lambrini, 2013; Lesho et al., 2013; Snitkin et al., 2013). Furthermore, the increasing reports of colistin-susceptible isolates harboring resistant subpopulations are of great concern (Meletis et al., 2011).

3.2. Veterinary medicine

Within the EU Member States, colistin and polymyxin B are authorised nationally. Main indications are infections caused by Enterobacteriaceae in rabbits, pigs, poultry, cattle, sheep and goats. Also, colistin is used in laying hens and cattle, sheep and goats producing milk for human consumption. Typically, colistin products are administered orally, as a drench, in feed, in drinking water or through milk replacer diets. Combinations of colistin with other antimicrobials are available for group treatments of food-producing animals in some European countries. Products for parenteral and intramammary administration are also available, and Gram-negative infections in ruminants including endotoxinaemia are claimed indications. Polymyxin B is on the list of substances essential for the treatment of equidae for systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases (Barton et al., 2004; Moore and Barton, 2003; Official Journal of the European Communities, 2013). As in human medicine, colistin and polymyxin B have been registered for topical administration to individual veterinary patients. In companion animals, prescription eye and eardrops are available as colistin alone, or in combination with other antimicrobials. Colistin tablets are available for calves for the prevention and treatment of neonatal colibacillosis. In some EU Member States, veterinary medicinal products containing colistin are not on the market and not used at all (ESVAC, 2012).

The 2010 and 2011 FDA reports on sales of veterinary antimicrobials show no sales of polymyxins, but there are reports of off label use of polymyxin B in horses in cases of endotoxemia (Moore and Barton, 2003).

In Europe, colistin has been used since the 1950s (Koyama et al., 1950), primarily for pigs including group treatments and prevention of diarrhoea caused by *E. coli* and *Salmonella* spp, as first choice treatments for neonatal diarrhoea in piglets (Timmerman et al., 2006) and veal calves (Pardon et al., 2012) caused by *E. coli* as well as for the therapy of mild colibacillosis in poultry. The median number of individuals treated with colistin per 1000 animals in Belgium was 41.3 (Callens et al., 2012b) and 58.9 (Pardon et al., 2012) for finishing pigs (50 farms) and for veal calves (15 farms), respectively. Based on the overall antimicrobial consumption, these studies demonstrate that colistin accounted for more than 30% of the antimicrobial use in swine and 15% in veal farming. Also, the Belgian use of colistin was for indications others than those for which it is authorised, e.g. respiratory disease, peritonitis (Pardon et al., 2012) and streptococcal infections (Callens et al., 2012b). Doses also varied between animal species, farm types and indications. Timmerman (2006) reported underdosing of oral

polymyxin E in piglets possibly due to dilution in food or water, since its administration was not weight-based. Studies on dairy farms have shown limited use of polymyxins (Catry et al., 2007; Menéndez González et al., 2010). In 32 broiler farms in Belgium, the use of colistin was not reported despite detailed consumption records (Persoons et al., 2012a), however colistin is used in medicated feed (www.belvetsac.ugent.be). Older studies from 2001-2003 in a limited number of Belgian cattle farms, have shown that starter rations with antibiotics were given for 6 to 13 days in all of 5 examined veal calves farms and 55 % of them contained colistin (Catry et al., 2007). In the same survey and in great contrast, the mean number of suckling beef (n= 5 farms) and dairy cattle (n= 5 farms) that received colistin was on average below 0.2 per 1000 animals daily (Catry et al., 2007).

Across 19 EU/EEA European countries for which sales data are available for 2010, polymyxins were the 5th most sold group of antimicrobials (4.5%), after tetracyclines (39%), penicillins (23%), sulphonamides (11%), and macrolides (9%). These data have been averaged and approximated for the animal population (Population Correction Unit, PCU). Four countries report zero sales. The percentage of polymyxin sold in veterinary medicine at the national level, according to the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC, 2012) is shown in Figure 3. The vast majority was for oral administration by different formulations (premix, powder, solution). Only a minority was given by injection, and the amount of tablets, pastes, intrauterine and intramammary preparations was negligible (Figure 4)(ESVAC, 2012).

Combinations of colistin with other antimicrobials are authorised in some Member States. The sales of those products represents less than 10% of the overall sales of colistin (data not published).

Colistin is used in aquaculture for the prevention of Gram-negative infections (Xu et al., 2012), consumption data are not available separately for this species. In the recent Danish monitoring programme, details on consumption do not refer to the use of colistin (Agersø et al., 2012).

Figure 3 Percentage of sales for food producing animals (including horses), in mg per population correction unit (mg/PCU), of polymyxins, by country, for 2010 (ESVAC, 2012). No sales reported in Finland, Iceland, Ireland or Norway.

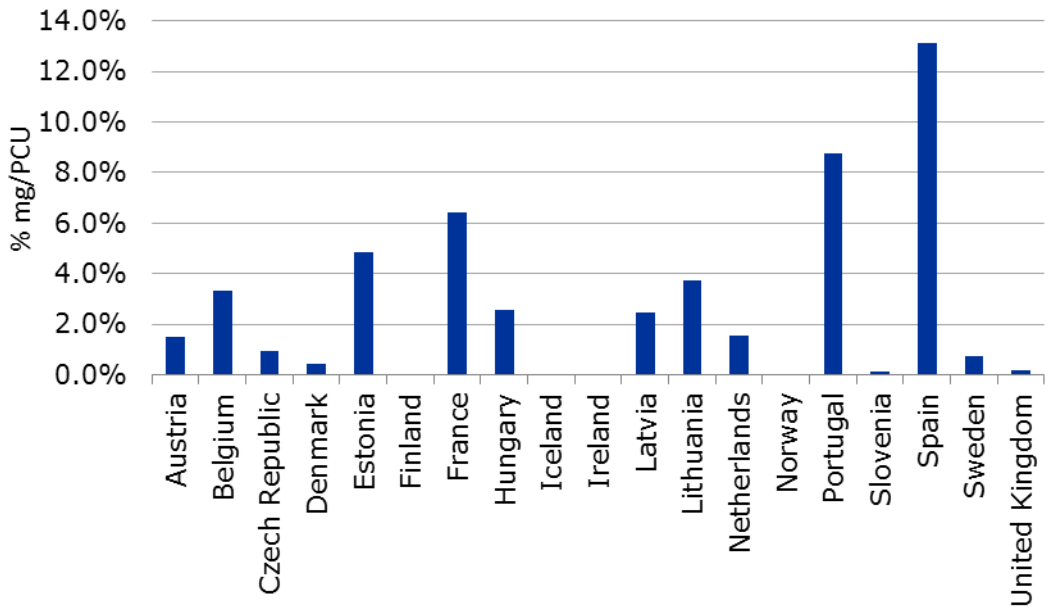
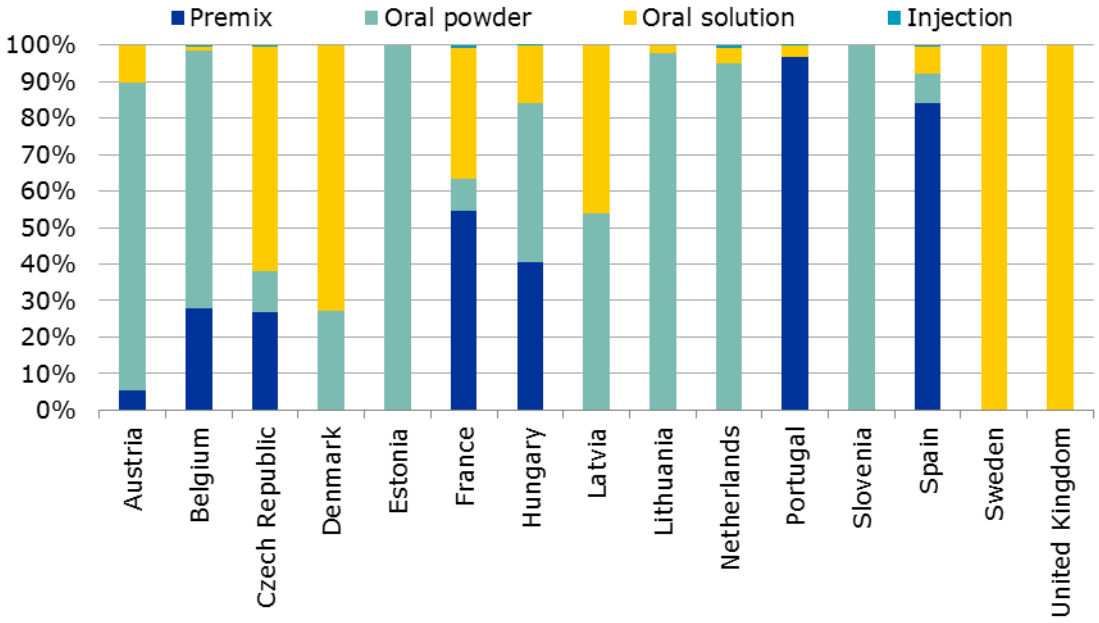


Figure 4 Distribution of sales by pharmaceutical form for polymyxins, in tonnes of active ingredient, by country, for 2010 (ESVAC, 2012). No sales reported in Finland, Iceland, Ireland or Norway. Negligible amounts sold as bolus, oral pastes, intramammary and intrauterine preparations.



Due to concerns that the differences in posology and withdrawal periods established across the European Union for veterinary medicinal formulations containing colistin at 2 000 000 IU per ml and intended for administration in drinking water to food producing species could present a potential serious risk to public and animal health, the United Kingdom referred the matter to the Agency on April 2009, under Article 35 of Directive 2001/82/EC as amended (EMA/CVMP, 2010). In their opinion the CVMP confirmed that the benefit risk balance remained positive for the use of colistin for treatment of gastrointestinal infections caused by non-invasive *E. coli* susceptible to colistin, when administered at dose of 100 000 IU colistin per kg body weight daily for calves, lambs, pigs and 75 000 IU colistin per kg body weight daily in poultry for 3-5 consecutive days. However, the benefit-risk balance regarding the use of colistin for treatment of gastrointestinal infections caused by *Salmonella* spp in calves, lambs, pigs and poultry was considered negative, and those indications were removed from the SPCs of the involved products. The scope of the mentioned referral was limited to veterinary medicinal products containing colistin for administration in drinking water; products administered in feed (or injectables) were not addressed.

3.3. Antibacterial effect

The bactericidal effect of colistin is the result of an electrostatic interaction with divalent cations of the outer bacterial membrane, which causes a disruption of the cell structure, leakage of the cell contents and thereby cell lysis (Lim et al., 2010; Schindler and Osborn, 1979). The polymyxin broad-spectrum of activity against Gram-negative bacteria involves binding to lipid A, the anchor for lipopolysaccharide, and the main constituent of the outer membrane of these bacteria. Time kinetic-kill *in vitro* studies have shown a concentration-dependent bactericidal action. Polymyxins are produced naturally by *Bacillus (Paenibacillus) polymyxa*. Polymyxins are particularly active against a wide range of species of Gram-negative bacilli (e.g. *E. coli*, *Salmonella* spp and *P. aeruginosa*) including those displaying carbapenem resistance, and certain *Mycobacterium* species. Colistin differs from polymyxin B, only by one amino acid in position 6 (D-leucine in colistin, phenylalanine in polymyxin B). However both compounds have the same mechanism-of-action and resistance development. Polymyxin B and colistin

(sulphate) have a similar spectrum of antibacterial activity against main Gram-negative pathogens (Gales et al., 2011).

Polymyxins have no clinically useful activity against Gram-positive bacteria, Gram-negative cocci, anaerobes and Molicutes including *Mycoplasma* (Falagas and Kasiakou, 2005). In addition, colistin lacks therapeutic activity against inherently resistant species, including organism of the genera *Serratia, Stenotrophomonas*, and *Proteus* (Pogue et al., 2011).

Colistin heteroresistance has been reported for *K. pneumoniae* (Poudyal et al., 2008), *P. aeruginosa* (Bergen et al., 2011) and *A. baumannii* (Hawley et al., 2008). The potential for under-dosing in relation to selecting subpopulations with higher MICs, during treatment with colistin has been illustrated for *A. baumannii* (David and Gill, 2008). The use of combination therapy would have the potential benefit to reduce the emergence of such subpopulations. Studies that included a moth infection model (*Galleria mellonella*) have found that vancomycin and doripenem might have a synergistic effect together with colistin in *A. baumanni* strains with decreased colistin susceptibility (O'Hara et al., 2013). For *P. aeruginosa*, synergistic effects have been shown *in vitro* between colistin and many other compounds (e.g. rifampicin and the anti pseudomonal agents azlocillin, piperacillin, aztreonam, ceftazidime, imipenem, doripenem, or ciprofloxacin) (Conway et al., 1997).

Recent studies have demonstrated that colistin is synergistic with drugs of the echinocandin family against *Candida* species, by increasing permeabilisation and attack by colistin on fungal membranes (Zeidler et al., 2013).

The pharmacokinetic/pharmacodynamics approach has been applied successfully to the selection of dose regimens for new antibacterial agents and the re-evaluation of efficacious dose regimens for several antimicrobial classes. Also, PK/PD has some potential to identify regimens that may minimise selection pressure for resistant strains. Although the vast majority have focused on the prevention of mutational resistance (Drlica and Zhao, 2007), some studies have shown a benefit for the containment of bacteria in which resistance is mediated mainly by horizontal gene transfer (McKinnon et al., 2008). The application of PK/PD for colistin has only recently re-gained attention due to its increasing systemic use to treat multidrug-resistant bacteria causing human infections. The PK/PD parameter to maximise antibactericidal activity and minimise resistance has been shown as the area under the inhibitory curve (AUIC, or fAUC/MIC) for target organisms such as *P. aeuruginosa* and *Acinetobacter* (Michalopoulos and Falagas, 2011).

4. Resistance mechanisms and susceptibility testing

4.1. Resistance mechanisms

Acquired resistance to colistin in normally susceptible bacteria has been characterised by chromosomal mutations and thus in theory is non-transferable by mobile genetic elements (Callens et al., 2012b; Landman et al., 2008). Polymyxin resistance is mediated by mutations in specific regions (pmrA/B and phoP/Q)(Moskowitz et al., 2012). Resistance is then associated with changes in the target components of the Gram-negative bacterial wall, namely a covalent addition of 4-amino-L-arabinose (LAra4N) to phosphate groups within the lipid A and oligosaccharide as elements from the lipopolysaccharide (LPS) (Boll et al., 1994; Moskowitz et al., 2012; Moskowitz et al., 2004; Nummila et al., 1995). The two-component regulatory ParR-ParS system with an identical modification of LPS is involved in the adaptive resistance at sub-inhibitory concentrations of cationic peptides, including colistin and the bovine peptide, indolicidin (Fernandez et al., 2010). Research has demonstrated that also the activity of lysozyme and other innate immune defence peptides (LL37) can be affected (Napier et al., 2013). Colistin resistance thus confers resistance to polymyxins and a range of other cationic peptides.

Decreased activity of polymyxins is due to structural LPS changes at both the cytosol and peri-plasmatic site of the cell membrane (Moskowitz et al., 2012). Studies indicate a similar (temperature dependent) mechanism in other bacteria including *A. baumannii, Yersina enterocolitica* and *Salmonella* (Beceiro et al., 2011a; Beceiro et al., 2011b; Guo et al., 1997; Reines et al., 2012). They found that the development of a moderate level of colistin resistance in *A. baumannii* requires distinct genetic events, including (i) at least one point mutation in pmrB, (ii) up-regulation of pmrAB, and (iii) expression of pmrC, which leads to the addition of phosphoethanolamine to lipid A (Beceiro et al., 2011a). Also, the phoP/Q system has been shown to be involved in strains with intrinsic resistance, for example pathogenic *Edwardsiella tarda* from fish (Lv et al., 2012). These systems are different from the mechanisms of colistin resistance in laboratory and clinical strains of *A. baumannii* as described by (Moffatt et al., 2010), whom noted – unexpectedly -the total loss of LPS production via inactivation of the biosynthesis pathway genes lpxA, lpxC, or lpxD. In *Yersinia* spp, polymyxin resistance can also be related to the existence of efflux pumps with potassium anti-porter systems (RosA/RosB) (Bengoechea and Skurnik, 2000).

With the exception of some well-examined clinical strains, many of the above mutation mechanisms are not stable after several passages *in vitro* (Moskowitz et al., 2012). This instability of polymyxin resistance, and the absence of horizontal gene transfer of these mutations, reduces the risk of rapid spread of resistance to colistin (Gentry, 1991; Landman et al., 2008). Investigations on consecutive samples of *Acinetobacter baumannii* from nosocomial infections have indicated that this in vitro instability of colistin resistance is also found in vivo during colistin therapy (Lesho et al., 2013; Snitkin et al., 2013; Yoon, 2013)REF. Out of 37 patients treated with colistin for <1 to 3 months, in 5 patients (13%) mutations in the *pmr* locus were found. Colistin susceptibilities returned soon after cessation of colisitin therapy (Snitkin et al., 2013), but in one of the isolates further examined an apparent more stable mutation was found (*pmrB*$_{L271R}$). Of notice,in this strain E-test and microbroth susceptibility tests were highly discordant (Snitkin et al., 2013).

Work on *Klebsiella pneumoniae* has indicated that colistin resistant mutants counteract horizontal gene transfer from multi-resistance gene clusters (Lamousin-White and O'Callaghan, 1986). This "conjugal deficiency" of colistin- resistant strains can be 1000-fold compared to colistin-susceptible strains. This aspect of colistin-resistant strains has been exploited successfully under clinical circumstances. Although stepwise mutational resistance has appeared following prolonged colistin use in certain hospital outbreaks, because plasmids were not present in the epidemic strains, whereby the colistin-resistant isolates remained susceptible to other antibiotics. Through the rotational use of colistin and aminoglycosides, the prevalence of resistant *Klebsiella* decreased during the latter outbreaks (O'Callaghan et al., 1978).

Multi-resistant *Klebsiella* strains remain a cause of hospital outbreaks and colistin resistance has been found in clones that are resistant to numerous unrelated antimicrobial agents. For example, often encountered in Europe is *K. pneumoniae* sequence types (ST) 258, resistant to all beta (β)-lactams, cephalosporins, carbapenems (KPC/class A; non-metallo), fluoroquinolones, macrolides, aminoglycosides, tigecycline, and colistin (Comandatore et al., 2013). This colistin-resistant variant of ST258 is circulating widely in Greece, with clinical cases also seen, possibly via importation, in Hungary the UK (Livermore, 2012) and USA (Bogdanovich et al., 2011). Other multi-resistant examples are *K. pneumoniae* ST 14 and ST17, reported in Asia (Balm et al., 2013). Despite the presence of many other horizontally- transferable extended spectrum resistance mechanisms (e.g. β-lactams and carbapenems), the colistin resistance determinants remain located on the chromosome and do not appear to be horizontally transferable. However, it is acknowledged that, as shown for the clone ST258 (Bogdanovich et al., 2011), these strains have high capability for successful spread.

Recent proteomic analysis by Chua and colleagues have shown that low intracellular c-di-GMP concentrations in bacteria (i.e. a secondary messenger required for adaptations in life style of bacteria) are also associated with polymyxin resistance. Biofilm formation by bacteria, which has long been regarded as leading to decreased susceptibility to antimicrobials, is systematically down-regulated at low intracellular c-di-GMP concentrations (Chua et al., 2013). Biofilms are protective layers around bacteria that are formed, for example, around inert invasive devices (e.g. implants) or in the digestive tract as mucosal biofilm communities (Fite et al., 2013). Whereas for many antimicrobial agents, resistance transfer is enhanced under biofilm conditions, this down-regulation of c-di-GMP might explain why this is not applicable for colistin resistance. In other words, colistin resistance, and maybe by extension colistin presence, might interfere with biofilm formation and therefore resistance transfer. To what extent conjugal deficiency and down-regulation of biofilm formation are related within the occurrence of colistin resistance, is not documented.

4.2. Susceptibility testing

Susceptibility testing methods and standards for colistin have been developed within the EU. Determination of minimal inhibitory concentrations (MICs) has long been applied to *in vitro* susceptibility testing of colistin. Colistin sulphate is recommended to be used by EUCAST and CLSI as the prodrug CMS is completely inactive as shown by Bergen et al (2006) and all its activity seen *in vitro* simply would derive from partial conversion of CMS to colistin over time. The breakpoints for susceptibility are based on colistin sulphate. Based on this, the microbiological testing of CMS *in vitro,* either for susceptibility testing, for PK or even potency determination, would be of disputable value.

Different techniques, such as broth microdilution (Gales et al., 2001), agar dilution, and the E-test (Boyen et al., 2010; Lo-Ten-Foe et al., 2007) have been used. The EUCAST clinical breakpoints for Enterobacteriaceae (*E. coli* and *Klebsiella* species, but excluding *Proteus* spp., *Morganella morganii*, *Providencia* spp., and *Serratia* spp.) and *A. baumannii* are based on testing using colistin sulphate and are currently ≤ 2 μg/ml for colistin susceptible strain; and >2 μg/ml for a colistin resistant strain (EUCAST, 2013). For *P. aeruginosa*, the values are ≤ 4 μg/ml for a colistin susceptible strain; and >4 μg/ml for a colistin resistance. These breakpoints are under continuous evaluation. For non-clinical surveillance purposes, the epidemiological cut-off value can deviate as proposed for certain intrinsically slightly-less susceptible *Salmonella* serovars, such as Dublin and Enteriditis (Agerso et al., 2012).

The disk diffusion test is routinely applied worldwide in human and veterinary medicine yet is seldom reliable due to the inability of colistin to diffuse gradually in the agar (Lo-Ten-Foe et al., 2007). More recently, the pre-disk diffusion test has been used successfully in which colistin tablets have an initial two hours pre-diffusion before the bacteria are added to the medium for another 18 hours of incubation. Both this pre-disk diffusion test and the E-test give comparable results to MIC determination using the agar dilution technique (Boyen et al., 2010).

Molecular identification of colistin resistance is so far not included in routine laboratory practice, and an appropriate phenotypic susceptibility test is necessary under laboratory conditions and for research purposes that involve screening of large quantities of bacteria. A number of new techniques for susceptibility testing and identification of resistance determinants is under development (van Belkum and Dunne, 2013). These techniques reduce the antimicrobial susceptibility testing time from 2 to 4 days to approximately 1 to 2 hours, which could reduce the empirical treatment and stimulate appropriate antimicrobial use.

The 2011 EFSA/ECDC EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food (EFSA/ECDC, 2013) details which countries voluntarily tested antimicrobial resistance (AMR) against colistin in zoonotic and indicator bacteria; seven Member States

tested *Salmonella*, one tested *Campylobacter* and seven tested *E. coli*. On the latest European Commission draft proposal for harmonized monitoring of AMR (not published) colistin should be tested for *Salmonella* and *E. coli* (epidemiological cut-off value [ECOFF] and clinical resistance breakpoint - >2 mg/l).

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

Despite the abundant use of colistin in veterinary medicine for over 50 years, from the available information colistin resistance transmission via horizontal gene transfer or sustained clonal expansion has not been observed for the target Gram-negative organisms.

However the rapid emergence of resistance in humans after oral use in the ICU for selective digestive tract decontamination shows that resistance in Enterobacteriaceae can emerge following oral use (Halaby et al., 2013).

Indeed, the lack of emergence of resistance should be addressed with caution since in depth epidemiological surveys in veterinary medicine are scarce. Large studies combining consumption and resistance are limited, because colistin susceptibility tests are not fully reliable.

Low antimicrobial consumption is found in dairy and beef cattle that have regular access to pasture. Under these conditions, 5-10 animals are treated on average with a standard antimicrobial dose per 1000 animals (equal to treatment incidence; TI), for colistin the TI was found to be lower than 0.2/1000 (Catry et al., 2007). For grazing animals, resistance in *E. coli* is low for most antimicrobials, but multi-resistance is encroaching slowly over consecutive years (Geenen et al.; MARAN, 2012).

In veal calves in central Europe, the average overall TI with antimicrobials was calculated to be 417 per 1000 animals per day (Pardon et al., 2012), and for colistin this daily incidence is approximately 60 per 1000. The evolution of multi-resistance is worrisome in veal calves (MARAN, 2012), yet colistin resistance in this production system is extremely low to absent (Di Labio et al., 2007).

In Belgium, the second highest antimicrobial-consuming livestock production system is that of fattening pigs, where on average over 200-250 per 1000 individuals are treated daily with antimicrobials (Callens et al., 2012b). Up to 30% of oral prophylactic and metaphylactic group treatments consist of colistin (Callens et al., 2012b). If appropriate testing is applied, resistance is only recent, but increasingly (10% in Belgium) being reported among porcine pathogenic *E. coli* strains (Boyen et al., 2010). Dutch, porcine *E. coli* and *Salmonella* isolates, as reported in 2009 (MARAN, 2009), remain fully susceptible.

In broilers, approximately 95 to 130 animals were reported to be treated daily with a standard antimicrobial dose per 1000 individuals (MARAN, 2009; Persoons et al., 2012a). Quantification of broiler consumption did not identify use of colistin in 50 randomly selected farms in Belgium (Persoons et al., 2012b), but it is used in many other EU Member States. The Dutch MARAN report covering 2009 showed a decrease in the use of intestinal anti-infectives (including colistin and neomycin) in broilers from 26.0 to 18.4 daily dosages per 1000 animals (conversion from daily dosages per animal year). Resistance in *E. coli* from broilers is increasingly becoming multi-resistance (Geenen et al., 2011), but colistin resistance reports remain scarce and limited to some broiler meat samples (2.1%, N=328) (MARAN, 2009). Care should be taken that technical difficulties can result in over-reporting of colistin resistance, in particular for *Salmonella* when contaminated with inherent resistant organisms such as *Proteus* species.

In Australian *Aeromonas* strains from fish have frequently been found to be resistant to colistin (55.5%), especially when retrieved from clinical cases (Aravena-Roman et al., 2012). Studies under European aquaculture conditions are not available.

Detailed studies relating colistin use with a reduction of multi-resistance plasmids at the farm level are currently unavailable. Surveillance data show low levels of colistin resistance despite considerable use especially in veal and fattening pigs (Callens et al., 2012a). Detailed accurate monitoring is needed in these confined production systems to follow up the emergence of clonal resistance strains and to demonstrate absence of multi-resistance plasmids or alternative structures that include efficient spreading mechanisms for polymyxin resistance.

6. Impact of use of colistin in food animals for animal and human health

Colistin is now regarded as a last line defence against infections caused by multidrug resistant Gram-negative bacteria such as *K. pneumoniae* and *A. baumanii.* Its clinical use has resurged in many parts of the world despite the limitations posed by its toxicity profile. The use of colistin in combination is also more frequently considered and clinical studies are on-going. Human nosocomial infections with colistin- resistant strains, particularly with carbapenem resistant *K. pneumoniae*, with high mortality have been reported (Capone et al., 2013; Kontopoulou et al., 2010; Zarkotou et al., 2010). The only independent risk factor demonstrated for colistin-resistant carbapenemase-producing Enterobacteriaceae (CPE) in matched, controlled studies, is the use of colistin itself (Brink et al., 2013; Halaby et al., 2013).

In European livestock, enteric diseases are treated with colistin, mainly in swine and poultry. The amount of colistin used varies significantly for those EU/EEA countries for which there are data on consumption. Differences in colistin use might result from amongst others; local bacterial resistance situation, management, production type and Marketing Authorisations. If colistin is no longer available then it could be speculated that other antimicrobials or medication (example zinc oxide in pig production) would replace its use if no other interventions are taken (biosecurity, vaccination, hygiene…). In the case of zinc oxide, other issues such as environmental impact should be taken into account. The alternatives to colistin, depending on the resistance situation in a particular country, are aminopenicillins, trimethroprim + sulfonamides, tetracyclines, aminoglyclosides, and the Critically Important Antimicrobial (CIA) cephalosporins and fluoroquinolones. The latter are of particular concern due to emerging ESBL (EFSA, 2011; EMA/CVMP/SAGAM, 2009). Although food producing animals are the main concern for the transmission of AMR from animals to man, also the risk of transmission of AMR via direct contact from companion animals should be taken into account.

There is no evidence that the use of colistin in veterinary medicine for food producing species has resulted in colistin resistance transference to humans. Nevertheless, based on current data, transmission of such resistance cannot be absolutely excluded. For other antimicrobial drug resistant organisms including *E. coli*, the emergence following antimicrobial consumption and the transfer via direct animal contact or via food has been documented (Angulo et al., 2004).

7. Conclusions

- Despite its high toxicity, colistin is a last resort antimicrobial for the treatment of severe infections caused by highly resistant bacteria in human medicine (among others carbapenemase-producing *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*). Polymyxins with a more favourable toxicological profile deserve attention for further research.

- Colistin is of therapeutic importance in veterinary medicine for the treatment of enteric diseases in certain food producing species for which there are few effective alternatives available for certain indication. Currently, there is no evidence of spread of colistin resistance from food producing animals to human patients, or vice versa.
- From the data available from 19 EU/EEA countries, colistin is the 5th most used antimicrobial for food producing animals. There is variation between Member States in the extent of use of colistin. From the data available the variation cannot be directly linked to specific animal species, category or husbandry system in an individual Member State with some Member States having a low level, or no use of the substance, suggesting that there is scope to decrease the overall use of colistin within the EU.
- Acquired resistance mechanisms are limited to a stepwise process *via* mutations in target bacteria. However, emerging data in humans show that this clonal resistance can develop rapidly and can spread efficiently under certain conditions in hospitals.
- Transfer of resistance either on mobile genetic elements (such as plasmids) between bacteria or from animals to humans has not been reported. Mutations coding for colistin resistance might even downregulate resistance spread by horizontal gene transfer.

8. Recommended Risk Management options:

8.1. Review of existing Marketing Authorisations:

The SPCs for currently authorised products should be reviewed to ensure consistency for measures to ensure responsible use in regards to protecting animal health and limiting the possibility of future risk to public health. Based on the current evidence, it is considered appropriate to maintain the use of colistin in veterinary medicine, but to restrict indications to therapy or metaphylaxis, and to remove all indications for prophylactic use in order to minimise any potential risk associated with a broader use. Reduction-of-use is expected to follow from elimination of prophylactic-use and other measures to implement responsible-use. This recommendation is made on the basis that it is prudent to minimise the possibility of resistance to colistin developing as a result of its use in animals and thereby also reduce the possibility that any resistance that does develop will be transferred to man.

8.1.1. Prophylactic use

Colistin should only be used for treatment (cure or metaphylaxis[1]) of disease, and not for prophylactic use.

8.1.2. Metaphylactic disease

Current indications such as colibacillosis, can spread rapidly through groups of animals/flocks if metaphylactic treatment is not available. Current indications should be reviewed and the appropriateness of metaphylaxis should be clarified.

[1] The recently published for consultation guidance for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances indicates: "Metaphylactic claims are when in addition to treatment of clinically affected animals there is a need for administration of an antimicrobial to other animals in the same group, still clinically healthy but likely to be infected due to close contact with diseased animals."

8.1.3. Indications

Indications for colistin should be revised according to current knowledge on rational antimicrobial therapy:

- In line with Regulation 1177/2006, antimicrobials should not be used for control and treatment of *Salmonella* in poultry except in exceptional circumstances. In addition, EFSA's Opinion on QMRA (EFSA, 2010) of *Salmonella* in pigs also advises that antimicrobials should not be used in control of this disease. Therefore, any indication for treatment of *Salmonella* spp. should be deleted.
- Indications should be for the treatment of named pathogens and general indications should be avoided.
- If any indications for production enhancement (e.g. increase of feed efficiency or growth promotion[2]) exist, they should be taken out of the SPC.

8.1.4. SPCs warnings of currently authorised products should be reviewed to ensure that use is in line with responsible use.

Some of the marketing authorisations for colistin have been in existence for decades without substantial revision or updating. Therefore, the SPCs should be examined to ensure that they are up to date on recommendations on responsible use. For example:

- Duration of treatment should be limited to the time needed for cure of diseases.
- Whenever possible, colistin should only be used based on susceptibility testing, although limitations on the susceptibility test are acknowledged.
- Use of the product deviating from the instructions given in the SPC may lead to treatment-failures and increase the prevalence of bacteria resistant to colistin.

8.1.5. Combination products (other than topical formulations)

The MAs for these products should be reviewed and unless sound justification can be provided that the combination is in line with responsible use principles, combination products should be withdrawn. This is particularly the case for any combination products of colistin with antimicrobials that are the highest priority on the WHO list of critically important antimicrobials i.e. 3rd and 4th generation cephalosporins, fluoroquinolones and macrolides.

8.2. New indications, formulations or species

New indications, formulations or species (e.g. fish) should be subject to full antimicrobial resistance risk assessment before approval. This is the standard procedure for any marketing authorisation application for an antimicrobial product for use in food producing animals, but in this case it is especially important that the relevance of colistin for human medicine is considered for any new marketing authorisation.

Studies that further examine the effect of different formulations of colistin (polymyxins) on duration of symptoms, and excretion of relevant bacteria and their antimicrobial susceptibilities would help to identify and to decrease inappropriate use.

[2] Use (and indications) of antimicrobials as growth promoters are banned in the EU.

8.3. Consumption and surveillance of colistin

The use of colistin in Member States is currently monitored as part of the ESVAC project in terms of overall use. The monitoring system should be enhanced to provide figures on use per species, production type and weight class.

The proposed revised European Commission harmonised monitoring of AMR requires all Member States to perform standardised and quality controlled susceptibility testing of colistin on representative samples of zoonotic and indicator bacteria (*Salmonella* and *E. coli)*. The findings from such testing are currently voluntarily reported by some Member States but should be made compulsory for all Member States. This monitoring system should preferably be linked to a system where a random selection of resistant isolates are screened for new resistance mechanisms, in particular for emergence of transferrable resistance genes.

Surveillance of target animal pathogens isolated from clinical cases should be implemented to ensure an early detection of any change on resistance patterns. Currently there is no official surveillance of target animal pathogens, therefore such a system should be implemented. The practical challenges for surveillance are recognised and are not restricted to colistin.

8.4. General considerations

Treatment of individual animals is preferred where possible.

Rapid, reliable diagnostic tests combining bacterial identification, susceptibility testing, and differentiation with non-bacterial diseases should be further developed.

Biosecurity measures should be implemented to reduce the need for use of antimicrobials in general (including colistin).

8.5. Follow up of scientific advice

This recommendation should be reviewed if there is a substantial increase of colistin resistance in animal bacteria and other new relevant information for public health.

Further studies on the transmission of colistin resistance from animals to humans could be useful to clarify the areas where information available is limited.

ANNEX

9. Risk Management options that were analysed and disregarded:

9.1. Withdrawal of Existing Marketing Authorisations

Considering the current information available, withdrawal of MAs is not considered to be proportionate taking into account the low levels of reported resistance in animals despite decades of veterinary use of colistin, and the current lack of evidence for transfer of resistance from animals to humans.

Withdrawal of marketing authorisations could have a negative overall impact on antimicrobial resistance development to other antimicrobial agents as it might increase their veterinary use, especially those used for the treatment of diseases where there are high levels of resistance to the first line alternatives, e.g. colibacillosis in poultry. Examples are, but are not limited to, cephalosporins, fluoroquinolones, betalactams, aminoglycosides, trimethoprim, sulphonamides and tetracyclines.

9.2. Use of colistin to be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials

The option or recommending that colistin should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials was discussed and disregarded for the following reasons:

- After decades of use of colistin in animals the detection of resistance to colistin is currently a rare event in surveillance programmes monitoring antimicrobial resistance in relation to veterinary use of antimicrobials.
- Limiting the use of colistin to the cases described in the heading would be likely to promote the use of other antimicrobials for which resistance might be of more relevance for public and animal health.

9.3. Group treatments

The option of placing restrictions to reduce the use of colistin for the treatment of groups of animals was discussed. Approximately 99% of use of colistin is in oral formulations which are mostly used for group treatment within herds/flocks. The same reasons as provided above for not recommending the withdrawal of existing marketing authorisations apply also for not placing restrictions on group treatment.

10. References

Agersø, Y., T. Hald, B. Helwigh, B. Høg, L. Jensen, V. Jensen, H. Korsgaard, L. Larsen, A. Seyfarth, and T. Struve. 2012. DANMAP 2011-use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. *DANMAP 2011-use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*

Agerso, Y., M. Torpdahl, C. Zachariasen, A. Seyfarth, A.M. Hammerum, and E.M. Nielsen. 2012. Tentative colistin epidemiological cut-off value for Salmonella spp. *Foodborne pathogens and disease* 9:367-369.

Angulo, F.J., V.N. Nargund, and T.C. Chiller. 2004. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health* 51:374-379.

Aravena-Roman, M., T.J. Inglis, B. Henderson, T.V. Riley, and B.J. Chang. 2012. Antimicrobial susceptibilities of Aeromonas strains isolated from clinical and environmental sources to 26 antimicrobial agents. *Antimicrobial agents and chemotherapy* 56:1110-1112.

Balm, M.N., G. Ngan, R. Jureen, R.T. Lin, and J.W. Teo. 2013. OXA-181-producing Klebsiella pneumoniae establishing in Singapore. *BMC infectious diseases* 13:58.

Barton, M.H., A. Parviainen, and N. Norton. 2004. Polymyxin B protects horses against induced endotoxaemia in vivo. *Equine veterinary journal* 36:397-401.

Beceiro, A., E. Llobet, J. Aranda, J.A. Bengoechea, M. Doumith, M. Hornsey, H. Dhanji, H. Chart, G. Bou, D.M. Livermore, and N. Woodford. 2011a. Phosphoethanolamine modification of lipid A in colistin-resistant variants of Acinetobacter baumannii mediated by the pmrAB two-component regulatory system. *Antimicrobial agents and chemotherapy* 55:3370-3379.

Beceiro, A., S. Maharjan, T. Gaulton, M. Doumith, N.C. Soares, H. Dhanji, M. Warner, M. Doyle, M. Hickey, G. Downie, G. Bou, D.M. Livermore, and N. Woodford. 2011b. False extended-spectrum {beta}-lactamase phenotype in clinical isolates of Escherichia coli associated with increased expression of OXA-1 or TEM-1 penicillinases and loss of porins. *The Journal of antimicrobial chemotherapy* 66:2006-2010.

Bengoechea, J.A., and M. Skurnik. 2000. Temperature-regulated efflux pump/potassium antiporter system mediates resistance to cationic antimicrobial peptides in Yersinia. *Molecular microbiology* 37:67-80.

Bergen, P.J., A. Forrest, J.B. Bulitta, B.T. Tsuji, H.E. Sidjabat, D.L. Paterson, J. Li, and R.L. Nation. 2011. Clinically relevant plasma concentrations of colistin in combination with imipenem enhance pharmacodynamic activity against multidrug-resistant Pseudomonas aeruginosa at multiple inocula. *Antimicrobial agents and chemotherapy* 55:5134-5142.

Bergen, P.J., J. Li, C.R. Rayner, and R.L. Nation. 2006. Colistin methanesulfonate is an inactive prodrug of colistin against Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy* 50:1953-1958.

Beringer, P. 2001. The clinical use of colistin in patients with cystic fibrosis. *Current opinion in pulmonary medicine* 7:434-440.

Bogaerts, P., A. Verroken, B. Jans, O. Denis, and Y. Glupczynski. 2010. Global spread of New Delhi metallo-beta-lactamase 1. *The Lancet infectious diseases* 10:831-832.

Bogdanovich, T., J.M. Adams-Haduch, G.B. Tian, M.H. Nguyen, E.J. Kwak, C.A. Muto, and Y. Doi. 2011. Colistin-resistant, Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae belonging to the international epidemic clone ST258. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 53:373-376.

Boll, M., J. Radziejewska-Lebrecht, C. Warth, D. Krajewska-Pietrasik, and H. Mayer. 1994. 4-Amino-4-deoxy-L-arabinose in LPS of enterobacterial R-mutants and its possible role for their polymyxin reactivity. *FEMS immunology and medical microbiology* 8:329-341.

Bosso, J.A., C.A. Liptak, D.K. Seilheimer, and G.M. Harrison. 1991. Toxicity of colistin in cystic fibrosis patients. *DICP : the annals of pharmacotherapy* 25:1168-1170.

Boyen, F., F. Vangroenweghe, P. Butaye, E. De Graef, F. Castryck, P. Heylen, M. Vanrobaeys, and F. Haesebrouck. 2010. Disk prediffusion is a reliable method for testing colistin susceptibility in porcine E. coli strains. *Veterinary microbiology* 144:359-362.

Brink, A.J., J. Coetzee, C. Corcoran, C.G. Clay, D. Hari-Makkan, R.K. Jacobson, G.A. Richards, C. Feldman, L. Nutt, J. van Greune, J.D. Deetlefs, K. Swart, L. Devenish, L. Poirel, and P. Nordmann. 2013. Emergence of OXA-48 and OXA-181 carbapenemases among Enterobacteriaceae in South Africa and evidence of in vivo selection of colistin resistance as a consequence of selective decontamination of the gastrointestinal tract. *Journal of clinical microbiology* 51:369-372.

Callens, B., F. Boyen, B. Catry, A. Ingenbleek, P. Butaye, F. Haesebrouck, D. Maes, D. Persoons, M. Postma, and M. Laanen. 2012a. Reply to letter to the Editor by Moore and Elborn (2012)

concerning the manuscript" Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds" by B. Callens et al.(2012). *Preventive veterinary medicine*

Callens, B., D. Persoons, D. Maes, M. Laanen, M. Postma, F. Boyen, F. Haesebrouck, P. Butaye, B. Catry, and J. Dewulf. 2012b. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. *Preventive veterinary medicine* 106:53-62.

Canton, R., M. Akova, Y. Carmeli, C.G. Giske, Y. Glupczynski, M. Gniadkowski, D.M. Livermore, V. Miriagou, T. Naas, G.M. Rossolini, O. Samuelsen, H. Seifert, N. Woodford, and P. Nordmann. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 18:413-431.

Capone, A., M. Giannella, D. Fortini, A. Giordano, M. Meledandri, M. Ballardini, M. Venditti, E. Bordi, D. Capozzi, M.P. Balice, A. Tarasi, G. Parisi, A. Lappa, A. Carattoli, and N. Petrosillo. 2013. High rate of colistin resistance among patients with carbapenem-resistant Klebsiella pneumoniae infection accounts for an excess of mortality. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 19:E23-30.

Carlet, J., V. Jarlier, S. Harbarth, A. Voss, H. Goossens, and D. Pittet. 2012. Ready for a world without antibiotics? The Pensieres Antibiotic Resistance Call to Action. *Antimicrobial resistance and infection control* 1:11.

Catry, B., J. Dewulf, G. Opsomer, M. Vanrobaeys, A. Decostere, F. Haesebrouck, and A. de Kruif. 2007. Antibioticumgebruik en antimicrobiële resistentie bij rundvee ontwikkeling van een surveillancesysteem op bedrijfsniveau. (Use of antibiotics and antimicrobial resistance in cattle as evaluated by a herd-level surveillance system). University of Ghent, Faculty of Veterinary Medicine,

Chua, S.L., S.Y. Tan, M.T. Rybtke, Y. Chen, S.A. Rice, S. Kjelleberg, T. Tolker-Nielsen, L. Yang, and M. Givskov. 2013. C-di-GMP regulates antimicrobial peptide resistance in Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy*

Comandatore, F., D. Sassera, S. Ambretti, M.P. Landini, D. Daffonchio, P. Marone, V. Sambri, C. Bandi, and P. Gaibani. 2013. Draft Genome Sequences of Two Multidrug Resistant Klebsiella pneumoniae ST258 Isolates Resistant to Colistin. *Genome announcements* 1:

Conway, S.P., M.N. Pond, A. Watson, C. Etherington, H.L. Robey, and M.H. Goldman. 1997. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. *Thorax* 52:987-993.

Cornaglia, G., H. Giamarellou, and G.M. Rossolini. 2011. Metallo-beta-lactamases: a last frontier for beta-lactams? *The Lancet infectious diseases* 11:381-393.

Cosgrove, S.E. 2006. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 42 Suppl 2:S82-89.

Couet, W., N. Gregoire, P. Gobin, P.J. Saulnier, D. Frasca, S. Marchand, and O. Mimoz. 2011. Pharmacokinetics of colistin and colistimethate sodium after a single 80-mg intravenous dose of CMS in young healthy volunteers. *Clinical pharmacology and therapeutics* 89:875-879.

Daikos, G.L., A. Markogiannakis, M. Souli, and L.S. Tzouvelekis. 2012. Bloodstream infections caused by carbapenemase-producing Klebsiella pneumoniae: a clinical perspective. *Expert Rev Anti Infect Ther* 10:1393-1404.

David, M.D., and M.J. Gill. 2008. Potential for underdosing and emergence of resistance in Acinetobacter baumannii during treatment with colistin. *The Journal of antimicrobial chemotherapy* 61:962-964.

Del Bono. 2013. Outcome of bloodstream infections due to carbapenem resistant Klebsiella pneumoniae in a teaching hospital in Italy. Focus on the emergence of colistin-resistant strains. In s.I.C.o. Polymixins, editor http://monash.edu/pharm/about/events/polymyxins/.

Di Labio, E., G. Regula, A. Steiner, R. Miserez, A. Thomann, and U. Ledergerber. 2007. Antimicrobial resistance in bacteria from Swiss veal calves at slaughter. *Zoonoses and public health* 54:344-352.

Drlica, K., and X. Zhao. 2007. Mutant selection window hypothesis updated. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 44:681-688.

Durante-Mangoni, E., G. Signoriello, R. Andini, A. Mattei, M. De Cristoforo, P. Murino, M. Bassetti, P. Malacarne, N. Petrosillo, N. Galdieri, P. Mocavero, A. Corcione, C. Viscoli, R. Zarrilli, C. Gallo, and R. Utili. 2013. Colistin and Rifampicin Compared With Colistin Alone for the Treatment of

Serious Infections Due to Extensively Drug-Resistant Acinetobacter baumannii: A Multicenter, Randomized Clinical Trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 57:349-358.

ECDC. 2012. Antimicrobial consumption rates by country. Consumption of antimicrobials in ATC group A (alimentary tract and metabolism) in the community (primary care sector) in Europe, reporting year 2012. In http://ecdc.europa.eu/en/activities/surveillance/ESAC-Net/database/Pages/consumption-rates-by-country.aspx.

ECDC/EMEA. 2009. ECDC/EMEA Joint technical report. The bacterial challenge: time to react. A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents. In Stockholm.

EFSA. 2010. EFSA Panel on Biological Hazards; Scientific Opinion on a Quantitative Microbiological Risk Assessment of *Salmonella* in slaughter and breeder pigs In EFSA Journal. http://www.efsa.europa.eu/en/efsajournal/doc/1547.pdf.

EFSA. 2011. Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. *EFSA Journal* 9:

EFSA/ECDC. 2013. The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2011. *EFSA Journal* 11(5):

EMA/CVMP. 2010. Opinion following an Article 35 referral for veterinary medicinal formulations containing colistin at 2 000 000 IU per ml and intended for administration in drinking water to food producing species. In http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Colistin_35/WC500093733.pdf.

EMA/CVMP/SAGAM. 2009. Revised reflection paper on the use of 3rd and 4th generation cephalosporins in food producing animals in the European Union: development of resistance and impact on human and animal health. In http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004307.pdf.

ESVAC. 2012. European Medicines Agency. Sales of veterinary antimicrobial agents in 19 EU/EEA countries in 2010 (EMA/88728/2012). In http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500133532.

EUCAST. 2013. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1, 2013. http://www.eucast.org. In http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf.

European Pharmacopoeia. 2013. Council of Europe. European Public Health Committee. European pharmacopoeia. Maisonneuve,

Falagas, M.E., and S.K. Kasiakou. 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 40:1333-1341.

Falagas, M.E., E.K. Vouloumanou, and P.I. Rafailidis. 2009. Systemic colistin use in children without cystic fibrosis: a systematic review of the literature. *International journal of antimicrobial agents* 33:503 e501-503 e513.

Fernandez, L., W.J. Gooderham, M. Bains, J.B. McPhee, I. Wiegand, and R.E. Hancock. 2010. Adaptive resistance to the "last hope" antibiotics polymyxin B and colistin in Pseudomonas aeruginosa is mediated by the novel two-component regulatory system ParR-ParS. *Antimicrobial agents and chemotherapy* 54:3372-3382.

Fite, A., S. Macfarlane, E. Furrie, B. Bahrami, J.H. Cummings, D.T. Steinke, and G.T. Macfarlane. 2013. Longitudinal analyses of gut mucosal microbiotas in ulcerative colitis in relation to patient age and disease severity and duration. *Journal of clinical microbiology* 51:849-856.

Gales, A.C., R.N. Jones, and H.S. Sader. 2011. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). *The Journal of antimicrobial chemotherapy* 66:2070-2074.

Gales, A.C., A.O. Reis, and R.N. Jones. 2001. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *Journal of clinical microbiology* 39:183-190.

Garonzik, S.M., J. Li, V. Thamlikitkul, D.L. Paterson, S. Shoham, J. Jacob, F.P. Silveira, A. Forrest, and R.L. Nation. 2011. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrobial agents and chemotherapy* 55:3284-3294.

Geenen, P., H. Blaak, M. Koene, A. Havelaar, M. Leverstein-van Hall, M. Mulders, A.d. Neeling, A.d. Roda Husman, E. Stobberingh, and J. Wagenaar. 2011. Antimicrobial resistance transmissible from food animals to humans–a risk profile. *Dutch Society for Veterinary Epidemiology and Economics* 18.

Gentry, L.O. 1991. Bacterial resistance. *The Orthopedic clinics of North America* 22:379-388.

Guo, L., K.B. Lim, J.S. Gunn, B. Bainbridge, R.P. Darveau, M. Hackett, and S.I. Miller. 1997. Regulation of lipid A modifications by Salmonella typhimurium virulence genes phoP-phoQ. *Science* 276:250-253.

Halaby, T., N. Al Naiemi, J. Kluytmans, J. van der Palen, and C.M. Vandenbroucke-Grauls. 2013. Emergence of colistin resistance in enterobacteriaceae after the introduction of selective digestive tract decontamination in an intensive care unit. *Antimicrobial agents and chemotherapy* 57:3224-3229.

Hawley, J.S., C.K. Murray, and J.H. Jorgensen. 2008. Colistin heteroresistance in acinetobacter and its association with previous colistin therapy. *Antimicrobial agents and chemotherapy* 52:351-352.

He, H., J.C. Li, R.L. Nation, J. Jacob, G. Chen, H.J. Lee, B.T. Tsuji, P.E. Thompson, K. Roberts, T. Velkov, and J. Li. 2013. Pharmacokinetics of four different brands of colistimethate and formed colistin in rats. *The Journal of antimicrobial chemotherapy*

Huang, T.D., P. Bogaerts, C. Berhin, B. Jans, A. Deplano, O. Denis, and Y. Glupczynski. 2011. Rapid emergence of carbapenemase-producing Enterobacteriaceae isolates in Belgium. *Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin* 16:

Koch-Weser, J., V.W. Sidel, E.B. Federman, P. Kanarek, D.C. Finer, and A.E. Eaton. 1970. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. *Annals of internal medicine* 72:857-868.

Kontopoulou, K., E. Protonotariou, K. Vasilakos, M. Kriti, A. Koteli, E. Antoniadou, and D. Sofianou. 2010. Hospital outbreak caused by Klebsiella pneumoniae producing KPC-2 beta-lactamase resistant to colistin. *The Journal of hospital infection* 76:70-73.

Koyama, Y., A. Kurosasa, A. Tsuchiya, and K. Takakuta. 1950. A new antibiotic 'colistin'produced by spore-forming soil bacteria. *J Antibiot* 3:457-458.

Kumarasamy, K.K., M.A. Toleman, T.R. Walsh, J. Bagaria, F. Butt, R. Balakrishnan, U. Chaudhary, M. Doumith, C.G. Giske, S. Irfan, P. Krishnan, A.V. Kumar, S. Maharjan, S. Mushtaq, T. Noorie, D.L. Paterson, A. Pearson, C. Perry, R. Pike, B. Rao, U. Ray, J.B. Sarma, M. Sharma, E. Sheridan, M.A. Thirunarayan, J. Turton, S. Upadhyay, M. Warner, W. Welfare, D.M. Livermore, and N. Woodford. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet infectious diseases* 10:597-602.

Lambrini. 2013. In the era of polymyxins use: Emergence of colistin resistance in *Klebsiella pneumoniae*. In s.I.C.o. Polymyxins, editor http://monash.edu/pharm/about/events/polymyxins/.

Lamousin-White, M., and R.J. O'Callaghan. 1986. Association between colistin resistance and broad-spectrum recipient deficiency in Klebsiella pneumoniae. *Antimicrobial agents and chemotherapy* 30:964-965.

Landman, D., C. Georgescu, D.A. Martin, and J. Quale. 2008. Polymyxins revisited. *Clinical microbiology reviews* 21:449-465.

Ledson, M.J., M.J. Gallagher, C. Cowperthwaite, R.P. Convery, and M.J. Walshaw. 1998. Four years' experience of intravenous colomycin in an adult cystic fibrosis unit. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* 12:592-594.

Lesho, E., E.J. Yoon, P. McGann, E. Snesrud, Y. Kwak, M. Milillo, F. Onmus-Leone, L. Preston, K. St Clair, M. Nikolich, H. Viscount, G. Wortmann, M. Zapor, C. Grillot-Courvalin, P. Courvalin, R.

Clifford, and P.E. Waterman. 2013. Emergence of Colistin-Resistance in Extremely-drug-resistant Acinetobacter baumannii Containing a Novel pmrCAB Operon During Colistin Therapy of Wound Infections. *The Journal of infectious diseases*

Li, J., R.L. Nation, J.D. Turnidge, R.W. Milne, K. Coulthard, C.R. Rayner, and D.L. Paterson. 2006. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *The Lancet infectious diseases* 6:589-601.

Lim, L.M., N. Ly, D. Anderson, J.C. Yang, L. Macander, A. Jarkowski, 3rd, A. Forrest, J.B. Bulitta, and B.T. Tsuji. 2010. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy* 30:1279-1291.

Livermore, D.M. 2012. Current epidemiology and growing resistance of gram-negative pathogens. *The Korean journal of internal medicine* 27:128-142.

Lo-Ten-Foe, J.R., A.M. de Smet, B.M. Diederen, J.A. Kluytmans, and P.H. van Keulen. 2007. Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant Enterobacter cloacae and Acinetobacter baumannii strains. *Antimicrobial agents and chemotherapy* 51:3726-3730.

Lu, Q., R. Luo, L. Bodin, J. Yang, N. Zahr, A. Aubry, J.L. Golmard, and J.J. Rouby. 2012. Efficacy of high-dose nebulized colistin in ventilator-associated pneumonia caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii. *Anesthesiology* 117:1335-1347.

Lv, Y., J. Xiao, Q. Liu, H. Wu, Y. Zhang, and Q. Wang. 2012. Systematic mutation analysis of two-component signal transduction systems reveals EsrA-EsrB and PhoP-PhoQ as the major virulence regulators in Edwardsiella tarda. *Veterinary microbiology* 157:190-199.

MARAN. 2009. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands In 2009. In http://edepot.wur.nl/165958.

MARAN. 2012. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands. In http://www.uu.nl/SiteCollectionImages/Fac_DGK/Nieuwsplaatjes/Nieuws/2012/NethmapMaran_Web.pdf.

McKinnon, P.S., J.A. Paladino, and J.J. Schentag. 2008. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. *International journal of antimicrobial agents* 31:345-351.

Meletis, G., E. Tzampaz, E. Sianou, I. Tzavaras, and D. Sofianou. 2011. Colistin heteroresistance in carbapenemase-producing Klebsiella pneumoniae. *The Journal of antimicrobial chemotherapy* 66:946-947.

Menéndez González, S., A. Steiner, B. Gassner, and G. Regula. 2010. Antimicrobial use in Swiss dairy farms: quantification and evaluation of data quality. *Preventive veterinary medicine* 95:50-63.

Michalopoulos, A., and M.E. Falagas. 2008. Colistin and polymyxin B in critical care. *Critical care clinics* 24:377-391, x.

Michalopoulos, A.S., and M.E. Falagas. 2011. Colistin: recent data on pharmacodynamics properties and clinical efficacy in critically ill patients. *Annals of intensive care* 1:1-6.

Moffatt, J.H., M. Harper, P. Harrison, J.D. Hale, E. Vinogradov, T. Seemann, R. Henry, B. Crane, F. St Michael, A.D. Cox, B. Adler, R.L. Nation, J. Li, and J.D. Boyce. 2010. Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. *Antimicrobial agents and chemotherapy* 54:4971-4977.

Mohamed, A.F., I. Karaiskos, D. Plachouras, M. Karvanen, K. Pontikis, B. Jansson, E. Papadomichelakis, A. Antoniadou, H. Giamarellou, A. Armaganidis, O. Cars, and L.E. Friberg. 2012. Application of a loading dose of colistin methanesulfonate in critically ill patients: population pharmacokinetics, protein binding, and prediction of bacterial kill. *Antimicrobial agents and chemotherapy* 56:4241-4249.

Moore, J.N., and M.H. Barton. 2003. Treatment of endotoxemia. *The Veterinary clinics of North America. Equine practice* 19:681-695.

Moskowitz, S.M., M.K. Brannon, N. Dasgupta, M. Pier, N. Sgambati, A.K. Miller, S.E. Selgrade, S.I. Miller, M. Denton, S.P. Conway, H.K. Johansen, and N. Hoiby. 2012. PmrB mutations promote polymyxin resistance of Pseudomonas aeruginosa isolated from colistin-treated cystic fibrosis patients. *Antimicrobial agents and chemotherapy* 56:1019-1030.

Moskowitz, S.M., R.K. Ernst, and S.I. Miller. 2004. PmrAB, a two-component regulatory system of Pseudomonas aeruginosa that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. *Journal of bacteriology* 186:575-579.

Napier, B.A., E.M. Burd, S.W. Satola, S.M. Cagle, S.M. Ray, P. McGann, J. Pohl, E.P. Lesho, and D.S. Weiss. 2013. Clinical Use of Colistin Induces Cross-Resistance to Host Antimicrobials in Acinetobacter baumannii. *mBio* 4:

Nation, R.L., and J. Li. 2009. Colistin in the 21st century. *Curr Opin Infect Dis* 22:535-543.

Nord, N.M., and P.D. Hoeprich. 1964. Polymyxin B and Colistin. A Critical Comparison. *The New England journal of medicine* 270:1030-1035.

Nummila, K., I. Kilpelainen, U. Zahringer, M. Vaara, and I.M. Helander. 1995. Lipopolysaccharides of polymyxin B-resistant mutants of Escherichia coli are extensively substituted by 2-aminoethyl pyrophosphate and contain aminoarabinose in lipid A. *Molecular microbiology* 16:271-278.

O'Callaghan, R.J., K.M. Rousset, N.K. Harkess, M.L. Murray, A.C. Lewis, and W.L. Williams. 1978. Analysis of increasing antibiotic resistance of Klebsiella pneumoniae relative to changes in chemotherapy. *The Journal of infectious diseases* 138:293-298.

O'Hara, J.A., L.A. Ambe, L.G. Casella, B.M. Townsend, M.R. Pelletier, R.K. Ernst, R.M. Shanks, and Y. Doi. 2013. Activity of Vancomycin-Containing Regimens against Colistin-Resistant Acinetobacter baumannii Clinical Strains. *Antimicrobial agents and chemotherapy*

Official Journal of the European Communities. 2013. Commission Regulation (EU) No 122/2013 of 12 February 2013 amending Regulation (EC) No 1950/2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of equidae. In http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:042:0001:0017:EN:PDF.

Pardon, B., B. Catry, J. Dewulf, D. Persoons, M. Hostens, K. De Bleecker, and P. Deprez. 2012. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. *The Journal of antimicrobial chemotherapy* 67:1027-1038.

Persoons, D., J. Dewulf, A. Smet, L. Herman, M. Heyndrickx, A. Martel, B. Catry, P. Butaye, and F. Haesebrouck. 2012a. Antimicrobial use in Belgian broiler production. *Preventive veterinary medicine*

Persoons, D., J. Dewulf, A. Smet, L. Herman, M. Heyndrickx, A. Martel, B. Catry, P. Butaye, and F. Haesebrouck. 2012b. Antimicrobial use in Belgian broiler production. *Preventive veterinary medicine* 105:320-325.

Petrosillo, N., M. Giannella, R. Lewis, and P. Viale. 2013. Treatment of carbapenem-resistant Klebsiella pneumoniae: the state of the art. *Expert review of anti-infective therapy* 11:159-177.

Plachouras, D., M. Karvanen, L.E. Friberg, E. Papadomichelakis, A. Antoniadou, I. Tsangaris, I. Karaiskos, G. Poulakou, F. Kontopidou, A. Armaganidis, O. Cars, and H. Giamarellou. 2009. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. *Antimicrobial agents and chemotherapy* 53:3430-3436.

Pogue, J.M., D. Marchaim, D. Kaye, and K.S. Kaye. 2011. Revisiting "older" antimicrobials in the era of multidrug resistance. *Pharmacotherapy* 31:912-921.

Poudyal, A., B.P. Howden, J.M. Bell, W. Gao, R.J. Owen, J.D. Turnidge, R.L. Nation, and J. Li. 2008. In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. *The Journal of antimicrobial chemotherapy* 62:1311-1318.

Qureshi, Z.A., D.L. Paterson, B.A. Potoski, M.C. Kilayko, G. Sandovsky, E. Sordillo, B. Polsky, J.M. Adams-Haduch, and Y. Doi. 2012. Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. *Antimicrobial agents and chemotherapy* 56:2108-2113.

Rattanaumpawan, P., J. Lorsutthitham, P. Ungprasert, N. Angkasekwinai, and V. Thamlikitkul. 2010. Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by Gram-negative bacteria. *The Journal of antimicrobial chemotherapy* 65:2645-2649.

Reed, M.D., R.C. Stern, M.A. O'Riordan, and J.L. Blumer. 2001. The pharmacokinetics of colistin in patients with cystic fibrosis. *Journal of clinical pharmacology* 41:645-654.

Reines, M., E. Llobet, C.M. Llompart, D. Moranta, C. Perez-Gutierrez, and J.A. Bengoechea. 2012. Molecular basis of Yersinia enterocolitica temperature-dependent resistance to antimicrobial peptides. *Journal of bacteriology* 194:3173-3188.

Ryan, K.J., L.I. Schainuck, R.O. Hickman, and G.E. Striker. 1969. Colistimethate toxicity. Report of a fatal case in a previously healthy child. *JAMA : the journal of the American Medical Association* 207:2099-2101.

Schindler, M., and M.J. Osborn. 1979. Interaction of divalent cations and polymyxin B with lipopolysaccharide. *Biochemistry* 18:4425-4430.

Schorr, C. 2009. Performance improvement in the management of sepsis. *Critical care clinics* 25:857-867, x.

Snitkin, E.S., A.M. Zelazny, J. Gupta, T.N. Palmore, P.R. Murray, and J.A. Segre. 2013. Genomic insights into the fate of colistin resistance and Acinetobacter baumannii during patient treatment. *Genome research* 23:1155-1162.

Timmerman, T., J. Dewulf, B. Catry, B. Feyen, G. Opsomer, A. de Kruif, and D. Maes. 2006. Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. *Preventive veterinary medicine* 74:251-263.

Tumbarello, M., P. Viale, C. Viscoli, E.M. Trecarichi, F. Tumietto, A. Marchese, T. Spanu, S. Ambretti, F. Ginocchio, F. Cristini, A.R. Losito, S. Tedeschi, R. Cauda, and M. Bassetti. 2012. Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: importance of combination therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 55:943-950.

Vaara, M., and T. Vaara. 2013. The novel polymyxin derivative NAB739 is remarkably less cytotoxic than polymyxin B and colistin to human kidney proximal tubular cells. *International journal of antimicrobial agents* 41:292-293.

van Belkum, A., and W.M. Dunne, Jr. 2013. Next Generation Antimicrobial Susceptibility Testing. *Journal of clinical microbiology*

Vicari, G., S.R. Bauer, E.A. Neuner, and S.W. Lam. 2013. Association between colistin dose and microbiologic outcomes in patients with multidrug-resistant gram-negative bacteremia. *Clinical Infectious Diseases* 56:398-404.

Xu, Y., X. Tian, C. Ren, H. Huang, X. Zhang, X. Gong, H. Liu, Z. Yu, and L. Zhang. 2012. Analysis of colistin A and B in fishery products by ultra performance liquid chromatography with positive electrospray ionization tandem mass spectrometry. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* 899:14-20.

Yoon, E.J.M., P.; Lesho, E.;Waterman, P.; Clifford, R.; Courvalin, P.; Grillot-Courvalin, C. 2013. Biological cost of colistin-resistance in clinical isolates of Acinetobacter baumannii. *ECCMID*

Zarb, P., B. Coignard, J. Griskeviciene, A. Muller, V. Vankerckhoven, K. Weist, M. Goossens, S. Vaerenberg, S. Hopkins, B. Catry, D. Monnet, H. Goossens, and C. Suetens. 2012. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. *Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin* 17:

Zarkotou, O., S. Pournaras, E. Voulgari, G. Chrysos, A. Prekates, D. Voutsinas, K. Themeli-Digalaki, and A. Tsakris. 2010. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing Klebsiella pneumoniae: a matched case-control study. *Journal of clinical microbiology* 48:2271-2274.

Zeidler, U., M.E. Bougnoux, A. Lupan, O. Helynck, A. Doyen, Z. Garcia, N. Sertour, C. Clavaud, H. Munier-Lehmann, C. Saveanu, and C. d'Enfert. 2013. Synergy of the antibiotic colistin with echinocandin antifungals in Candida species. *The Journal of antimicrobial chemotherapy*