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

Draft

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1. Executive summary

Colistin is an antibacterial agent of the polymyxin class. Following the discovery of a new colistin horizontally transferable resistance mechanism (MCR-1), the European Commission requested the European Medicines Agency to update the previous advice on the impact of and need for colistin use for human and animal health (EMA, 2013). This updated advice provides an analysis of the colistin toxicity, susceptibility testing, activity and resistance mechanisms, risk profile (based upon the consumption patterns and epidemiology), and risk management options.

Soon after its introduction in the 1950s, the use of colistin in human medicine was predominantly restricted to topical administrations due to its toxicity if given systemically. Severe nosocomial infections due to multidrug-resistant (MDR) Gram-negative bacteria increasingly account for high morbidity and mortality and colistin is therefore nowadays a last resort drug in human medicine in the context of systemic treatment of infections caused by MDR *Pseudomonas aeruginosa, Acinetobacter baumannii* and Enterobacteriaceae (*Escherichia coli, Klebsiella pneumoniae*). The prospect of novel alternative antimicrobials for treatment of infections due to MDR pathogens in the near future is limited. The main indications for systemic use in human medicine are treatment and control of infections in cystic fibrosis patients and treatment of severe systemic infections. In some countries oral colistin is in addition used in prophylaxis of healthcare-associated infections through selective digestive tract decontamination (SDD). Total consumption of colistin in humans (reflecting topical, inhalational and systemic routes of administration combined) varies widely between European Union/European Economic Area (EU/EEA) countries but has doubled in some of EU/EEA countries between 2010 and 2014 following the rise in MDR Gram negative pathogens involved in healthcare-associated infections.

Under routine laboratory conditions a broth dilution methodology is recommended to determine colistin resistance. Care should be taken for proper identification to avoid overestimation of acquired colistin resistance due to some intrinsically less susceptible bacteria (*Salmonella* spp.) Bacteria containing antimicrobial resistance genes can be selected through the use of colistin. Spread may be *via* passing on chromosomal genes to daughter colonies (vertical transmission) or *via* mobile genetic elements (horizontal transmission).

In isolates from humans, colistin resistance due to chromosomal mechanisms has increased dramatically in some countries including Greece and Italy but resistance levels are now also increasing in most other EU/EEA countries. Mobile (transferable) colistin resistance, mediated by the *mcr-1* gene, has been documented in several EU/EEA countries. This is of great concern due to the rapidly increasing use of colistin in EU/EEA hospitals leading to increased selection pressure. Furthermore, other antimicrobial classes can further stimulate the spread of colistin resistance via co-selection when there is simultaneous presence of such resistance genes (i.e. beta-lactamases, including carbapenemases). The *mcr-1* gene was found in similar plasmids in the same bacterial species isolated from food-producing animals, food, humans and the environment indicating a possible transmission between these compartments. Nevertheless the overall prevalence of colistin resistance in animals remains - so far and with some exceptions - low in food and in animals in the EU/EEA. Even though retrospective studies on collections of isolates have shown that the *mcr-1* gene has been present in some bacterial species for decades, data from China indicate that the situation is changing and that the prevalence of such strains is increasing. The *mcr-1* gene is present both in isolates from clinical cases of veterinary colibacillosis and in invasive human pathogens. Human carriers can become negative within one month in the absence of a selection pressure. The relative proportion amid human clinical isolates in the EU/EEA remains fairly low (less than 1%), so far.

Colistin has been used regularly in veterinary medicine for decades, both as curative treatment and for prevention of disease. It is of therapeutic importance for the treatment of Gram-negative gastrointestinal infections in certain food-producing species. Colistin is predominantly administered as group treatment using the oral route of administration. In 2013, polymyxins (mainly colistin) were the 5th most sold group of antimicrobials (6.1 %) based on the total sales of polymyxins in 26 EU/EEA countries reporting data. The possible alternatives to colistin, depending on the resistance situation in a particular country, include aminopenicillins, trimethoprim, sulphonamides, tetracyclines, aminoglycosides, cephalosporins and fluoroquinolones. If colistin is no longer available in veterinary medicine it could be speculated that other antimicrobials or medication would replace its use if no concomitant interventions such as vaccination or improved biosecurity measures are taken.

The larger abundance of the *mcr-1* gene in veterinary isolates compared to human isolates, together with the much higher use of colistin in livestock compared to human medicine, and the finding of the *mcr-1* gene along with genetic determinants typically seen in animal environments, has been considered suggestive of a flow from animals to humans.

In December 2014 the CVMP recommended to restrict the indications for use of colistin to treatment of enteric infections caused by susceptible non-invasive *E. coli* only, that any indications for prophylactic use should be removed and that the treatment duration should be limited to the minimum time necessary for the treatment of the disease and not exceed 7 days. In addition, it was recommended to remove horses from the Summary of Product Characteristics (SPCs) on the grounds of target species safety concerns. In April 2016 the CVMP recommended the withdrawal of the marketing authorisations for all veterinary medicinal products containing colistin in combination with other antimicrobial substances.

There is a wide variation between European Union (EU) Member States (MS) in the extent of veterinary use of colistin. From the data available the variation cannot be directly linked to the predominance of specific animal species, a category or husbandry system in an individual MS, with some MS 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.

Antimicrobial use in both human and veterinary medicine must be rationalised and reserved for clinical conditions. Further to previous advice, the Antimicrobial Advice *ad hoc* Expert Group (AMEG) main recommendations, which were endorsed by the CVMP and the CHMP are that colistin sales for use in animals should be reduced to the minimum feasible (see below) and that colistin should be added to a higher risk category (category 2) of the AMEG classification (EMA, 2014a).

There are wide variations in the use of colistin adjusted for the biomass under exposure (kg livestock, expressed as population correction unit (PCU))[1], between countries and these are largely unexplained. Countries with intensive livestock production can have a level of usage below 1 mg/PCU (e.g. Denmark and the UK) or much higher, up to 20 to 25 mg/PCU (Italy and Spain). Considering the rapidly increasing importance of colistin for treatment of critically ill human patients, all countries should strive to reduce the use of polymyxins as much as possible.

For the current "high and moderate consumers" the target and desirable levels are set at 5 mg/PCU and 1 or below 1 mg/PCU, respectively, based on the observations on the level of use in other countries. Meanwhile more information should be gathered to determine the minimum level of colistin

[1] The population correction unit (PCU) corresponds to the food-producing animal population that can be subject to treatment with antimicrobial agents, for further details see: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp&mid=WC0b01ac0580153a00&jsenabled=true

use that can be achieved while maintaining animal welfare and preventing the increased use of other Critically Important Antimicrobials (CIAs).

Reduction in use of colistin should be achieved without an increase in the use (in mg/PCU) of fluoroquinolones, 3rd- and 4th-generation cephalosporins or overall consumption of antimicrobials.

The above targets for reduction in sales of colistin should be achieved in a period of 3 to 4 years.

If the situation regarding colistin resistance in animals or humans further deteriorates, it may be necessary to lower the proposed targets.

2. Introduction

The global emergence and steady increase in bacteria that are resistant to multiple antimicrobials has become a public health threat (Carlet et al., 2012). Human infections with MDR bacteria are associated with higher patient morbidity and mortality, higher costs and longer length of hospital stay (Cosgrove, 2006; Hauck et al., 2016; Schorr, 2009). In the current state of increasing resistance coupled with a 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 antimicrobial 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).

In human medicine, colistin was early on predominately restricted to topical use due to its systemic toxicity (Nord and Hoeprich, 1964). The last 10 years, increasing numbers of hospital outbreaks with carbapenemase-producing Enterobacteriaceae (*E. coli, Klebsiella* species), and multidrug-resistant (MDR) *Pseudomonas* and *Acinetobacter* species (i.e. non-fermentative Gram-negative bacteria), have forced clinicians to re-introduce systemic colistin treatment, as a last resort drug for the treatment of healthcare-associated infections in which these organisms are involved. Colistin therefore increasingly has 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, colistimethate sodium (CMS) is used by inhalation for the treatment of *Pseudomonas aeruginosa* lung infections in patients with cystic fibrosis. In certain countries prophylaxis of healthcare-associated infections by means of selective digestive tract decontamination (SDD) also includes the use of colistin in the antimicrobial regimen.

Colistin has been used for decades in veterinary medicine, especially in swine and veal calves. Based on SPCs (prior to the last referral procedures, see chapter 3.2. for further details) Gram-negative infections of the intestinal tract, due to *E. coli* and *Salmonella* spp. were the primary indications. Most of the colistin applications in animals are for oral group treatments.

In July 2013 the AMEG was convened on behalf of the European Commission (EC) by the European Medicines Agency (EMA) and concluded that 'for colistin use in particular, detailed monitoring of colistin resistant bacteria is required to confirm horizontal gene transfer is not involved and that 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' (EMA, 2013).

In light of this recommendation, and following the recent discovery of *mcr*-1, a horizontal transferable resistance gene in bacteria of food animal origin (Liu et al., 2015), the impact of the current or future use of colistin products in veterinary medicine for animal health and welfare has been re-assessed.

3. The use of colistin in human and veterinary medicine

3.1. Human medicine

Due to the major concerns for neuro- and nephrotoxicity (Koch-Weser et al., 1970; Ryan et al., 1969), parenteral use of polymyxins has until recently been limited and polymyxins were mainly for ophthalmic and topical use (Falagas and Kasiakou, 2005; Koch-Weser et al., 1970). Cystic fibrosis patients have been an exception to this practice for decades, and such patients have received systemic or nebulised colistin to control lower airway bacterial infections and complications (Beringer, 2001; Tappenden et al., 2013). During the last five years two major indications have renewed the interest for polymyxin in human medicine, namely as part of surgical prophylaxis via selective digestive tract decontamination (SDD) and for MDR Gram-negative healthcare-associated infections.

For human patients, two salt forms of polymyxin E (colistin) have been widely commercially available, namely colistin sulphate and colistimethate sodium (CMS, syn colistin methanesulphate, colistin sulphonyl methate, pentasodium colistimethanesulphate). CMS is a prodrug of colistin 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 parts of the world, in the EU/EEA polymyxin B is used only for topical administration in humans. Polymyxin B has been reported to be associated with a similar or even worse toxicity pattern than colistin 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.

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 multidrug-resistant (MDR), 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 increasingly have to resort to colistin to treat nosocomial infections in critically ill patients, such as bacteraemia and ventilator-associated pneumonia (VAP), 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 Metallo-β-Lactamase 1, NDM-1 and the Verona integron-encoded metallo-β-lactamase , VIM) (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 (ECDC, 2016; Huang et al., 2011).

Colistin in combination with other antibiotics such as tigecycline or carbapenems is used in some countries as limited available treatment options for carbapenemase-producing Enterobacteriaceae*, Acinetobacter* spp. *and Pseudomonas* spp*.* (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 extremely drug-resistant (XDR) *Acinetobacter baumannii*, despite synergism was shown *in vitro* (Durante-Mangoni et al., 2013).

The use of colistin by inhalation as adjunctive therapy or as monotherapy for treatment of VAP has also been explored (Lu et al., 2012; Michalopoulos and Falagas, 2008; Rattanaumpawan et al., 2010); 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 MDR Gram-negative infections (Falagas et al., 2009). For MDR and XDR Gram-negative infections, a recent survey among 94 children has found colistin to be non-inferior to a non-colistin treatment group (Ozsurekci et al., 2016), although in both groups infection-related mortality was high (11% and 13.3%, respectively).

The 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 neurotoxicity – and occasionally irreversible – nephrotoxicity (approximately 7%), compared to more recent studies. The exception is cystic fibrosis patients in whom up to 29% adverse (neurological) effects have been reported (Bosso et al., 1991; Reed et al., 2001). The need for higher doses of CMS to achieve adequate colistin concentrations for therapeutic effect, as shown in recent studies (Garonzik et al., 2011; Plachouras et al., 2009), raises concerns around the consequent further increase in nephrotoxicity (Pogue et al., 2011). To contain toxic side-effects following systemic use of colistin, 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 was hampered in the past by remaining uncertainties 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) and by the uncertainty regarding what is actually delivered as active substance to the patient (Garonzik et al., 2011; Mohamed et al., 2012; Vicari et al., 2013). In the context of a recent article 31 referral procedure of Directive 2001/83/EC, the EMA Committee for Human Medicinal products (CHMP) reviewed the existing evidence and decided to revise the approved indication so that colistin can be used without age restrictions, but only for the treatment of infections with limited treatment options. The posology and method of administration section of the Summary of Product Characteristics (SmPC) were revised, and the need of a loading dose was agreed upon. No firm recommendations could be nevertheless made for patients with hepatic or renal impairment and for patients on renal replacement therapy, due to the scarcity of data for these subpopulations (EMA, 2014b)[2,3]. Within the same framework, the CHMP also reviewed the optimal way of expressing the strength and dose of colistin and agreed that the EU product information for CMS will continue to be expressed in International Units (IU). At the same time, a dose

[2] http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Polymyxin_31/WC500179663.pdf
[3] http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Polymyxin_31/WC500176332.pdf

content conversion table between CMS (expressed in IU and in mg) and colistin base activity (expressed in mg) was introduced to help the prescribers.

Colistin is used in human medicine both in the community and hospital sectors, and there is a growing need in specific settings like intensive care units (Ingenbleek et al., 2015) and for treatment of healthcare-associated infections due to carbapenemase-producing Gram-negative bacteria (ECDC, 2016). Medical doctors often have to rely on colistin for the treatment of these infections. Alternative antibacterials such as tigecycline, fosfomycin and temocillin also have limitations and are sometimes authorized only in a limited number of countries across EU MSs. Few new antimicrobials for systemic infections with MDR Gram-negative pathogens are expected in the future. Of notice, a new beta (β-)lactam- β-lactamase inhibitor combination product (ceftazidime-avibactam), which is active against organisms that produce serine-based but not metallo-based carbapenemases, was approved by the Food and Drug Administration of the USA (FDA) in 2015 and received a positive opinion from the CHMP in April 2016.

Total consumption (reflecting topical, inhalational and systemic routes of administration combined) varies widely between EU/EEA countries and doubled between 2010 and 2014 (ECDC, 2015) following the rise in MDR Gram-negative pathogens involved in healthcare-associated infections (Skov and Monnet, 2016). **Table 1** shows the distribution of and trends in the consumption of polymyxins (mainly colistin) for systemic use in EU/EEA countries.

Table 1. Trends in consumption of polymyxins in EU/EEA countries, 2010-2014 (expressed in DDD per 1 000 inhabitants and per day)

Source: European Centre for Disease Prevention and Control (ECDC): "*Summary of the latest data on antibiotic consumption in the European Union, ESAC-Net surveillance data, November 2015*" (ECDC, 2015)

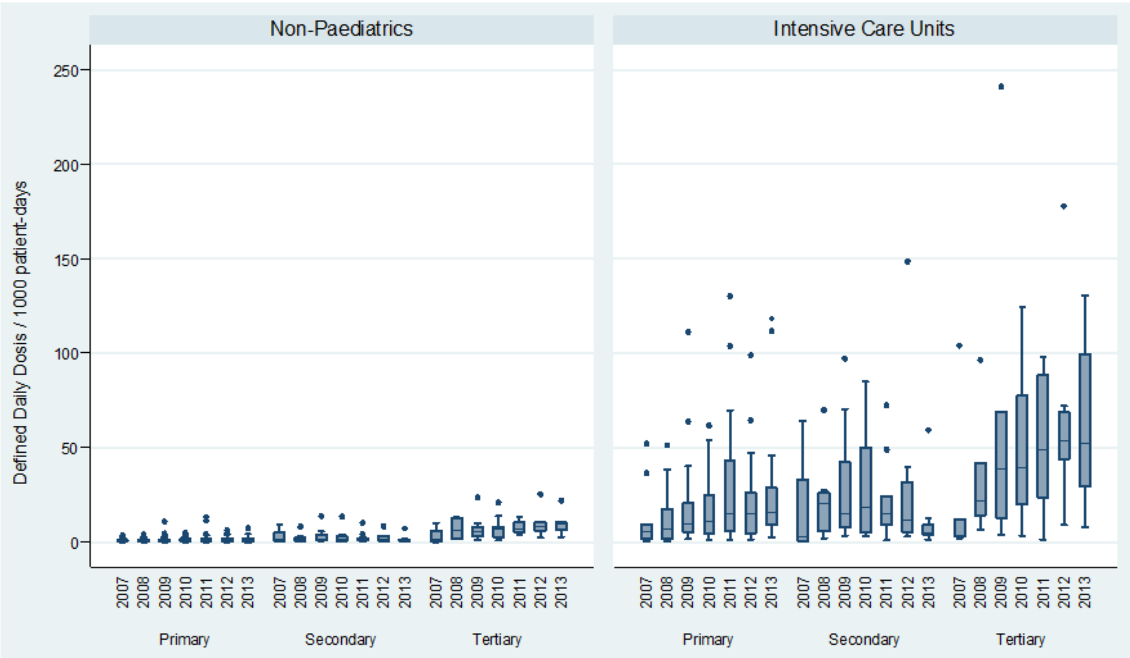
Country	2010	2011	2012	2013	2014	Trends in consumption of polymyxins, 2010–2014	Average annual change 2010–2014	Statistical significance
Finland (b)	0	0	0	0	0			n.a.
Lithuania (a)			0	0	0			n.a.
Norway	0.0002	0.0004	0.0006	0.0006	0.0006		<0.001	significant
Poland (a)					0.001			n.a.
Latvia	0	0	0.003	0.002	0.001		<0.001	n.s.
Sweden	0.000	0.001	0.001	0.001	0.001		<0.001	n.s.
Netherland	0.006	0.003	0.002	0.003	0.002		-0,001	n.s.
Bulgaria	0	0	0	0	0.002		<0.001	n.s.
Estonia	<0.001	<0.001	0.002	0	0.002		<0.001	n.s.
Denmark	0.002	0.002	0.002	0.001	0.003		<0.001	n.s.
Luxembourg	0.005	0.005	0.005	0.006	0.003		<0.001	n.s.
Slovenia	0.001	0.002	0.003	0.003	0.005		0.001	n.s.
United Kingdom (a)(d)				0.005	0.006			n.a.
Hungary	0.002	0.004	0.005	0.006	0.007		0.001	significant
France	0.008	0.008	0.008	0.008	0.008		<0.001	n.s.
Malta	0.026	0.004	0.002	0.006	0.011		0.003	n.s.
EU/EEA	**0.008**	**0.011**	**0.014**	**0.012**	**0.012**		**<0.001**	**n.s.**
Ireland	0.014	0.014	0.015	0.015	0.013		<0.001	n.s.
Portugal (c)	0.013	0.018	0.019	0.020	0.019		0.001	n.s.
Croatia	0.055	0.010	0.029	0.003	0.019		0.008	n.s.
Slovakia (a)			0.020	0.023	0.025			n.a.
Italy	0.012	0.011	0.019	0.023	0.025		0.004	significant
Greece (a)		0.078	0.085	0.084	0.095			n.a.
Belgium	0.008	0.009	0.006	0.008				n.a.

The number for EU/EEA refers to the corresponding population-weighted mean consumption, calculated by summing the products of each country's consumption in DDD per 1 000 inhabitants an per day x country population as in Eurostat, and then dividing this sum by the total EU/EEA population.

a) These countries did not report data for all years during the period 2010-2014.
b) Finland: data include consumption in remote primary healthcare centres and nursing homes.
c) Portugal: data relate to public hospitals only.
d) United Kingdom: data do not include consumption from UK-Wales (2013) or UK-Northern Ireland (2014).
n.a.: not applicable; linear regression was not applied due to missing data.
n.s.: not significant.

Long-term, detailed surveillance is needed to monitor the evolution at the country level and stratified by speciality. For example in Belgium, the use of colistin has more than doubled in intensive care units according to the latest surveillance data, in particular in university hospitals (**Figure 1**).

Figure 1. Evolution of colistin use (J01XB01) in Belgian acute care hospitals, 2007-2013, stratified by type of care (Primary = general hospitals; Secondary = general hospital with teaching missions; Tertiary = teaching/university hospital), modified from (Ingenbleek et al., 2015).



Evolution is expressed in DDD (defined daily dose) per 1000 patient-days for hospital wide non-paediatrics wards (left) and intensive care units (right). Participation rates exceed on average >85% among 110 acute care hospitals over consecutive years.

Virulent clones of *K. pneumoniae* or other difficult to treat Gram-negative bacteria are becoming resistant during therapy and associated with hospital outbreaks within the EU/EEA and worldwide (Balm et al., 2013; Brink et al., 2013; Comandatore et al., 2013; Del Bono, 2013; Lambrini, 2013; Lesho et al., 2013; Monaco et al., 2014; Onori et al., 2015; Snitkin et al., 2013). Analysis of nosocomial outbreaks with *Acinetobacter baumannii* indicated that prior carbapenem and colistin consumption may have acted as triggering factors for the development of resistance (Agodi et al., 2014; Wright et al., 2016). As outlined below, the *mcr-1* gene has now been shown in different human isolates including invasive pathogens both in hospital and ambulatory care (**Table 9**) (Meletis et al., 2011), and outbreaks due to MDR pathogens expressing the *mcr-1* gene might occur in the near future.

Colistin resistance thus has been emerging rapidly following its reintroduction in human medicine, 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, colistin resistance rates rose from 0% in 2007 to 8.1% in 2008 and to 24.3% in 2009 (Meletis et al., 2011). The latest estimates from Italy show a rise of colistin resistance in *K. pneumoniae* from 1 to 2% in 2006 to 33% in 2009 (Monaco et al., 2014). Prior to the discovery of the *mcr-1* gene, a Dutch survey has demonstrated that colistin resistance, shown to be clonal in nature after oral use in the ICU for selective digestive tract decontamination (SDD), can rapidly spread in a hospital and therefore SDD should be discouraged in outbreak settings (Halaby et al., 2013). Since *mcr-1*-producing bacteria already have been isolated from a limited number of human patients (**Table 9**) Poirel et al. (2016) expressed similar concerns and requested an urgent review of SDD, given the occurrence of horizontally transferable colistin resistance.

3.2. Veterinary medicine

Within the EU MSs, colistin and polymyxin B are authorised nationally. Main indications are infections caused by Enterobacteriaceae in pigs, poultry, cattle, sheep, goats and rabbits. Colistin is also used in laying hens and cattle, sheep and goats producing milk for human consumption. Typically, colistin products are administered orally, in feed, in drinking water, as a drench, or through milk replacer diets. Combinations of colistin with other antimicrobials are available for group treatments of food-producing animals in some EU countries. Products for parenteral and intramammary administration are also available, and Gram-negative infections in ruminants including endotoxaemia are claimed indications. Polymyxin B is on the list of substances essential for the treatment of equidae for systemic treatment for endotoxaemia (antitoxigenic effect, not antibacterial as such) associated with severe colic and other gastrointestinal diseases (Barton et al., 2004; Moore and Barton, 2003; Official Journal of the European Union, 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 with 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 MSs, veterinary medicinal products (VMPs) containing colistin are not on the market, i.e. not commercialised (EMA/ESVAC, 2015).

Colistin products (polymyxin E) have never been marketed for use in animals in the United States (US Food and Drug Administration, 2016). Sources from the FDA have indicated that there is only one polymyxin B product (ophthalmic ointment, combination of polymyxin B and oxytetracycline) approved for use in food-producing species. In recent years, this product has been marketed in 2009 and 2012-2015, although it has been marketed in small quantities. Polymyxin B is also available in the US as a component of approved ophthalmic products (for use in dogs and cats) and otic products (for use in dogs). There is documented legal off label use in other non-food-producing species, such as horses. Sources from the Public Health Agency of Canada have indicated that there are no approved colistin products (polymyxin E) for use in animals in Canada (Public Health Agency of Canada, 2016).

In the EU/EEA, colistin has been used in veterinary medicine 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 and per day 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. 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 varied between animal species, farm types and indications. Timmerman (2006) reported underdosing (sub-dosing) of oral colistin 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., 2016; Catry et al., 2007; Menéndez González et al., 2010). In 32 broiler farms in Belgium, the use of colistin was not reported despite detailed antimicrobial consumption records (Persoons et al., 2012), although colistin has been used in medicated feed (www.belvetsac.ugent.be). Older studies from 2001-2003 in a limited number of Belgian cattle farms, have shown that feed (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).

In 2013, the total sales of polymyxins in the 26 EU/EEA countries reporting data to the ESVAC project, including tablets but excluding topical forms, polymyxins were the 5th most sold group of antimicrobials (6.1%), after tetracyclines (36.7%), penicillins (24.5%), sulphonamides (9.6%), and macrolides (7.4%). Total sales in weight summed up 495 tonnes. Of those 99.7% were for oral forms as follows: 43.2% were oral solution (powder for use in drinking water), 42.4% were premix (premixes for medicated feeding stuff) and 14.0% were oral powder (powder to be administered with the feed). Small amounts were sold as: injectables (0.2%), tablets (0.1%) and intramammaries, intrauterines and oral paste (less than 0.0% for each of the three forms). Of the group of polymyxins, colistin represented more than 99.9% of the sales. In addition combinations of colistin with other antimicrobials are authorised in some MSs. The sales of those combination products represents less than 10% of the overall sales of colistin (data not published).

Some MSs with high consumption of polymyxins have shown a decrease in consumption between 2011 and 2013, whereas others have shown a stable situation or even an increase (**Figure 2**). In Belgium, polymyxin use showed a 28.1% decrease in 2014. This reduction seen for the second year in a row has been attributed due to start of the use of zinc oxide as an alternative for colistin use in the treatment of post-weaning diarrhoea in piglets (BelVetSac, 2015). The last ESVAC report shows an overall decrease of 19% of sales of polymyxins in 23 countries over the last year (EMA/ESVAC, 2015).

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

Figure 2. Consumption estimates based upon sales for food-producing animals (including horses) of polymyxins, adjusted for biomass under exposure (in mg/PCU), by country, for 2011-2013 (EMA/ESVAC, 2015). No sales reported in Finland, Iceland and Norway.

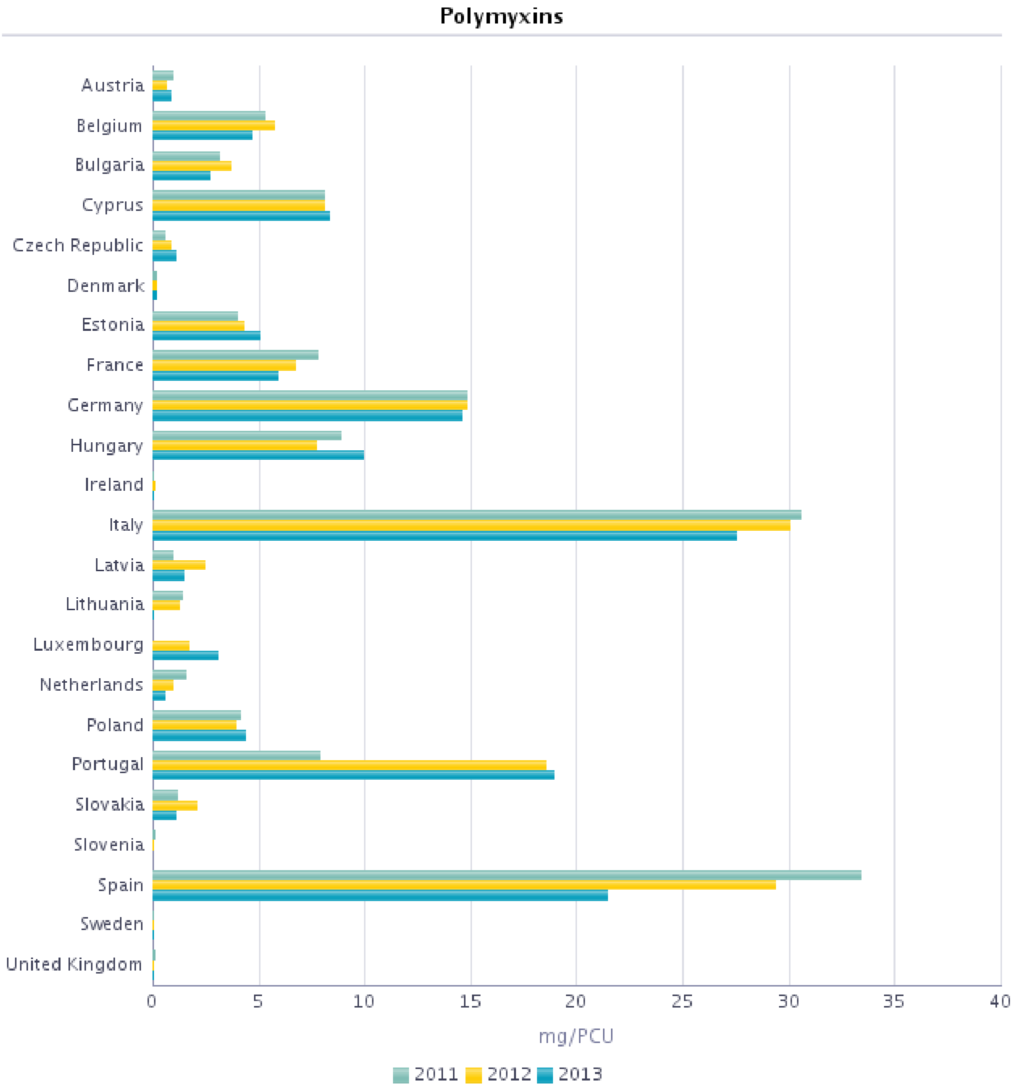
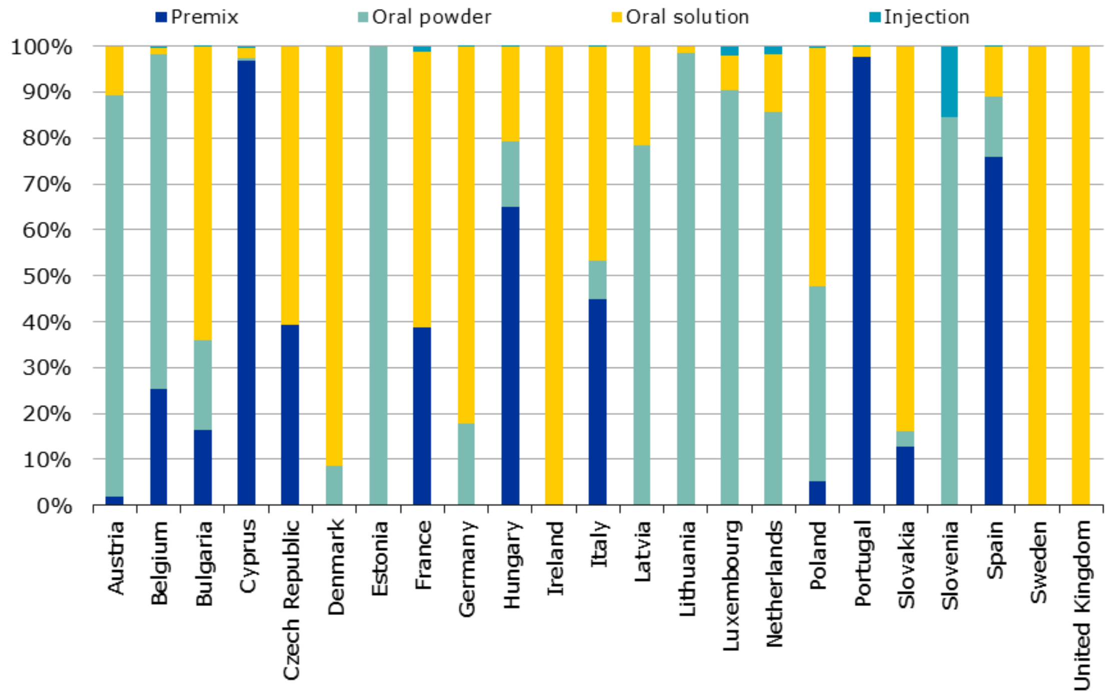


Figure 3 Distribution of veterinary sales for polymyxins by pharmaceutical form, adjusted for biomass under exposure (in mg/PCU), by country for 2013. No sales in Finland, Iceland and Norway. In addition, negligible amounts were sold as bolus, oral paste, intramammaries and/or intrauterine preparations in some countries (EMA/ESVAC, 2015).



Due to concerns that the differences in posology and withdrawal periods established across the EU 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. The risk-benefit 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.

Subsequent to the AMEG's previous advice in 2013, a further referral was concluded under Article 35 of Directive 2001/82/EC for all VMPs containing colistin as a sole substance administered orally (including premixes) to food-producing animals (EMA/CVMP, 2015).

In December 2014 the CVMP recommended to restrict the indications for use of colistin to treatment of enteric infections caused by susceptible non-invasive *E. coli* only, that any indications for prophylactic use should be removed and the treatment duration should be limited to the minimum time necessary for the treatment of the disease and not exceeding 7 days. In addition, it was recommended to remove horses from the SmPCs on the grounds of target species safety concerns.

In April 2016 the CVMP recommended the withdrawal of the marketing authorisations for all veterinary medicinal products containing colistin in combination with other antimicrobial substances.

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 broad-spectrum of activity of polymyxins 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 (Guyonnet et al., 2010). 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). 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 Mollicutes including *Mycoplasma* spp. (Falagas and Kasiakou, 2005). In addition, colistin lacks therapeutic activity against intrinsically (inherently) resistant species, including bacteria of the genera *Serratia, Stenotrophomonas*, and *Proteus* (Pogue et al., 2011).

Colistin heteroresistance, (i.e. cultures where both susceptible and resistant subpopulations are present), has been reported for *K. pneumoniae* (Poudyal et al., 2008), *P. aeruginosa* (Bergen et al., 2011) , *A. baumannii* and *E. cloacae* (Hawley et al., 2008; Lo-Ten-Foe et al., 2007). 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 *(Galleria mellonella)* infection model 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/pharmacodynamic (PK/PD) 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. 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 bactericidal activity and minimise resistance has been shown as the area under the inhibitory curve (AUIC, or fAUC/MIC) for target organisms such as *P. aeruginosa* and *Acinetobacter* spp. (Michalopoulos and Falagas, 2011). In veterinary medicine, similar estimates have been found to be reliable for preclinical studies for colibacillosis in piglets (Guyonnet et al., 2010). It is, however, unlikely that the diversity of gut microbiota and their intrinsic difference in antibiotic susceptibilities will ever allow a PK/PD approach to be sustainable in limiting the spread of (multi)resistance in non-target bacteria. Some subpopulations among wild type strains (e.g. 3 % of wild type *P. aeruginosa* strains) have a slightly increased MIC (4 µg/ml) and thereby jeopardising safe PK/PD targeting if such bacteria are clinically involved (Skov Robert, personal communication).

4. Resistance mechanisms and susceptibility testing

4.1. Resistance mechanisms

Acquired resistance to colistin in normally susceptible bacteria has for long been characterised by chromosomal mutations and thus in theory was non-transferable by mobile genetic elements (Callens et al., 2012b; Landman et al., 2008; Olaitan et al., 2014).

Chromosomal 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 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, Yersinia enterocolitica* and *Salmonella* spp. (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). 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) and *Klebsiella pneumoniae* (Wright et al., 2015). 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 be related to the existence of efflux pumps with potassium anti-porter systems (RosA/RosB) (Bengoechea and Skurnik, 2000). In *K. pneumoniae* mutations in *crrAB*, present in many multidrug resistant virulent strains (ST258, see below), a histidine kinase gene as part of a two-component regulatory system (TCRS), have been found involved in decreased colistin susceptibility (Wright et al., 2015).

Colistin-resistant mutants of *E. coli*, *K. pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* can be selected *in vitro* from cultures progressively grown in medium containing 0.5 to 16 µg/ml colistin (Lee et al., 2016). With the exception of some well-examined clinical strains (*K. pneumoniae*), many of the above mutation mechanisms are not stable after several passages *in vitro* (Moskowitz et al., 2012). This instability of polymyxin resistance by mutation, has been for long and prior to the *mcr-1* discovery, stated to reduce 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). Out of 37 patients treated with colistin for less than one to three months, in five patients (13%) mutations in the *pmr* locus were found. Colistin susceptibilities returned soon after cessation of colistin therapy (Snitkin et al., 2013), but in one of the isolates an apparently more stable mutation was found (*pmrB*^{L271R}). Of note is that this strain's gradient diffusion (E-test) and microbroth dilution susceptibility tests were highly discordant (Snitkin et al., 2013).

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 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. An exhaustive update on chromosomal colistin resistance mechanisms (vertical transmissible) was done by Olaitan et al. (2014).

In the 1980's, work on *Klebsiella pneumoniae* did indicate 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 was found to be 1000-fold compared to colistin-susceptible strains under laboratory conditions. No later reports have confirmed these findings and underlying mechanisms. This aspect of colistin-resistant isolates has been nevertheless at that time 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, the colistin-resistant isolates remained susceptible to other antibiotics. Through the rotational use of colistin and aminoglycosides, the prevalence of resistant *Klebsiella* spp. decreased during the latter outbreaks (O'Callaghan et al., 1978). More recently genomic analysis have suggested a possible fitness cost due to colistin resistant mutations with loss of β-lactamase-encoding plasmids (Wright et al., 2016). Whereas some *mcr-1* harbouring plasmids do not show so far identified resistance genes (Suzuki et al., 2016), many *E. coli* harbour β-lactamases together with *mcr-1* (**Table 9**) including decreased susceptibility for carbapenems as well as resistance determinants for other antimicrobial classes (Poirel et al., 2016).

In November 2015, Liu et al. (2015) reported that a transferable plasmid-mediated colistin resistance gene, *mcr-1*, had been found in *E. coli* isolates from animals, food and bloodstream infections from human patients in China. Subsequent retrospective analysis of strain collections showed the *mcr-1* gene was already circulating in the 1980's (Shen et al., 2016) and the EU/EEA in a variety but low absolute number of Gram-negative organisms (Doumith et al., 2016). Although the exact mechanism is under examination, the *mcr-1* gene encodes a membrane-anchored phosphoethanolamine transferase that likely confers resistance to colistin by a modifying lipid A (Thanh et al., 2016). The *mcr-1* gene is often associated with transposable elements located on different types of plasmids (pHNSHP45, IncI2, IncX4, IncHI2 and IncP2…). (Liu et al., 2015; Thanh et al., 2016; Zeng et al., 2016). These plasmids have been shown to have high *in vitro* transfer rates (10^{-1} to 10^{-2}) or absent, depending on the conditions and strains involved. Conjugation has been shown from *E. coli* and *Salmonella* spp. into other Enterobacteriaceae, not only *K. pneumoniae*, *Enterobacter aerogenes* and *Enterobacter* spp. but also *P. aeruginosa*. (Callens et al., 2016; Quesada et al., 2016; Zeng et al., 2016). Linked resistance genes have been shown in many isolates (**Table 9**). The MIC observed in strains carrying *mcr-1* has ranged from 0.5 to 32 mg/l and is stated to be associated with the diversity of lipid A structures found in Enterobacteriaceae (Thanh et al., 2016). The *mcr-1* positive *E. coli* strains can have other colistin resistance genes due to mutations in chromosomal DNA present (PmrA/B), and of notice these strains failed to transfer the *mcr-1* gene in conjugation mating experiments (Quesada et al., 2016). The occurrence of the *mcr-1* gene in *E. coli* and also across different *Salmonella* serovars has been recently confirmed in different EU MSs like Belgium (Botteldoorn, 2016 (in press)), Spain (Quesada et al., 2016), the Netherlands (Veldman, 2016), and France (Perrin-Guyomard et al., 2016) with special relevance for turkeys.

4.2. Susceptibility testing

4.2.1. Methodological approaches

Susceptibility testing of colistin is performed by testing colistin sulphate since 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. In the last couple of years there has been intensive research under the auspices of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) to delineate methods that could produce reliable and reproducible susceptibility results. Presently only broth dilution can be recommended for susceptibility testing, i.e. for the time being neither disk diffusion, agar dilution nor gradient test should be used for testing of colistin. Broth microdilution (BMD) should be performed using uncoated polystyrene microtiter plates; cation adjusted Mueller-Hinton broth without any other additives (in particular no polysorbate 80 or other surfactants) (EUCAST homepage www.eucast.org)

The EUCAST clinical breakpoints for Enterobacteriaceae (*E. coli* and *Klebsiella* spp., but excluding *Proteus* spp., *Morganella morganii*, *Providencia* spp., and *Serratia* spp.), *P. aeruginosa,* and *A. baumannii* are ≤2 µg/ml for a colistin susceptible isolate; and >2 µg/ml for a colistin resistant isolate (EUCAST, 2013). For non-clinical surveillance purposes, the epidemiological cut-off value (ECOFF) can be difficult to determine given certain *Salmonella* serovars, such as Dublin and Enteriditis demonstrate subpopulations that are (intrinsically) slightly-less susceptible (Agersø et al., 2012b).

A number of new techniques for susceptibility testing and identification of resistance determinants have been developed (Jung et al., 2014; Review on antimicrobial resistance (conference), 2015; van Belkum and Dunne, 2013). These techniques reduce the antimicrobial susceptibility testing time from two to four days to approximately one to two hours, which could reduce the empirical treatment and stimulate appropriate antimicrobial use. The utility of colistin resistance determinations has recently been demonstrated for *E. coli* (Liu et al., 2016), with a method called SERS-AST (simple surface-enhanced Raman – antimicrobial susceptibility testing).

For the interpretation of **Table 9**, it is of importance to stress that in the absence of research into the specificity and sensitivity of the *mcr-1* PCR (test characteristics identifying false positive/negative results), and estimation of the true (absolute prevalence) prevalence is difficult. In particular only isolates with elevated MICs according to the latest EUCAST/CLSI recommendations might have been included.

4.2.2. Monitoring results

4.2.2.1. Occurrence of microbiological resistance to colistin

A summary of an extraction of all available phenotypic data on colistin resistance from the "European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014" (EFSA, 2016) is given here:

Twenty fourteen was the first year of mandatory EU monitoring for colistin resistance in *Salmonella* and indicator *E. coli* from animals. Although some MSs encountered technical difficulties in accurately determining colistin susceptibility, the monitoring data obtained are being considered to a baseline in poultry (animal species targeted for 2014) against which future changes can be measured. The reported occurrence of colistin resistance is unlikely to equate directly to the occurrence of *mcr-1* gene, because a number of different resistance mechanisms can confer colistin resistance as indicated in a previous section of this report. In the case of *Salmonella*, data were reported and is presented for broilers, layers, fattening turkeys, meat from broilers and meat from turkeys. For *E. coli* data were reported and is only available for broilers and fattening turkeys. The ECOFF value applied for the analysis of the occurrence of 'microbiological' resistance to colistin in both Salmonella and *E. coli* was >2 mg/l.

EU harmonised monitoring data indicated that 0.9% of *E. coli* from broilers (total tested equal to 4037, colistin-resistance found in 24 MSs) and 7.4% of *E. coli* from fattening turkeys (total tested equal to 1663, colistin-resistance found in 11 MSs) were colistin-resistant according to the interpretative criteria applied.

In the case of *Salmonella* spp., 8.3% of isolates from broilers (total tested=1683, colistin-resistance found in 10 MSs), 2% of isolates from fattening turkeys (total tested=757, colistin-resistance found in 6 MS), 14.1% of isolates from laying hens (total tested=822, colistin-resistance found in 13 MS), 24.7% of isolates from turkey meat (total tested equal to 279, colistin-resistance found in 2 MS), and 4.4% of isolates from broiler meat (total tested equal to 911, colistin-resistance found in nine MSs) were colistin-resistant according to the interpretative criteria applied. Resistance was detected in a diversity of *Salmonella* serovars, although a large proportion of the colistin-resistant *Salmonella* from broilers and laying hens were *S.* Enteritidis. There are studies showing that the distribution of the wild type differs between serovars. A general epidemiological cut off value (ECOFF) therefore can lead to false positive resistance interpretation for some serovars or subpopulations herein (Agersø et al., 2012b).

4.2.2.2. Multidrug resistance in colistin resistant isolates

Data on multidrug resistance in *E. coli* isolates from poultry populations and meat thereof, reported in the EU from harmonised surveillance as resistant to colistin are presented in **Table 2**. In this analysis we included the *E. coli* spp. isolates originating from laying hens, broilers, and fattening turkeys flocks; and isolates from broilers and turkey meat, for which antimicrobial resistance (AMR) data to the following 10 antimicrobials were reported: ampicillin (AMP), cefotaxime (CTX), ceftazidime (CAZ), nalidixic acid (NAL), ciprofloxacin (CIP), tetracycline (TET), gentamicin (GEN), trimethoprim (TMP), sulphonamide (SUL), chloramphenicol (CHL), meropenem (MERO) and colistin. For the purpose of this analysis, resistance to CIP/NAL and CTX/CAZ have been addressed together. Data on 'microbiological' and 'clinical' co-resistance to colistin and in addition to critically important antimicrobials (CIP and/or CTX) in *E. coli* from poultry populations and meat thereof are presented in **Table 3** and **Table 4**.

Data on multidrug resistance, in *Salmonella* isolates from poultry populations and meat thereof, reported in the EU as resistant to colistin are presented in

Table 5. Data on 'microbiological' and 'clinical' co-resistance to colistin and in addition to critically important antimicrobials (CIP and/or CTX) in *Salmonella* spp. from poultry populations and meat thereof are presented in **Table 6** and **Table 7**.

Table 2. Percentage of MDR isolates in *E. coli* from poultry populations and meat thereof, reported as resistant to colistin

N	Res. colistin	Res 0	Res 1	Res 2	Res 3	Res 4	Res 5	Res 6	Res 7	Res 8	Res 9
6259	162	2	2	10	18	21	42	52	14	1	0
100%	*2.6%*	*1.2%*	*1.2%*	*6.2%*	*11.1%*	*13.0%*	*25.9%*	*32.1%*	*8.6%*	*0.6%*	*0%*

N: total number of *E. coli* spp. isolates from poultry origin and meat derived thereof tested against 9 classes of antimicrobials; Res0: number (%) of isolates resistant to colistin only and to none of the 9 additional antimicrobial classes. Res1-Res9: number (%) of isolates resistant to colistin being also resistant to one antimicrobial class/resistance to nine antimicrobial classes.

Table 3. 'Microbiological' co-resistance to colistin and CIP and/or CTX in *E. coli* from poultry populations and meat thereof – resistance assessed against ECOFFs (COL: MIC >2 mg/l, CIP: MIC >0.064 mg/l, CTX: MIC >0.25 mg/l)

N	Res. colistin	Not Res. to CIP nor CTX	Res. to CIP or CTX	Res. to both CIP and CTX
6259	162 (2.6%)	33 (20.4%)	120 (74.1%)	9 (5.6%)

N: total number of *E. coli* spp. isolates from poultry origin and meat derived thereof tested against 9 antimicrobial classes.

Table 4. 'Clinical' co-resistance to colistin and CIP and/or CTX in *E. coli* from poultry populations and meat thereof – resistance assessed against CBPs (COL: MIC >2 mg/l, CIP: MIC >1 mg/l, CTX: MIC >2 mg/l)

N	Res. colistin	Not Res. to CIP nor CTX	Res. to CIP or CTX	Res. to both CIP and CTX
6259	162 (2.6%)	87 (53.7%)	73 (45.1%)	2 (1.2%)

N: total number of *E. coli* spp. isolates from poultry origin and meat derived thereof tested against 9 antimicrobial classes.

Table 5. Percentage of multidrug-resistant (MDR) isolates in *Salmonella* spp. from poultry populations and meat thereof, reported as resistant to colistin

In this analysis we included the *Salmonella* spp. isolates originating from laying hens, broilers, and fattening turkeys flocks; and isolates from broilers and turkey meat, for which antimicrobial resistance data to the following 10 antimicrobials were reported: AMP, CTX, CAZ, NAL, CIP, TET, GEN, TMP, SUL, CHL, MERO and colistin. For the purpose of this analysis, resistance to CIP/NAL and CTX/CAZ have been addressed together.

N	Res. colistin	Res0	Res1	Res 2	Res 3	Res 4	Res 5	Res 6	Res 7	Res 8	Res 9
4432	377	236	101	5	12	13	8	2	0	0	0
100%	*8.5%*	*62.6%*	*26.8%*	*1.3%*	*3.2%*	*3.5%*	*2.1%*	*0.5%*	*0%*	*0%*	*0%*

N: total number of *Salmonella* spp. isolates from poultry origin and meat derived thereof tested against 9 classes of antimicrobials; Res0: number (%) of isolates resistant to colistin only and to none of the 9 additional antimicrobial classes. Res1-Res9: number (%) of isolates resistant to colistin being also resistant to one antimicrobial class/resistance to nine antimicrobial classes.

Table 6. 'Microbiological' co-resistance to colistin and CIP and/or CTX in *Salmonella* spp. from poultry populations and meat thereof - resistance assessed against ECOFFs (COL: MIC >2 mg/l, CIP: MIC >0.064 mg/l, CTX: MIC >0.5 mg/l)

N	Res. colistin	Not Res. to CIP nor CTX	Res. to CIP or CTX	Res. to both CIP and CTX
4432	377 (8.5%)	309 (82.0%)	67 (17.8%)	1 (0.3%)

N: total number of *Salmonella* spp. isolates from poultry origin and meat derived thereof tested against 9 antimicrobial classes.

Table 7. 'Clinical' co-resistance to colistin and CIP and/or CTX in *Salmonella* spp. from poultry populations and meat thereof - resistance assessed against CBPs (COL: MIC >2 mg/l, CIP: MIC >1 mg/l, CTX: MIC >2 mg/l)

N	Res. colistin	Not Res. to CIP nor CTX	Res. to CIP or CTX	Res. to both CIP and CTX
4432	377 (8.5%)	373 (98.9%)	4 (1.1%)	0 (0%)

N: total number of *Salmonella* spp. isolates from poultry origin and meat derived thereof tested against 9 antimicrobial classes.

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, a retrospective analysis of bacterial collections showed that transmission of colistin resistance in Gram-negative bacteria *via* horizontal gene transfer or sustained clonal expansion has not been substantial in the EU/EEA. Following the first Asian reports, confirmation of the *mcr-1* gene in large databases in UK (Doumith et al., 2016) among 15 out of 24,000 isolates of *Salmonella* species, *E. coli*, *Klebsiella spp. Enterobacter* spp. and *Campylobacter* spp. from food and human isolates from between 2012 and 2015 has been done, while the number of reports is ever growing in the EU/EEA and worldwide (**Table 9**). In the latest reports, *mcr-1*-postive isolates from clinical specimens so far remain uncommon (Cannatelli et al., 2016). To date the earliest animal isolates were in the 1980s in China and were from poultry (Shen et al., 2016); the earliest human isolate was a *Shigella sonnei* strain in 2008 from Vietnam (Skov and Monnet, 2016; Thanh et al., 2016). More research is needed because of the diversity of plasmids and occurrences of the *mcr-1* gene in different ecosystems including surface water (**Table 9**).

The larger abundance in veterinary isolates compared to human cases, together with the by far exceeding quantities of colistin use in livestock (ECDC/EFSA/EMA, 2015) has been considered suggestive of a flow from animals to humans (Skov and Monnet, 2016). Nordmann & Poirel (2016) recently have listed further arguments for this rationale aside from the difference in colistin use and resistance prevalence. First, the occurrence of isolates with simultaneous resistance for florfenicol which is only authorised for used in animals (Poirel et al., 2016), and the co-presence of extended-spectrum β-lactamases typical of animal origin, CMY-2 (Falgenhauer et al., 2016). Homologies in the genetic organisation of *mcr-1* with insertion sequences in an important ubiquitous animal pathogen *Pasteurella multocida* (Poirel et al., 2016). Given that the *mcr-1* gene is present in isolates that often harbour other resistance determinants like those encoding β-lactamase production (**Table 9**), co-selection of these isolates by other antimicrobials than polymyxins should be considered. A review of antimicrobial consumption in livestock at large is therefore provided in the next paragraphs.

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-drug resistance is worrisome in veal calves (MARAN, 2012), yet colistin resistance in this production system has historically been extremely low to absent (Di Labio et al., 2007). Latest findings have however demonstrated the presence of *mcr-1* in clinical isolates from veal (Haenni et al., 2016; Malhotra-Kumar et al., 2016a). The latest figures from Belgium show a gradual decrease in colistin resistance in *E. coli* from veal calves, from 14.7% in 2011 to 6.7% in 2014 (CODA-CERVA, 2015).

In Belgium, the second highest antimicrobial-consuming livestock production system is that of fattening pigs, where on average over 200 to 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). With the exception of a very slight increase in 2013, colistin resistance is considered very low in *E. coli* from Belgian pigs over the period 2011-2014 (CODA-CERVA, 2015). Dutch, porcine *E. coli* and *Salmonella* isolates, as reported in 2009 (MARAN, 2009), remain fully susceptible.

Large studies combining consumption and resistance are limited, because colistin susceptibility tests as routinely performed are not fully reliable or available. A large surveillance study in Polish livestock revealed 0.9% of *E. coli* (n=1728) to be resistant to colistin for the period 2011-2012 (Wasyl et al., 2013). In central European 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., 2012). Quantification of broiler consumption did not identify use of colistin in 50 randomly selected farms in

Belgium (Persoons et al., 2012), but it is used in many other EU MSs. 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).

Colistin resistance in *E. coli* from broilers is increasingly becoming associated with multi-resistance (Geenen et al., 2011). Nevertheless reports of colistin resistance remain scarce and limited to some broiler meat samples (2.1%, N=328) (MARAN, 2009) and more recently turkey (4.5%) (MARAN, 2015). A retrospective study from the Netherlands demonstrated a presence of 10% *mcr-1* in *E. coli* from turkey meat (Veldman, 2016). In Italy, Battisti and coworkers found a high prevalence while screening turkey isolates (*E. coli, Salmonella* from monitoring). In the non-selective monitoring, prevalence of *mcr-1* in *E. coli* from fattening turkeys was 22%, and in isolates from ESBL-screening 25% (Battisti, 2016b). A recent report from Germany has revealed that, in particular, turkey and turkey-derived food (6-18%) frequently contained colistin-resistant *E. coli* compared to broilers and broiler derived food (2-8%) (Alt et al., 2015). Care should be taken that technical difficulties can result in over-reporting of colistin resistance, in particular for *Salmonella* spp. when contaminated with inherent resistant organisms such as *Proteus* species. Studies on antimicrobial consumption and further processed in the production chain of turkeys should be done in the future to investigate the reasons for the relative high prevalence of colistin resistance, particularly in turkey and meat thereof compared with other production types.

In Australian *Aeromonas* strains from fish have frequently been found to have decreased susceptibility to colistin (55.5%), especially when retrieved from clinical cases (Aravena-Roman et al., 2012), although this might be intrinsically present. Studies under EU/EEA aquaculture conditions are not available.

Surveillance data until 2014 show low levels of colistin resistance despite considerable colistin use especially in veal and fattening pigs (Callens et al., 2012a) with even a decrease or low steady state during the last couple of years in Belgium (Hanon et al., 2015), and Sweden (Swedres-Svarm, 2014). Detailed accurate monitoring is needed in these confined production systems to follow up the emergence of clonally resistant strains and to demonstrate absence of multi-resistance plasmids or alternative structures that include efficient spreading mechanisms for polymyxin resistance. In China (Shen et al., 2016) and Taiwan (Kuo et al., 2016), and France (Perrin-Guyomard et al., 2016) the occurrence of *mcr-1* from food-producing animals shows an increase of colistin resistance during the most recent years which might be of importance for prediction of potential for the further global spread (Grami et al., 2016).

The Netherlands (SDa, 2015) and Belgium (BelVetSac, 2015) have set and attained targets to reduce the consumption of antimicrobials in veterinary medicine over a limited number of years. In the Netherlands for instance, a 58% (50% in fattening pigs) has been demonstrated over the period from 2009 to 2014. Along, a decrease of overall resistance in faecal bacteria has been found in *E. coli* in livestock in the Netherlands (MARAN, 2015). In Belgium, after two consecutive years of substantial reduction in consumption adjusted for kg biomass in 2012 (-6.9%) and 2013 (-6.3%), disappointing results were found for 2014 (+1.1%) (BelVetSac, 2015). A decrease in resistance in indicator *E. coli* from different Belgian livestock species has also been found (CODA-CERVA, 2015).

An increase in Chinese livestock production (broilers, i.e. chicken raised for meat, and swine) by nearly 5% in upcoming years (2016-2020) is anticipated as is a subsequent increase in colistin use (Liu et al., 2015). Doses given for growth promotion outside the EU/EEA can be several times lower than the doses given for metaphylaxis and curative purposes to EU/EEA livestock, and subsequent concerns for a different selection pressure of *mcr-1* have been raised (Richez and Burch, 2016). A large retrospective analysis showed the presence of this gene in the early 1980's in China, and rather quickly after the use of colistin in animal production (Shen et al., 2016). In the EU/EEA details on the chronology and occurrence of *mcr-1* in animals and ways of administrations are lacking to investigate to what extent differences in selection pressure have an impact on the occurrence and spread of the *mcr-1* gene. From the retrospective analysis of databanks worldwide so far (**Table 9**), it is clear that transferable colistin resistance was out there but only "detected" within weeks, and highest prevalence have been demonstrated only in the most recent years of interests. Based upon the prevalence of colistin resistance and *mcr-1* in turkey or turkey meat in particular (Battisti, 2016b; Perrin-Guyomard et al., 2016; Veldman, 2016), e.g. from 0 in 2007 to 6% in 2014 in French turkey isolates, detailed investigations in this livestock production sector on colistin consumption and antimicrobials at large are lacking to demonstrated associations with these findings.

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

Colistin is now regarded as a last line defence against infections caused by MDR Gram-negative bacteria such as *K. pneumoniae* and *A. baumannii.* 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 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).

Often encountered in the EU/EEA 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; Dhar et al., 2016). 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. It is acknowledged that, as shown for the clone ST258 (Bogdanovich et al., 2011), these strains have high capability for successful spread.

In EU/EEA 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 available 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 a recent prospective experimental study, zinc oxide (ZnO) showed to be as effective as colistin (compared to oral and in feed groups) on piglet health and production parameters the control of weaning diarrhoea, with a better daily weight gain during the supplemented period and a reduced diarrhoea score (Van den Hof et al., submitted). In the case of zinc oxide, other issues such as environmental impact and co-selection of resistance as for example livestock associated MRSA should be taken into account (Amachawadi et al., 2015; Cavaco et al., 2011). The alternatives to colistin, depending on the resistance situation in a particular country, are aminopenicillins, trimethoprim-sulphonamides, tetracyclines, aminoglycosides, and the critically important antimicrobial cephalosporins and fluoroquinolones. The latter are of particular concern due to emerging ESBL resistance (EMEA/CVMP/SAGAM, 2009) (EFSA BIOHAZ Panel, 2011). Although food-producing animals are the main concern for the transmission of antimicrobial resistance from animals to man, the risk of transmission of antimicrobial resistance *via* direct contact from companion animals should be taken into account.

Until recently there was no evidence that the use of colistin in veterinary medicine for food-producing species has resulted in the transfer of colistin resistance from animals to humans. Nevertheless, based on current data, transmission of such resistance is likely to have taken place in the EU/EEA, albeit at low frequency, with the exception of specific cohorts from Asian origin. The results from China (Liu et al., 2015; Shen et al., 2016) indicate that a rapid increase cannot be excluded (Skov and Monnet, 2016). For other drug resistant organisms including *E. coli*, the emergence following antimicrobial consumption and the transfer *via* direct animal contact or *via* food has already been documented (Angulo et al., 2004). The increasing use of colistin in humans, in particular in well-defined settings will lead to increased selection pressure which may be the catalyst for dispersal of zoonotic colistin resistance mediated by *mcr-1* (Skov and Monnet, 2016). Multifactorial cycling of these reservoirs of genes via hotspots of colistin use in e.g. intensive care medicine (Ingenbleek et al., 2015), via the environment at large (Zurfuh et al., 2016) and fattening poultry, pigs and veal calves (Callens et al., 2016) need to be considered in the analysis of the epidemiology and for targeted interventions.

The *mcr-1* gene has been found in clinical cases of veterinary colibacillosis in veal calves and pigs (Haenni et al., 2016; Malhotra-Kumar et al., 2016a; Richez and Burch, 2016) and in human invasive pathogens (Skov and Monnet, 2016). The *mcr*-1 genes were found in similar plasmids in the same bacteria species isolated from food-producing animals, food humans and environment indicating a possible transmission between these compartments.

Data from 2012 compared after controlling for biomass in a joint report from ECDC, EFSA and EMA, has shown that consumption of polymyxins, mainly colistin, was on average more than 600 times higher in food-producing animals than in humans for the included 19 Member States in the EU and EEA. (ECDC/EFSA/EMA, 2015; Olaitan et al., 2015). Since *mcr-1* is substantially more sparse in humans compared to animal isolates (Kluytmans–van den Bergh et al., 2016) the hypothesis that it might have originated from animals and then attain humans is plausible (Skov & Monnet, 2016). The fairly low presence in humans so far, might be due to absence of selection in a non-favourable environment as indicated by the fact that all travellers that were tested positive for *mcr-1* upon return were negative after one month (Arcilla et al., 2015). According to Skov & Monnet, the presence of plasmid-mediated colistin resistance in foods and asymptomatic human carriers combined with increasing colistin use in EU/EEA hospitals may be a game changer and the EU/EEA may face hospital outbreaks of infections with colistin resistant MDR (Skov & Monnet, 2016).

7. Conclusions on updated literature review

- 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.

- Following its discovery of the horizontally transferable colistin gene *mcr-1* in 2015, the number of reports is very rapidly increasing with a recent increase in animal sources although the relative proportion amid human clinical isolates in the EU/EEA remains fairly low (less than 1%), so far.

- Despite the recent nature of the *mcr-1* discovery, this is an indication of limited spread of colistin resistance from food-producing animals to human patients, and to a lesser extent vice versa. *Mcr-1* genes were found in similar plasmids in the same bacteria species isolated from food-producing animals, food humans and environment indicating a possible transmission between these compartments.
- Transfer of resistance either on mobile genetic elements (such as plasmids) between bacteria or from animals to humans has been suggested based upon prevalence studies but appears to remain at an overall low incidence in the EU/EEA.
- It is of therapeutic importance for the treatment of Gram-negative gastrointestinal infections in certain food-producing species.
- From the data available from 26 EU/EEA countries, colistin is the 5th most used antimicrobial for food-producing animals (6.1%). There is large variation between MSs 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 MS with some MSs 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 no longer limited to a stepwise process *via* mutations in target bacteria and plasmid mediated spread is emerging. In humans the clonal resistance (mutations) forms can develop rapidly and can spread efficiently under certain conditions in hospitals.
- Since resistance to other antimicrobial classes are frequently found in the same bacteria that harbour *mcr-1*, this form can easily spread due to both the use of colistin and co-selection of other antibiotic classes.
- The mechanisms and evolutionary pathways resulting in decreased susceptibility for colistin in certain *Salmonella* serovars remain to be fully understood.

8. Profiling of the risk to public health resulting from the use of colistin in animals in the EU

Due to the major data gaps relating to risk factors, particularly in relation to a lack of information about the historical and current prevalence of colistin resistance and the *mcr-1* gene and its evolution in bacteria in animals, humans and food, this risk profiling is based substantially on expert opinion. As new evidence becomes available, this profiling may need to be revised.

8.1. Hazard identification

Use of colistin in animals can select for colistin-resistant Enterobacteriaceae which have the potential to be transmitted to humans. In addition to chromosomal mechanisms of resistance to colistin, a plasmid-borne mechanism has recently been identified (MCR-1). The *mcr-1* gene is associated with transposable elements located on different types of plasmids (Kuo et al., 2016; Skov and Monnet, 2016) and has been shown to be present in strains that harbour genes encoding for ESBLs and carbapenemases and for resistance to many other antimicrobial classes (Kuo et al., 2016; Poirel et al., 2016). Therefore the use of other antimicrobial classes both in human and veterinary medicine could maintain *mcr-1* colistin resistance. The potential for co-selection is high and colistin-resistant organisms may also be multi-drug resistant.

8.2. Exposure

Release of resistance genes from animals treated with colistin: colistin is used extensively in food-producing animals, especially as group treatments for pigs, poultry and veal calves. It is mostly administered via the oral route and has low bioavailability, even among experimentally-infected animals (Rhouma et al., 2015), so direct exposure of the gastrointestinal microbiota is high. The colistin dose used in the EU is bactericidal limiting the selection of resistant target organisms (Guyonnet et al., 2010); the impact on commensals is less clear. The transfer of *mcr-1* plasmids between commensal Enterobacteriaceae has been shown to be very high *in vitro*. This has yet to be demonstrated *in vivo* but has the potential to lead to an increase in the previously stable levels of colistin resistance. The prevalence of colistin-resistant *Salmonella* and *E. coli* organisms in food-producing animals appears to be low overall in major species. Based on the new mechanism of resistance including the presence of linked resistance genes, the overall risk for release of resistance genes is now assessed as potentially high.

Exposure of humans to resistance genes *via* bacteria from animals: The consumption of pork and poultry products in the EU is high (consumption of veal is relatively low). Contamination of meat with *Salmonella* spp. is low, but as with other foodborne organisms, dependent on hygiene and food type amongst other factors. Although data are limited, general prevalence of colistin resistance in *E. coli* and *Salmonella* spp. from EU produced meat appears to be low, although prevalence in poultry and turkey should be investigated further based upon individual country reports (Italy, Germany, France and the Netherlands). Exposure to resistance genes may occur via other routes, e.g. direct contact with animals and manure in the environment.

8.3. Consequences to human health/ hazard characterisation

Colistin is an antimicrobial of last resort in human medicine that is used systemically to treat serious infections caused by carbapenem-resistant bacteria that are generally also multi-drug resistant. As there are often no alternative treatments for these patients, the consequences of colistin- resistant infections are serious (death). Across the EU, with clear exceptions in defined areas, very low numbers of human patients require treatment with colistin each year and prevalence of colistin resistance is low. In recent years colistin use has been increasing rapidly in southern European regions as a consequence of increasing carbapenem resistance and this will increase the selection pressure for colistin resistance. The prospect of new alternative antimicrobial substances coming forward in the near future is very limited, and alternative antimicrobials (e.g. temocillin) are not available across all countries in the EU/EAA region.

8.4. Overall risk estimation/characterisation

A plasmid-borne mechanism of resistance to colistin (MCR-1) has recently been identified in Enterobacteriaceae from food-producing animals. Colistin is used extensively in pigs, poultry and veal calves, administered to groups of animals predominantly via the oral route. At present, levels of colistin-resistance in Enterobacteriaceae from animals are estimated as low; although data on the prevalence of colistin resistance, including the *mcr-1* gene, and its progression over time are limited. Taking into account the nature of veterinary use of colistin, the characteristics of the newly identified mechanism of resistance and the opportunity for co-selection (**Table 2**-**Table 7** and **Table 9**), suggests that colistin resistance has the potential to spread rapidly and to be associated with MDR organisms which could transfer to humans, for example via food, litter, or surface water. Colistin is used in human medicine as an antimicrobial of last resort for the treatment of serious MDR infections that are also resistant to carbapenems. The occurrence of carbapenem resistance, subsequent use of colistin, and therefore its importance to human medicine have increased substantially in regions of southern Europe in recent years. The prospect of novel alternative antimicrobials for treatment of these infections in the near future is limited. In conclusion, although there are limited data on the evolution of colistin resistance, the newly identified mechanism has the potential for rapid spread and, coupled with the recent increasing importance of colistin to human medicine, this leads to an increased risk to human health from the use of colistin in animals.

9. Risk Management options

9.1. Recommended risk management options for colistin

The main recommendation is that colistin sales for use in animals should be reduced to the minimum feasible and that colistin should be added to a more critical category (category 2) of the AMEG classification (**Table 8**).

Category 2 includes those antimicrobial classes listed as critically important antimicrobials by the WHO for which the risk to public health from veterinary use is considered only acceptable provided that specific restrictions are placed on their use. These reserved antimicrobials should only be used when there are no effective alternative antimicrobials from category 1 authorised for the respective target species and indication. Use of colistin should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to antimicrobials in category 1.

Table 8. Classification of antimicrobial classes according to their probability of transfer of resistance genes and resistant bacteria

Antimicrobial class	Mobile genetic element-mediated transfer of resistance[a]	Vertical transmission of resistance gene(s)[b]	Co-selection of resistance[c]	Potential for transmission of resistance through zoonotic and commensal food-borne bacteria[d]	Evidence of similarity of resistance: genes / mobile genetic elements / resistant bacteria[e]	Overall probability of resistance transfer	References
Assessment 2013							
Polymyxins (e.g. colistin)	1	1	2	1	1	Low	(EMA, 2013)
Assessment 2016							
Polymyxins (e.g. colistin)	3	1	2	3	3	High	UPDATE 2016

[a]Mobile genetic element-mediated transfer of resistance. Defined as a resistance gene that is transmitted by means of mobile genetic elements (horizontal transmission of the gene occurs). Probability (1 to 3): 1, no gene mobilization described; 2, gene is exclusively on the core bacterial chromosome; 3, gene is on a mobile genetic element, e.g. plasmid.

[b]Vertical transmission of resistance gene. Defined as the vertical transfer of a resistance gene through the parent to the daughter bacteria in a successful, highly disseminated resistant clone of bacteria through a bacterial population, e.g. *E. coli* ST131 clone, MRSP CC(71) clone, MRSA ST398 clone. Probability (1 to 3): 1, no vertical transmission of gene described as associated with in a particular successful resistant clone; 2, gene is exclusively on the core bacterial chromosome in a particular successful resistant clone; 3, gene is on a mobile genetic element, e.g. plasmid, in a particular successful resistant clone.

[c]Co-selection of resistance. Defined as selection of resistance which simultaneously selects for resistance to another antimicrobial. Probability (1 to 3): 1, no co-mobilization of the gene or risk factor described; 2, gene is either co-mobilized or a risk factor has been described; 3, gene is co-mobilized and a risk factor has been described.

[d]Transmission of resistance through zoonotic and commensal food-borne bacteria. Defined as transmission of resistance through food-borne zoonotic pathogens (e.g. *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *E. coli* VTEC) or transmission of resistance through commensal food-borne bacteria (e.g. *E. coli*, *Enterococcus* spp.). Probability (1 to 3): 1, no transmission of resistance through food-borne zoonotic pathogens or commensal food-borne

bacteria; 2, transmission of resistance through food-borne zoonotic pathogens or commensal food-borne bacteria; 3, transmission of resistance through food-borne zoonotic pathogens and commensal food-borne bacteria.

[e]Evidence of similarity of resistance: genes/mobile genetic elements/resistant bacteria. Genes - Defined as similar resistance gene detected in bacterial isolates of animal and human origin; Mobile genetic elements - Defined as a similar resistance mobile genetic element detected in bacterial isolates of animal and human origin; Resistant bacteria - Defined as a similar bacterium harboring a resistance gene (either chromosomally or mobile genetic element-encoded) of animal and human origin. Probability (1 to 3): 1, unknown resistance similarity; 2, genes or mobile genetic elements or resistant bacteria similar between animals and humans; 3, genes and mobile genetic elements similar between animals and humans; 4, genes and mobile genetic elements and resistant bacteria similar between animals and humans.

The scoring of the table above is based on the expert opinion of the members of the Working Group.

9.1.1. Considerations when proposing risk management measures

- A balance should be found between the need to protect public health and the potential impact of risk management measures on animal health (One Health approach).
- Colistin is mainly used in pigs, poultry, and veal calves to treat *E. coli* which causes serious diseases with potential for high morbidity and mortality. Resistance to category 1 antibiotics is common.
- Alternatives to the use of colistin for treatment of the indicated diseases include other critically important antimicrobials and removal of colistin from the market could increase the selection pressure for resistance to these substances through increased use.
- Because of the high potential for co-selection with other classes, as well as reducing the use of colistin it is important that there is an overall reduction in the use of antimicrobials of all classes.
- Eliminating any prophylactic use will be essential to achieve a significant reduction of sales of colistin for veterinary use.
- In December 2014 the CVMP recommended to restrict the indications for use of colistin to treatment of enteric infections caused by susceptible non-invasive *E. coli* only, that any indications for prophylactic use should be removed and the treatment duration limited to the minimum time necessary for the treatment of the disease and not exceeding 7 days. In addition, it was recommended to remove horses from the SPCs on the grounds of target species safety concerns. Commission Decision (2015)1916 of 16 March 2015 translated the CVMP recommendation into legislation.
- In April 2016 the CVMP recommended the withdrawal of the marketing authorisations for all veterinary medicinal products containing colistin in combination with other antimicrobial substances.
- As colistin is used in all the major food-producing species, measures in only one animal species would not provide the expected results in terms of reduction of use.
- Use of colistin as reported to ESVAC (26 countries) decreased 19% between 2011 and 2013 in terms of tonnes of colistin sold.
- Countries with a low consumption of colistin should be encouraged not to increase such use.
- Targets should ideally be established by animal species, but as comparable consumption data per animal species across the EU are not available, this is not possible.

9.1.2. Recommendation on target for use of colistin and considerations on impact on use of other antimicrobials

In order to reduce the exposure of Enterobacteriaceae in animals to colistin and hence the possibility of further selection of colistin-resistance genes which have the potential to be transmitted to humans, the use of colistin in mg/PCU should be reduced. This reduction in use should be achieved without a consequential increase in the consumption (in mg/PCU) of fluoroquinolones, 3rd- and 4th-generation cephalosporins or the overall use of antimicrobials.

The consumption of antimicrobials (amount in mg) can be compared over countries by adjusting for the biomass under exposure (kg livestock), which is expressed by the population correction unit (PCU). Use of colistin in the EU/EEA countries varies significantly; some EU countries have reported a high consumption of colistin per kg of biomass produced, whilst others have reported little or no use. Taking into account the current use of colistin, the possible alternatives to its use, impacts on animal health and welfare and the tendency over recent years to reduced consumption of colistin, it is proposed that there is a target for MSs to reduce use to a maximum of 5 mg colistin/PCU (as reported by ESVAC). Further reasoning for the target is provided under "justification for the target".

If successfully applied at an EU level, the above threshold would result in an overall reduction of approximately 65% of the current sales of colistin for veterinary use; this decrease should build upon the decrease of colistin sales for veterinary use already seen between 2011 and 2013.

For those countries with a colistin consumption below 5 mg/PCU, the recommendation should not result in an increase of the colistin consumption. For those countries with a consumption that is well below the proposed 5 mg/PCU, the trends on colistin consumption should be analysed case by case in the concerned country. In some countries with high pig and poultry production, e.g. Denmark (0.5 mg/PCU) and the Netherlands (0.9 mg/PCU), the level of consumption of colistin is below 1 mg/PCU. Member states should consider the possibility of setting stricter national targets therefore, ideally a lower level than 5 mg/PCU of colistin, e.g. below 1 mg/PCU, is desirable. There is insufficient information to establish the feasibility of such a measure in all countries, and the impact of those intended reductions on colistin resistance.

The above target for sales reduction of colistin should be achieved in a period of 3 to 4 years. Through the EU surveillance programmes, the impact of the measures should be closely monitored and assessed to conclude on their impact on antimicrobial resistance, including on the presence of the *mcr-1* gene in animals and humans, if data are available.

Because of the possibility of co-selection, an overall reduction of all antimicrobials use should be achieved, especially for those countries for which the antimicrobial consumption, expressed as mg/PCU, is very high. The reduction of sales of colistin should not be compensated by increase in the use of other classes; it should be achieved by other measures such as improved farming conditions, biosecurity in between production cycles, and vaccination.

9.1.3. Further considerations

Antimicrobial sales data are not available to ESVAC for Greece and Malta. Those MSs would need to start such collection in order to provide the results of colistin sales in mg/PCU.

As indicated above, in case circumstances lead to a significant increase (*or decrease*) in the risk to public health due to the use of colistin in animals the recommended measures should be revised.

The levels of resistance to colistin in humans, animals and derived foods and prevalence of *mcr-1* herein should be measured in order to establish a baseline from which to assess the impact of the measures.

The use of colistin, fluoroquinolones and 3rd- and 4th-generation cephalosporins and the reasons for use, should be recorded by the prescribing veterinarian and provided to the authorities as requested. MSs are encouraged to set up systems to request and analyse these data.

9.1.4. Justification for the target

One of the objectives when establishing the target was to ensure that from the current experience from EU countries with a high production of pigs and poultry, it is possible to produce those animals with a consumption of colistin that is below the proposed target. The proposed target is higher than the current sales of colistin in some countries with high production of pigs and poultry (i.e. above 50% PCU). Although the target will demand a very important reduction in the use of colistin for some high using countries (more than 80% reduction in the most extreme case), it should still allow for the treatment of animals in those cases where colistin would remain the best option. It was considered if the target should be reduced to below 5 mg/PCU, but reducing the consumption of colistin in high using countries before they have had time to implement compensatory strategies could result in an increase of use of other critically important antimicrobials (e.g. fluoroquinolones), or overall use, which could be counterproductive for public health.

9.1.5. Summary of the risk mitigation recommendations

Colistin should be added to category 2 of the AMEG's classification; the risk to public health from veterinary use is considered only acceptable provided that specific restrictions are placed on its use. Colistin should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to antimicrobials in category 1.

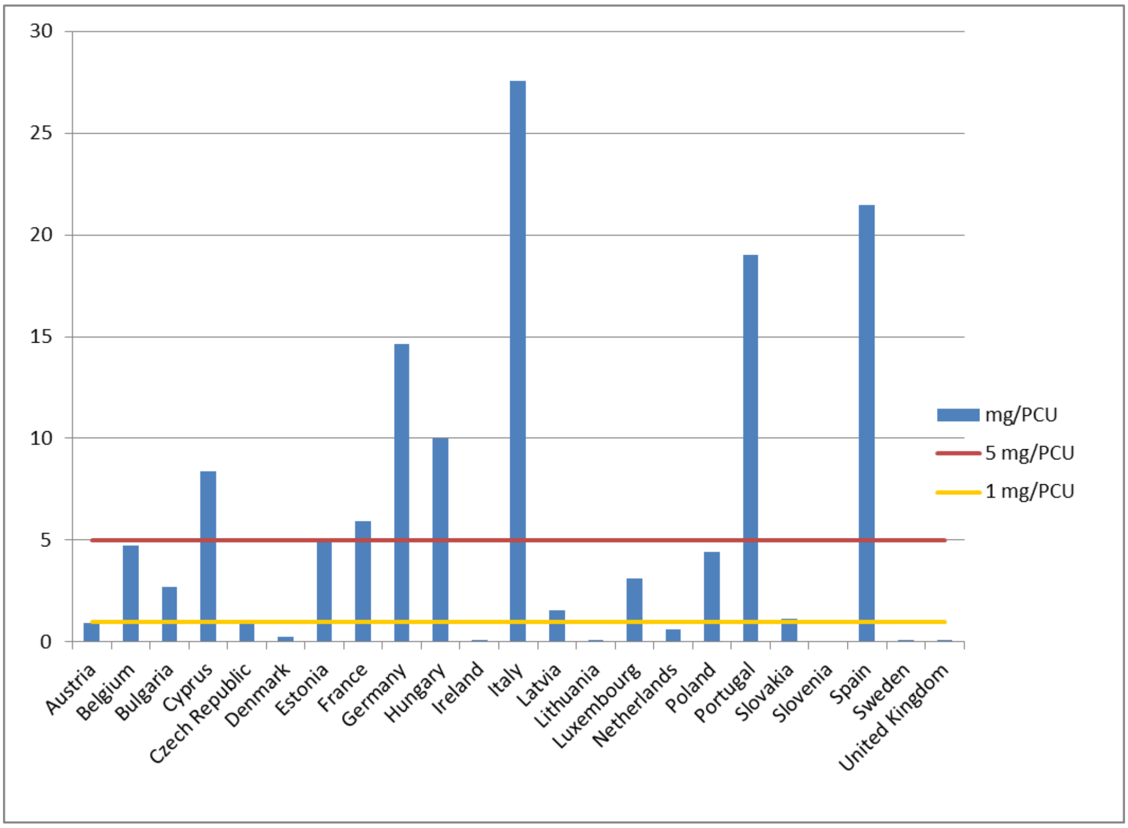
There are wide variations in the use of colistin between countries which are largely unexplained. Countries with intensive livestock production can have a level of usage below 1 mg/PCU (e.g. Denmark and the UK) and much higher, up to 20-25 mg/PCU (Italy and Spain). Considering the rapidly increasing importance of colistin for treatment of critically ill human patients, all countries should strive to reduce the use of polymyxins as much as possible.

For the current "high and moderate consumers" the target and desirable levels are set at 5 and 1 or below 1, mg/PCU, respectively, based on the observations on the level of use in other countries. Meanwhile more information should be gathered to determine the minimum level of colistin use that can be achieved while maintaining animal welfare and preventing the increased use of other critically important antimicrobials.

If the situation regarding colistin resistance in animals or humans deteriorates further it may be necessary to lower the level proposed targets.

Reduction in use of colistin should be achieved without an increase in the use (in mg/PCU) of fluoroquinolones, 3rd- and 4th-generation cephalosporins or overall consumption of antimicrobials.

Figure 4. Sales of colistin in for use in animals in mg/PCU in 2013 (ESVAC data), including the 5 and 1 mg/PCU levels. No sales reported in Finland, Iceland and Norway.



9.2. Strategies for responsible use and alternatives to the use of colistin

Strategies for the responsible use of colistin in veterinary medicine, can be subdivided into approaches that limit or fine-tune the use, and approaches that replace the use of the substance.

To limit or fine-tune use, a better identification of animals that are diseased versus animals that do not need treatment is required. Appropriate diagnostics should be undertaken to establish the cause of disease and identify the appropriate antimicrobial treatment for the group, if needed.

Secondly improving the antibiotic regimen by applying PK/PD analyses to assist in dose regimen selection (Guyonnet et al., 2010), along with identifying a minimum number of days under exposure is another option. In a recent systematic review (Burow et al., 2014) it was concluded that orally administered antimicrobials increase the risk of antimicrobial resistance in *E. coli* from swine, although it was noted that more research is needed into the impact of dosage and the longitudinal effects of treatment.

Further improved herd management, in particular biosecurity through well controlled cleaning and disinfection strategies (biocides) (Carlsson et al., 2009), in between production cycles should be encouraged to limit the accumulation of resistance genes over consecutive production cycles (Dorado-García et al., 2015; Geenen et al., 2011; Schmithausen et al., 2015). Good farming practices and herd health planning including animal quarantine, restrictions on movements before freedom of disease certification, among others, prevent spread of infections and therefore reduce the need for antimicrobials (EFSA/EMA, foreseen 2016). Vaccination, voluntary and later mandatory, has been proved in broilers to reduce the occurrence of *Salmonella* spp. and thereby the need for antimicrobial consumption (Dewaele et al., 2012). Vaccines are available in the EU to reduce the incidence of enteric *E. coli* infections in piglets.

Pro- and prebiotics, and in a broader sense faecal transplants have shown in human medicine to be extremely useful for the control of antibiotic associated diarrhoea *(Clostridium difficile)* (Cammarota et al., 2015). Instead of giving long-term doses of antibiotics via feed or water, the digestive tract content can be replaced with healthy bacteria. Given the high number of indications for antimicrobials related to the digestive tract in pigs (Stege et al., 2003), veal calves (Pardon et al., 2012) and broilers (Persoons et al., 2012), this approach must receive consideration for further research. These historically named 'transfaunations' have been used for gastrointestinal disorders in horses. Organic acids and metals (Cu, Zn) are alternatives to reduce the use of antimicrobials at large and colistin in particular although attention should be paid to environmental concerns relating to the use of metals.

For an exhaustive review on alternatives to replace or to reduce the selection pressure exerted by antimicrobials in animal husbandry, we refer to the RONAFA working group (Reduction of Need for Antimicrobials in Food-producing Animals) document, to be completed by the end of 2016 (EFSA/EMA, foreseen 2016).

9.3. Previously applied risk management options

Following the previous AMEG recommendations in 2013, the SPCs for authorised products were 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. As detailed in Section 3.2. a referral was concluded under Article 35 of Directive 2001/82/EC for all VMPs containing colistin as a sole substance administered orally (including premixes) to food-producing animals (EMA/CVMP, 2015). Indications were restricted to therapy or metaphylaxis, all indications for prophylactic use removed and indications restricted to the treatment of enteric infections caused by susceptible non-invasive *E. coli* only.

The treatment duration was limited to the minimum time necessary for the treatment of the disease and not exceeding 7 days. Horses were removed from the SPCs on the grounds of target species safety concerns.

In April 2016 the CVMP recommended the withdrawal of the marketing authorisations for all veterinary medicinal products containing colistin in combination with other antimicrobial substances.

9.4. 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.

9.5. Surveillance of colistin consumption and of colistin resistance

The use of colistin in MSs is 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 revised EU/EEA harmonised monitoring of antimicrobial resistance now requires all MSs to perform standardised and quality controlled susceptibility testing of colistin on representative samples of zoonotic and indicator bacteria (*Salmonella* spp. and *E. coli*). The findings from such testing are reported by MSs as phenotypic data on colistin resistance. This monitoring system could be enhanced by selecting a random sample of resistant isolates that are subsequently screened for resistance mechanisms, this would facilitate in particular the detection of emerging 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. As 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.

9.6. General considerations

Treatment of individual animals is preferred.

Rapid, reliable diagnostic tests combining accurate bacterial identification (e.g. mass spectrometry) and colistin susceptibility testing (Liu et al., 2016) should be explored and tested under routine laboratory conditions.

The rapid accumulation of a considerable amount of additional information following the first report of *mcr-1* in November 2015, together with insights in mutations responsible for decreased colistin susceptibility (Wright et al., 2016) highlights the strength of whole genome sequencing (WGS) and publicly-available sequence databases (Skov and Monnet, 2016).

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

9.7. Follow up of the advice

This recommendation should be reviewed after 3 to 4 years to determine (i) if the targets on antimicrobial consumption have been achieved, (ii) if possible, if there has been any impact on the prevalence of colistin resistance in food-producing animals, although acknowledging that there are limited data, especially in regards to the *mcr-1* gene and that more time might be required to observe changes in resistance levels. At this time, further consideration should be given to any changes in the need for and use of colistin in human medicine and the occurrence of colistin resistance in humans. The effectiveness of the proposed measures should then be reviewed taking a 'One Health' approach, and further considerations on the measures as detailed in section 11 should be addressed.

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

ANNEX

10. Risk Management options that were analysed and disregarded

10.1. Withdrawal of existing marketing authorisations

The withdrawal of marketing authorisations was considered but it was noted that, in addition to potential animal health and welfare impacts, this could increase the use of other CIAs, in particular fluoroquinolones, as there are high levels of resistance to category 1 alternative substances for the given indications. It could be speculated that due to the high potential for co-selection by other antimicrobial classes, the *mcr-1* gene would still be maintained in animal populations after withdrawal of colistin.

10.2. 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 for not banning group treatment.

It was also considered if premix formulation should be withdrawn since these could have greater tendency to be used off-label for prolonged duration of (preventive) treatment. ESVAC data suggest that in those MSs where use of medicated feeds is limited, this does not necessarily impact colistin sales and oral powder and solution formulations are used instead. In addition, due to differences in use of premix and other oral formulations that may be associated with availability and national legislation, this measure would be inconsistent across the EU.

10.3. Restriction on use for metaphylaxis

As 99% of use of colistin is in oral formulations which are mostly used for simultaneous group treatment and metaphylaxis within herds/flocks, and it is difficult to separate medication of clinically ill and "in-contact" animals in intensive husbandry systems, it was considered that this measure would not be practical to implement effectively.

10.4. Restriction from use in certain species

Sufficient species-specific data are not available to perform the risk assessment required.

10.5. Injectable, intramammary and topical formulations

Taking into account the fact that these formulations account for less than 1% of colistin sales, are mostly used for individual animal treatment and via non-enteral routes of administration, it was considered that restrictions on these colistin formulations would have minimal impact on the risk to public health.

11. Figures

Figure 5. Spatial distribution of sales of polymyxins in veterinary medicine, in mg/kg biomass, in 26 EU/EEA countries, for 2013. No sales reported in Finland, Iceland and Norway. (EMA/ESVAC, 2015)

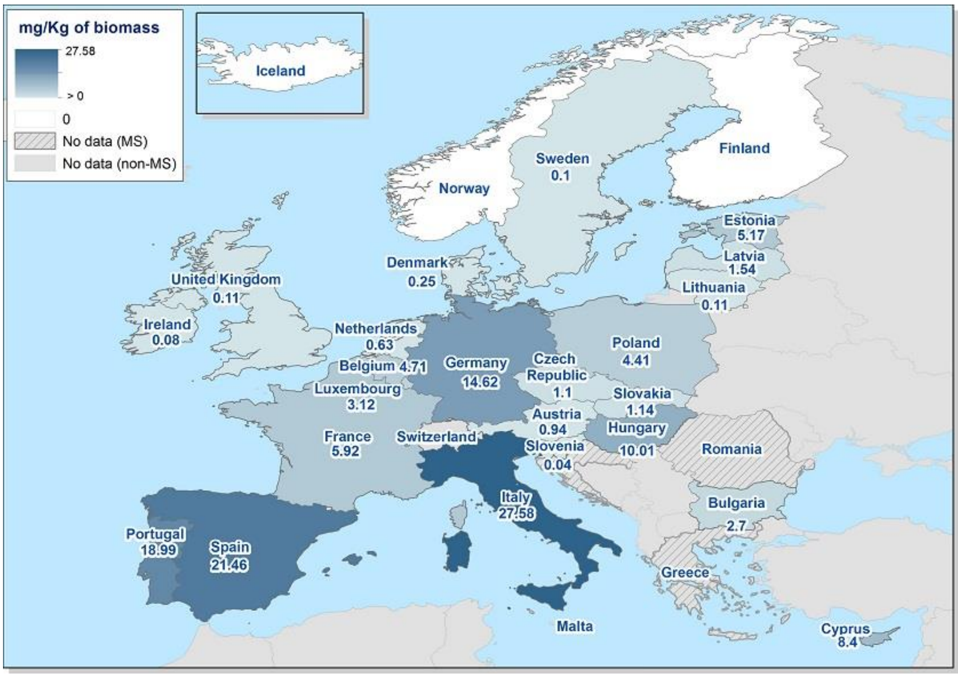
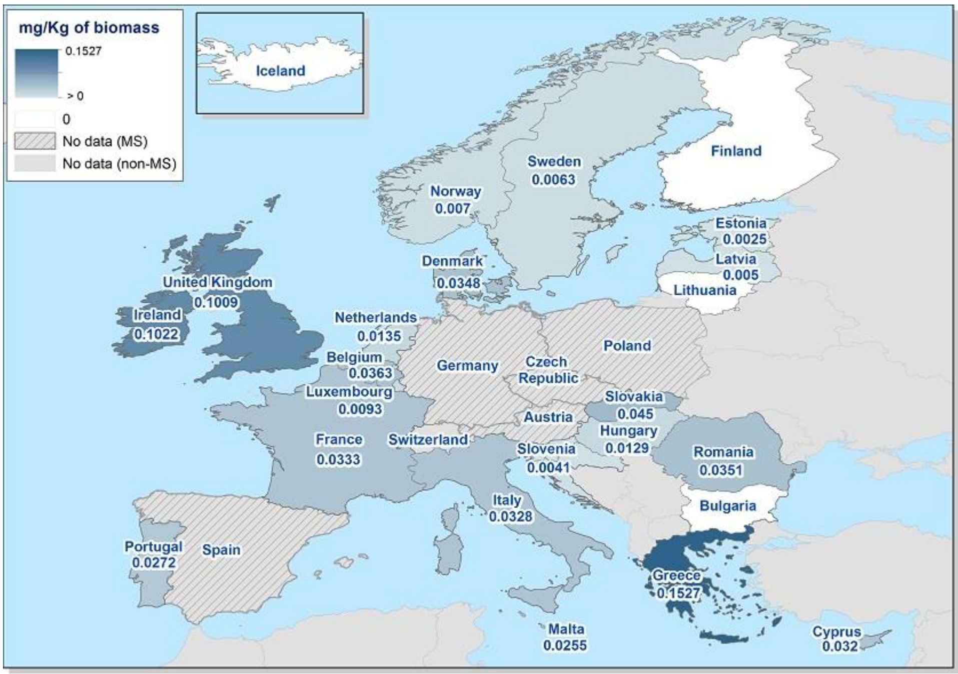
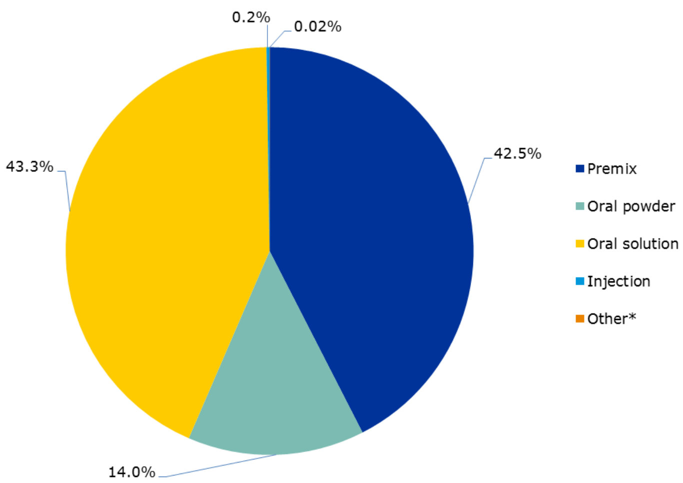


Figure 6. Spatial distribution of sales of polymyxins in human medicine, in mg/kg biomass, in 25 EU/EEA countries, for 2013 (data shown only for countries reporting on total consumption in the country; i.e. reporting for antibiotic consumption in the community (outside hospitals) and in the hospital sector) (ECDC, 2015)



Please note that **Figure 5** and **Figure 6** show polymyxin consumption expressed in mg/kg biomass with a different scale because consumption is much lower in humans than in animals.

Figure 7. Percentage of veterinary sales in mg/PCU for food-producing animals, by pharmaceutical form of polymyxins, in the EU/EEA for 2013. No sales reported in Finland, Iceland and Norway. (EMA/ESVAC, 2015) (unpublished ESVAC data 2013)



*Negligible amount of polymyxins were sold as oral paste, bolus, intramammary and intrauterine preparations.

Figure 8. Copy of the February 2016 call for scientific data for the update of advice

Advice on the impact on public health and animal health of the use of antibiotics in animals (colistin) following the recent discovery of the first mobile colistin resistance gene (mcr-1)

Call for scientific data for the update of advice

Submission period: 29 February – 15 March 2016

Dear colleagues,

The CVMP and CHMP invites all interested parties to submit any scientific data which might have impact on public and animal health that should be considered when updating the previously published advice on ***colistin****.*

The answers should address some of the following points:

- *The importance of colistin to human and veterinary medicine (e.g. estimated frequency of use, target indications, including selective digestive tract decontamination, estimation of the use per animal species).*
- *Any information on colistin resistance mediated by the mcr-1 gene in isolates from humans and animals, including animal pathogens.*
- *The effectiveness and availability of alternative treatments to the use of colistin in human and animals especially if restrictions on the use of colistin would be applied.*

- *Experiences on colistin resistance risk management measures such as changes in indications, restrictions of use, husbandry practices or controls of imported food for the protection of public and animal health in Europe.*

For further details see http://www.ema.europa.eu/docs/en_GB/document_library/Other/2016/02/WC500202544.pdf and http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000639.jsp&mid=WC0b01ac058080a585

The call is open until 15 March 2016.

Scientific contributions should be sent by email to: vet-guidelines@ema.europa.eu

Table 9. Prevalence and characteristics of *mcr-1*-positive isolates from food-producing animals, the environment, food and humans, 1980s–2016 (updated from Skov & Monnet, 2016)

Source	Year*	Country	Type of specimen/animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
Food-producing animals	1980s–2014	China	Chickens	[a]	104	*E. coli*	NA	NA	(Shen et al., 2016)
	2005–2014	France	Veal calves	[a]	106	*E. coli*	CTX-M-1 (n = 7)	No	(Haenni et al., 2016)
	2008–10	Japan	Pigs	[a]	2	*E. coli*	NA	NA	(Suzuki et al.)
	2009-2011	Spain	Pigs	[a]	4	*S. Typhimurium; S. rissen*	NA	NA	(Quesada et al., 2016)
	2010-2014	Spain	Pigs, turkeys	[a]	5	*E. coli, Salmonella*	NA	NA	(Quesada et al., 2016)
	2010–2011	Germany	Pigs	[a]	3	*E. coli*	CTX-M-1 (n = 3)	No	(Falgenhauer et al., 2016)
	2010-2015	The Netherlands	Chickens, veal calves, turkeys	[a]	4 (< 1%)	*E. coli*	NA	NA	(Bonten, 2014)
	2011	France	Pigs	[a]	1 (<1%)	*E. coli*	NA	NA	(Perrin-Guyomard et al., 2016)
	2011–12	Belgium	Pigs	[a]	6	*E. coli*	No	No	(Malhotra-Kumar et al., 2016a)
	2011–12	Belgium	Veal calves	[a]	7	*E. coli*	No	No	(Malhotra-Kumar et al., 2016a)
	2012	Laos	Pigs	[a]	3	*E. coli*	NA	NA	(Olaitan et al., 2015)
	2012	China	Pigs	[a]	31 (14%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2013-2014	Vietnam	Chicken and pig	[a]	37 (21%)	*E. coli*	NA	NA	(Nguyen et al., 2016)
	2012–13	Japan	Cattle	[a]	4	*E. coli*	CTX-M-27	No	(Suzuki et al.)
	2012-2015	Taiwan	Chicken, Pigs	[a]	18 (5.9%)	*E. coli*	CTX-M-		(Kuo et al., 2016)
	2013	Japan	Pigs	[a]	1	*Salmonella* Typhimurium	NA	NA	(Suzuki et al.)
	2013	China	Pigs	[a]	68 (25%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2013	Malaysia	Chickens	[a]	3	*E. coli*	NA	NA	(Petrillo et al., 2016)
	2013	Malaysia	Pigs	[a]	1	*E. coli*	NA	NA	(Petrillo et al., 2016)
	2013	France	Pigs	[a]	1 (<1%)	*E. coli*	No	No	(Perrin-Guyomard et al., 2016)
	2013	France	Chickens	[a]	3 (2%)	*E. coli*	No	No	(Perrin-Guyomard et al., 2016)
	2013	France	Chickens (farm)	[a]	1	*Salmonella* 1,4 [5],12:i:-	NA	NA	(Webb et al., 2015)
	2013	Italy	Turkeys	[a]	3 (1%)	*Salmonella*	No	NA	Alba et al., 2016 ECCMID
	2013	Italy	Turkeys	[a]	58 (19.3%)%	E. coli	No	NA	Alba et al., 2016 ECCMID
	2014	France	Broilers	[a]	4 (2%)	*E. coli*	No	No	(Perrin-Guyomard et al., 2016)
	2014	France	Turkeys	[a]	14 (6%)	*E. coli*	CMY-2	No	(Perrin-Guyomard et al.,

Source	Year*	Country	Type of specimen/animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
									2016)
	2014	Italy	Turkeys	[a]	1	*E. coli*	No	No	(Battisti, 2016a)
	2014	China	Pigs	[a]	67 (21%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2014–15	Vietnam	Pigs	[a]	9 (38%)	*E. coli*	CTXM-55	No	(Malhotra-Kumar et al., 2016b)
	2014-15	South Africa	Chickens	[a]	9%	*E. coli*	NA	NA	(Keeton, 2016)
	2015	Tunisia	Chickens	France /Tunisia	37 (67%)	*E.coli*	CTX-M-1	NA	(Grami et al., 2016)
	2015	Algeria	Chickens	[a]	1	*E. coli*	NA	NA	(Olaitan et al., 2015)
Environment	2012	Switzerland	River water	[a]	1	*E. coli*	SHV-12	NA	(Zurfuh et al., 2016)
	2013	Malaysia	Water	[a]	1	*E. coli*			(Petrillo et al., 2016)
Food	2009	The Netherlands	Chicken meat	Unknown	1	*E. coli*	CTX-M-1	No	(Kluytmans–van den Bergh et al., 2016)
	2009-2016	The Netherlands	Retail meat (mostly chicken and turkey)	Dutch fresh meat and imported frozen meat	47 (2%)	*E. coli*	NA	NA	(Bonten, 2014)
	2010	Canada	Ground beef	Unknown	2	*E. coli*	No	No	(Mulvey et al., 2016)
	2011	Portugal	Food product	NA	1	*Salmonella* Typhimurium	CTX-M-32	No	(Tse and Yuen, 2016)
	2011	China	Chicken meat	[a]	10 (5%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2011	China	Pork meat	[a]	3 (6%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2012	France	Chicken meat, guinea fowl pie	NA	2	*Salmonella* Paratyphi B	NA	NA	(Webb et al., 2015)
	2012	Thailand	Faecal carriage	[a]	2	*E. coli*	NA	NA	(Olaitan et al., 2015)
	2012	Laos	Faecal carriage	[a]	6	*E. coli*	NA	NA	(Olaitan et al., 2015)
	2012	Cambodia	Faecal carriage	[a]	1	*E. coli*	CTX-M-55	No	(Stoesser et al., 2016)
	2012–2014	Denmark	Chicken meat	Germany	5	*E. coli*	CMY-2, SHV-12	No	(Hasman et al., 2015)
	2012-2015	Belgium	Poultry meat	[a]	2	*Salmonella*	AmpCipColNalSmxTmp , AmpColStrSmxTet	NA	(Botteldoorn, N, in press)
	2012–2015	United Kingdom	Poultry meat	European Union, non-United Kingdom	2	*Salmonella* Paratyphi B var Java	NA	NA	(Doumith et al., 2016)
	2012-2015	Taiwan	Beef, Chicken, Pork	[a]	5.9%	*E. coli*	CTX-M	NA	(Kuo et al., 2016)
	2013	France	Pork sausage	NA	1	*Salmonella* Derby	NA	NA	(Webb et al., 2015)
	2013	China	Chicken meat	[a]	4 (25%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2013	China	Pork meat	[a]	11 (23%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2014	China	Chicken meat	[a]	21 (28%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2014	China	Pork meat	[a]	29 (22%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2014	The Netherlands	Chicken meat	Europe, non-Dutch (n = 1), origin unknown	2	*E. coli*	SHV-12	No	(Kluytmans–van den Bergh et al., 2016)

Source	Year*	Country	Type of specimen/animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
				(n = 1)					
	2014	Switzerland	Vegetables	Thailand, Vietnam	2	*E. coli*	CTX-M-55, CTX-M-65	No	(Zurfuh et al., 2016)
	2014	China	Chickens	[a]	1	*E. coli*	CTX-M-65	NDM-9	(Yao et al., 2016)
	NA	The Netherlands	Turkey meat	[a]	10%	*TBA*			(Veldman, 2016)
Humans	2008	Vietnam	Dysentery	Vietnam	1	*Shigella sonnei*	NA	NA	(Thanh et al., 2016)
	Before 2010	China	Faecal carriage	[a]	27 (7%)	NA	NA	NA	(Hu et al., 2015; Ruppé et al., 2016)
	2010-2014	Taiwan	Sterile sites	[a]	20 0.3%	*E. coli*	CTX-M	NA	(Kuo et al., 2016)
	2011	Canada	Gastrostomy tube	Egypt (previous healthcare)	1	*E. coli*	NA	OXA-48	(Mulvey et al., 2016)
	2011	The Netherlands	Bloodstream infection	[a]	1 (0.08%)	*E. coli*	NA	NA	(Bonten, 2014)
	2011& 2015	Denmark	Bloodstream infection		2 (*<0.001%*)	*E. coli*	ESBL	NA	(Hasman, 2015) Skov, R. personal communication
	2012	Thailand	Faecal carriage	[a]	2	*E. coli*	NA	NA	(Olaitan et al., 2015)
	2012	Laos	Faecal carriage	[a]	6	*E. coli*	NA	NA	(Olaitan et al., 2015)
	2012	Cambodia	Faecal carriage	[a]	1	*E. coli*	CTX-M-55	No	(Stoesser et al., 2016)
	2012-2013	Vietnam	Chicken farmers Sub+rural inhabitants	[a]	(25.1%) (14.9%)	*Coliforms*	NA	NA	(Nguyen, 2016)
	2012–2013	The Netherlands	Faecal carriage	China (n = 2), South America (n = 2), Tunisia, South-East Asia	6	*E. coli*	CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-55 (2), CTX-M-65	No	(Arcilla et al., 2015)
	2012–2015	United Kingdom	Salmonellosis	Asia (n = 2)	8	*Salmonella* Typhimurium	No	No	(Doumith et al., 2016)
	2012–2015	United Kingdom	Salmonellosis	Asia	1	*Salmonella* Paratyphi B var Java	No	No	(Doumith et al., 2016)
	2012–2015	United Kingdom	Salmonellosis	[a]	1	*Salmonella* Virchow	No	No	(Doumith et al., 2016)
	2012–2015	United Kingdom	NA	NA	3	*E. coli*	CTX-M-type	No	(Doumith et al., 2016)
	2012-2015	Italy	Urine, SSI	[a]	8 (<0.02%)	*E. coli*	ESBL (2/8)	No	(Cannatelli et al., 2016)
	2012-2015	Spain	Clinical isolates	[a]	15 (0.15%)	*E. coli*	ESBL (3/15), 7 non MDR	No	(Prim et al., 2016)
	2012-2016	Argentina	Blood,urine, abscess, abdominal, bone	[a]	9+10	*E. coli*	4(CTX-M2,14,15)	No	(Rapoport et al., 2016)
	2014	Germany	Wound infection	NA	1	*E. coli*	No	KPC-2	(Falgenhauer et al.,

Source	Year*	Country	Type of specimen/animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
			(foot)						2016)
	2014	China	Inpatient	[a]	13 (1%)	*E. coli*	NA	NA	(Liu et al., 2015)
	2014	China	Urogenital tract	[a]	2	*E. aerogenes* *E. cloaca**	*bla*CTX-M-15, *bla*TEM-1, *qnrS*, *aac(6')-Ib*-cr, *armA**	NA	(Zeng et al., 2016)
	2014–2015	China	Bloodstream infection	[a]	2	*E. coli*	CTX-M-1	No	(Du et al., 2016)
	2014-2015	Denmark	Salmonellosis		4 (total 8397)	*Salmonella*			R. Skov, personal communication
	2015	Switzerland	Urinary tract infection	NA	1	*E. coli*	No	VIM	(Poirel et al., 2016)
	2015	China	Diarrhoea	[a]	3	*E. coli*	NA	NA	(Ye et al., 2016)
	2015	China	Inpatient	[a]	3 (<1%)	*K. pneumoniae*	NA	NA	(Liu et al., 2015)
	2015	China	Surgical site infection, peritoneal fluid	[a]	2	*K. pneumoniae*	CTX-M-1	NDM-5	(Du et al., 2016)
	2015	China	Faecal carriage (children)	[a]	5 (2%)	*E. coli*	CTX-M-15	No	(Zhang et al., 2016)
	NA	Sweden	Faecal carriage	Asia	2	*E. coli*	NA	NA	(Folkhalsomyndigheten, 2016)

NA: not available;
SSI: surgical site infection
*: year of isolation is not synonym for study period
[a]:Same as reporting country

12. Acknowledgement

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13. References

Agersø, Y., T. Hald, B. Helwigh, B. Høg, L. Jensen, V.F. Jensen, H. Korsgaard, L. Larsen, A. Seyfarth, and T. Struve, 2012a. '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, Vol.

Agersø, Y., M. Torpdahl, C. Zachariasen, A. Seyfarth, A.M. Hammerum, and E.M. Nielsen, 2012b. 'Tentative colistin epidemiological cut-off value for Salmonella spp', Foodborne pathogens and disease, Vol. 9 (4), pp.367-369.

Agodi, A., E. Voulgari, M. Barchitta, A. Quattrocchi, P. Bellocchi, A. Poulou, C. Santangelo, G. Castiglione, L. Giaquinta, and M. Romeo, 2014. 'Spread of a carbapenem-and colistin-resistant Acinetobacter baumannii ST2 clonal strain causing outbreaks in two Sicilian hospitals', Journal of Hospital Infection, Vol. 86 (4), pp.260-266.

Alt, K., B.-A. Tenhagen, and A. Käsbohre. 2015. Resistenzmonitoring in der Lebensmittelkette - Daten für Deutschland 2011-2014. In BfR - Antibiotikaresistenz in der Lebensmittelkette. Berlin.

Amachawadi, R.G., H.M. Scott, S. Nitikanchana, J. Vinasco, M.D. Tokach, S.S. Dritz, J.L. Nelssen, R.D. Goodband, and T.G. Nagaraja, 2015. 'Nasal Carriage of mecA-Positive Methicillin-Resistant Staphylococcus aureus in Pigs Exhibits Dose–Response to Zinc Supplementation', Foodborne pathogens and disease, Vol. 12 (2), pp.159-163.

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, Vol. 51 (8-9), pp.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, Vol. 56 (2), pp.1110-1112.

Arcilla, M.S., J.M. van Hattem, S. Matamoros, D.C. Melles, J. Penders, M.D. de Jong, and C. Schultsz, 2015. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet infectious diseases, Vol.

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, Vol. 13 p.58.

Barton, M.H., A. Parviainen, and N. Norton, 2004. 'Polymyxin B protects horses against induced endotoxaemia in vivo', Equine veterinary journal, Vol. 36 (5), pp.397-401.

Battisti, A., 2016a. 'Antibiotic resistance–Italy: colistin, MCR-1, E. coli, turkeys 2014. ProMEDmail 20160113.3933461 (12 January 2016)', http://www.promedmail.org/post/3933461

Battisti, A. 2016b. Transferable colistin resistance mediated by the mcr-1 gene is widespread among Escherichia coli and is emerging in Salmonella in the Italian fattening turkey industry - ePoster #PLB30. In 26th ECCMID (European Congress of Clinial Microbiology and Infectious Diseases). Amsterdam.

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, Vol. 55 (7), pp.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 β-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, Vol. 66 (9), pp.2006-2010.

BelVetSac, 2015. 'Belgian Veterinary Surveillance of Antimicrobial Consumption (BelVetSac) - National consumption report 2014', http://www.belvetsac.ugent.be/pages/home/BelvetSAC_report_2014%20finaal.pdf

Bengoechea, J.A., and M. Skurnik, 2000. 'Temperature-regulated efflux pump/potassium antiporter system mediates resistance to cationic antimicrobial peptides in Yersinia', Molecular microbiology, Vol. 37 (1), pp.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, Vol. 55 (11), pp.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, Vol. 50 (6), pp.1953-1958.

Beringer, P., 2001. 'The clinical use of colistin in patients with cystic fibrosis', Current opinion in pulmonary medicine, Vol. 7 (6), pp.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, Vol. 10 (12), pp.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, Vol. 53 (4), pp.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, Vol. 8 (4), pp.329-341.

Bonten, M., 2014. 'Antimicrobial resistance: is it really all going wrong now?', Nederlands tijdschrift voor geneeskunde, Vol. 160 p.D81.

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, Vol. 25 (11), pp.1168-1170.

Botteldoorn, N., 2016 (in press). 'La résistance antimicrobienne chez les E. coli commensaux, Campylobacter spp. et Salmonella spp. isolés des carcasses et de la viande de volaille, de bœuf et de porc en 2015', Report for the Belgian Federal Food Agency, WIV-ISP report D/2016/2505/07, Vol.

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, Vol. 144 (3-4), pp.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, Vol. 51 (1), pp.369-372.

Burow, E., C. Simoneit, B.-A. Tenhagen, and A. Käsbohrer, 2014. 'Oral antimicrobials increase antimicrobial resistance in porcine E. coli–A systematic review', Preventive veterinary medicine, Vol. 113 (4), pp.364-375.

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, Vol. 107 (3-4), pp.288-290.

Callens, B., F. Haesebrouck, J. Dewulf, F. Boyen, P. Butaye, B. Catry, P. Wattiau, and E. de Graef, 2016. 'Risk of colistin resistance on the rise', Vlaams Diergeneeskundig Tijdschrift, Vol. 85 (1), pp.36-40.

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, Vol. 106 (1), pp.53-62.

Cammarota, G., L. Masucci, G. Ianiro, S. Bibbò, G. Dinoi, G. Costamagna, M. Sanguinetti, and A. Gasbarrini, 2015. 'Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection', Alimentary pharmacology & therapeutics, Vol. 41 (9), pp.835-843.

Cannatelli, A., T. Giani, A. Antonelli, L. Principe, F. Luzzaro, and G.M. Rossolini, 2016. 'First detection of the mcr-1 colistin resistance gene in Escherichia coli, Italy', Antimicrobial agents and chemotherapy, Vol. 60 (5), pp.3257-3258.

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, Vol. 18 (5), pp.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, Vol. 19 (1), pp.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, Vol. 1 (1), p.11.

Carlsson, U., P. Wallgren, L. Renström, A. Lindberg, H. Eriksson, P. Thoren, L. Eliasson-Selling, N. Lundeheim, E. Nörregard, and C. Thörn, 2009. 'Emergence of porcine reproductive and respiratory syndrome in Sweden: detection, response and eradication', Transboundary and emerging diseases, Vol. 56 (4), pp.121-131.

Catry, B., J. Dewulf, D. Maes, B. Pardon, B. Callens, M. Vanrobaeys, G. Opsomer, A. de Kruif, and F. Haesebrouck, 2016. 'Effect of Antimicrobial Consumption and Production Type on Antibacterial Resistance in the Bovine Respiratory and Digestive Tract', PloS one, Vol. 11 (1),

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,

Cavaco, L.M., H. Hasman, F.M. Aarestrup, J.A. Wagenaar, H. Graveland, K. Veldman, D. Mevius, A. Fetsch, B.-A. Tenhagen, and M.C. Porrero, 2011. 'Zinc resistance of Staphylococcus aureus of animal origin is strongly associated with methicillin resistance', Veterinary microbiology, Vol. 150 (3), pp.344-348.

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. 'Bis-(3′-5′)-Cyclic Dimeric GMP Regulates Antimicrobial Peptide Resistance in Pseudomonas aeruginosa', Antimicrobial agents and chemotherapy, Vol. 57 (5), pp.2066-2075.

CODA-CERVA, 2015. 'Antimicrobial Resistance in commensal E. coli from livestock in Belgium: Trend Analysis 2011-2014', http://www.amcra.be/en/coda-cerva-antimicrobial-resistance-commensal-e-coli-livestock-belgium-trend-analysis-2011-2014

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, Vol. 1 (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, Vol. 52 (11), pp.987-993.

Cornaglia, G., H. Giamarellou, and G.M. Rossolini, 2011. 'Metallo-beta-lactamases: a last frontier for beta-lactams?', The Lancet infectious diseases, Vol. 11 (5), pp.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, Vol. 42 (2), pp.S82-89.

Daikos, G.L., A. Markogiannakis, M. Souli, and L.S. Tzouvelekis, 2012. 'Bloodstream infections caused by carbapenemase-producing Klebsiella pneumoniae: a clinical perspective', Expert review of anti-infective therapy, Vol. 10 (12), pp.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, Vol. 61 (4), pp.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', http://monash.edu/pharm/about/events/polymyxins/

Dewaele, I., G. Rasschaert, C. Wildemauwe, H. Van Meirhaeghe, M. Vanrobaeys, E. De Graef, L. Herman, R. Ducatelle, M. Heyndrickx, and K. De Reu, 2012. 'Polyphasic characterization of Salmonella Enteritidis isolates on persistently contaminated layer farms during the implementation of a national control program with obligatory vaccination: A longitudinal study', Poultry science, Vol. 91 (11), pp.2727-2735.

Dhar, S., E.T. Martin, P.R. Lephart, J.P. McRoberts, T. Chopra, T.T. Burger, R. Tal-Jasper, K. Hayakawa, H. Ofer-Friedman, and T. Lazarovitch. 2016. Risk Factors and Outcomes for Carbapenem-Resistant Klebsiella pneumoniae Isolation, Stratified by Its Multilocus Sequence Typing: ST258 Versus Non-ST258. In Open forum infectious diseases. Oxford University Press, ofv213.

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, Vol. 54 (9-10), pp.344-352.

Dorado-García, A., H. Graveland, M.E. Bos, K.M. Verstappen, B.A. Van Cleef, J.A. Kluytmans, J.A. Wagenaar, and D.J. Heederik, 2015. 'Effects of Reducing Antimicrobial Use and Applying a Cleaning and Disinfection Program in Veal Calf Farming: Experiences from an Intervention Study to Control Livestock-Associated MRSA', PloS one, Vol. 10 (8), p.e0135826.

Doumith, M., G. Godbole, P. Ashton, L. Larkin, T. Dallman, M. Day, M. Day, B. Muller-Pebody, M.J. Ellington, E. de Pinna, A.P. Johnson, H. K.L, and W. N., 2016. 'Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales', Journal of Antimicrobial Chemotherapy, Vol.

Drlica, K., and X. Zhao, 2007. 'Mutant selection window hypothesis updated', Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, Vol. 44 (5), pp.681-688.

Du, H., L. Chen, Y.-W. Tang, and B.N. Kreiswirth, 2016. 'Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae', The Lancet infectious diseases, Vol. 16 (3), pp.287-288.

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, Vol. 57 (3), pp.349-358.

ECDC, 2015. 'Summary of the latest data on antibiotic consumption in the European Union. ESAC-Net data', http://ecdc.europa.eu/en/eaad/antibiotics-news/Documents/antimicrobial-consumption-ESAC-Net-summary-2015.pdf

ECDC, 2016. 'Rapid risk assessment: Carbapenem-resistant Enterobacteriaceae', http://ecdc.europa.eu/en/publications/Publications/carbapenem-resistant-enterobacteriaceae-risk-assessment-april-2016.pdf

ECDC/EFSA/EMA, 2015. 'First joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (JIACRA)', EFSA Journal Vol. 13(1):4006

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', Stockholm

EFSA, 2016. 'The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014', EFSA Journal, Vol. 14(2):4380

EFSA BIOHAZ Panel, 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, Vol. 9(8):2322

EFSA/EMA, foreseen 2016. 'Request for a joint EFSA and EMA scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety',

EMA, 2013. 'Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health (EMA/755938/2012)', http://www.ema.europa.eu/docs/en_GB/document_library/Report/2013/07/WC500146813.pdf

EMA, 2014a. 'Answers to the requests for scientific advice on the impact on public health and animal health of the use of antibiotics in animals - Answer to the second request from the EC (ranking of antibiotics); Answer to the third request from the EC (new antibiotics); Answer to the fourth request from the EC (risk mitigation options) (EMA/381884/2014)', http://www.ema.europa.eu/docs/en_GB/document_library/Other/2014/07/WC500170253.pdf

EMA, 2014b. 'Polymyxin Article-31 referral - European Medicines Agency completes review of polymyxin-based medicines - Recommendations issued for safe use in patients with serious infections resistant to standard antibiotics (EMA/785229/2014 corr1)', http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Polymyxin_31/WC500176333.pdf

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 (EMA/189829/2010)', http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Colistin_35/WC500093733.pdf

EMA/CVMP, 2015. 'Opinion following an Article 35 referral for veterinary medicinal products containing colistin to be administered orally (EMA/160340/2015)', http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Colistin_oral_35/WC500185448.pdf

EMA/ESVAC, 2015. 'European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013 (EMA/387934/2015). Fifth ESVAC report.', http://www.ema.europa.eu/docs/en_GB/document_library/Report/2015/10/WC500195687.pdf

EMEA/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 (EMEA/CVMP/SAGAM/81730/2006-Rev.1)', http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004307.pdf

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', http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf

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, Vol. 40 (9), pp.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, Vol. 33 (6), p.503.

Falgenhauer, L., S.-E. Waezsada, Y. Yao, C. Imirzalioglu, A. Käsbohrer, U. Roesler, G.B. Michael, S. Schwarz, G. Werner, and L. Kreienbrock, 2016. 'Colistin resistance gene mcr-1 in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany', The Lancet infectious diseases, Vol. 16 (3), pp.282-283.

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, Vol. 54 (8), pp.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, Vol. 51 (3), pp.849-856.

Folkhalsomyndigheten, 2016. 'Bakterie resistent mot sista behandlingsalternativet funnen [Bacteria resistant to the last treatment option found]. Press release. ', http://www.folkhalsomyndigheten.se/nyheter-och-press/nyhetsarkiv/2016/februari/bakterie-resistent-mot-sista-behandlingsalternativet-funnen/

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, Vol. 66 (9), pp.2070-2074.

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, Vol. 55 (7), pp.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, Vol. p.18.

Gentry, L.O., 1991. 'Bacterial resistance', The Orthopedic clinics of North America, Vol. 22 (3), pp.379-388.

Grami, R., W. Mansour, W. Mehri, O. Bouallègue, N. Boujaâfar, J. Madec, and M. Haenni, 2016. 'Impact of food animal trade on the spread of mcr-1-mediated colistin resistance, Tunisia, July 2015', Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, Vol. 21 (8), p.30144.

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, Vol. 276 (5310), pp.250-253.

Guyonnet, J., B. Manco, L. Baduel, V. Kaltsatos, M. Aliabadi, and P. Lees, 2010. 'Determination of a dosage regimen of colistin by pharmacokinetic/pharmacodynamic integration and modeling for treatment of GIT disease in pigs', Research in veterinary science, Vol. 88 (2), pp.307-314.

Haenni, M., L. Poirel, N. Kieffer, P. Châtre, E. Saras, V. Métayer, R. Dumoulin, P. Nordmann, and J.-Y. Madec, 2016. 'Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids', The Lancet infectious diseases, Vol. 16 (3), pp.281-282.

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, Vol. 57 (7), pp.3224-3229.

Hanon, J.-B., S. Jaspers, P. Butaye, P. Wattiau, E. Méroc, M. Aerts, H. Imberechts, K. Vermeersch, and Y. Van der Stede, 2015. 'A trend analysis of antimicrobial resistance in commensal Escherichia coli from several livestock species in Belgium (2011–2014)', Preventive veterinary medicine, Vol. 122 (4), pp.443-452.

Hasman, H., A. Hammerum, F. Hansen, R.S. Hendriksen, B. Olesen, Y. Agersø, E. Zankari, P. Leekitcharoenphon, M. Stegger, and R.S. Kaas, 2015. 'Detection of mcr-1 encoding plasmid-mediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015', Eurosurveillance (Online Edition), Vol. 20 (49), pp.1-5.

Hauck, C., E. Cober, S. Richter, F. Perez, R. Salata, R. Kalayjian, R. Watkins, N. Scalera, Y. Doi, and K. Kaye, 2016. 'Spectrum of excess mortality due to carbapenem-resistant Klebsiella pneumoniae infections', Clinical Microbiology and Infection, Vol.

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, Vol. 52 (1), pp.351-352.

Hu, Y., F. Liu, I.Y. Lin, G.F. Gao, and B. Zhu, 2015. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet infectious diseases, Vol. 16 (2), pp.146-147.

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, Vol. 16 (26),

Ingenbleek, A., E. Van Gastel, M. Costers, M. Goossens, B. Catry, and K. Magerman, 2015. 'National report on consumption of systemic antimicrobial agents in Belgian hospitals (2007-2013)', http://www.nsih.be/download/GM/BeH-SAC_Report-2015_EN.docx

Jung, J., T. Eberl, K. Sparbier, C. Lange, M. Kostrzewa, S. Schubert, and A. Wieser, 2014. 'Rapid detection of antibiotic resistance based on mass spectrometry and stable isotopes', European journal of clinical microbiology & infectious diseases, Vol. 33 (6), pp.949-955.

Keeton, C., 2016. 'Mutant superbug threat to SA poultry', http://www.timeslive.co.za/sundaytimes/stnews/2016/03/20/Mutant-superbug-threat-to-SA-poultry

Kluytmans–van den Bergh, M., P. Huizinga, M. Bonten, M. Bos, K. De Bruyne, A. Friedrich, J. Rossen, P. Savelkoul, and J. Kluytmans, 2016. 'Presence of mcr-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009', Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, Vol. 21 (9), p.30149.

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, Vol. 72 (6), pp.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, Vol. 76 (1), pp.70-73.

Koyama, Y., A. Kurosasa, A. Tsuchiya, and K. Takakuta, 1950. 'A new antibiotic ‘colistin’produced by spore-forming soil bacteria', J Antibiot, Vol. 3 pp.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, Vol. 10 (9), pp.597-602.

Kuo, S.-C., W.-C. Huang, H.-Y. Wang, Y.-R. Shiau, M.-F. Cheng, and T.-L. Lauderdale, 2016. 'Colistin resistance gene mcr-1 in Escherichia coli isolates from humans and retail meats, Taiwan', Journal of Antimicrobial Chemotherapy, Vol.

Lambrini, 2013. 'In the era of polymyxins use: Emergence of colistin resistance in *Klebsiella pneumoniae*', 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, Vol. 30 (6), pp.964-965.

Landman, D., C. Georgescu, D.A. Martin, and J. Quale, 2008. 'Polymyxins revisited', Clinical microbiology reviews, Vol. 21 (3), pp.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, Vol. 12 (3), pp.592-594.

Lee, J.-Y., M.-J. Choi, H.J. Choi, and K.S. Ko, 2016. 'Preservation of acquired colistin resistance in gram-negative bacteria', Antimicrobial agents and chemotherapy, Vol. 60 (1), pp.609-612.

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, Vol. 208 (7), pp.1142-1151.

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, Vol. 6 (9), pp.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, Vol. 30 (12), pp.1279-1291.

Liu, C.-Y., Y.-Y. Han, P.-H. Shih, W.-N. Lian, H.-H. Wang, C.-H. Lin, P.-R. Hsueh, J.-K. Wang, and Y.-L. Wang, 2016. 'Rapid bacterial antibiotic susceptibility test based on simple surface-enhanced Raman spectroscopic biomarkers', Scientific reports, Vol. 6

Liu, Y.-Y., Y. Wang, T.R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, and X. Huang, 2015. 'Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study', The Lancet infectious diseases, Vol. 16 (2), pp.161-168.

Livermore, D.M., 2012. 'Current epidemiology and growing resistance of gram-negative pathogens', The Korean journal of internal medicine, Vol. 27 (2), pp.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, Vol. 51 (10), pp.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, Vol. 117 (6), pp.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, Vol. 157 (1-2), pp.190-199.

Malhotra-Kumar, S., B.B. Xavier, A.J. Das, C. Lammens, P. Butaye, and H. Goossens, 2016a. 'Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid', The Lancet infectious diseases, Vol. 16 (3), pp.283-284.

Malhotra-Kumar, S., B.B. Xavier, A.J. Das, C. Lammens, H.T.T. Hoang, N.T. Pham, and H. Goossens, 2016b. 'Colistin-resistant Escherichia coli harbouring mcr-1 isolated from food animals in Hanoi, Vietnam', The Lancet infectious diseases, Vol. 16 (3), pp.286-287.

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

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

MARAN, 2015. 'Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2014', http://www.wageningenur.nl/upload_mm/2/2/2/0ab4b3f5-1cf0-42e7-a460-d67136870ae5_NethmapMaran2015.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, Vol. 31 (4), pp.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, Vol. 66 (4), pp.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, Vol. 95 (1), pp.50-63.

Michalopoulos, A., and M.E. Falagas, 2008. 'Colistin and polymyxin B in critical care', Critical care clinics, Vol. 24 (2), pp.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, Vol. 1 (1), pp.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, Vol. 54 (12), pp.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, Vol. 56 (8), pp.4241-4249.

Monaco, M., T. Giani, M. Raffone, F. Arena, A. Garcia-Fernandez, S. Pollini, S.-I.C. Network Eu, H. Grundmann, A. Pantosti, and G. Rossolini, 2014. 'Colistin resistance superimposed to endemic carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014', Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, Vol. 19 (42),

Moore, J.N., and M.H. Barton, 2003. 'Treatment of endotoxemia', The Veterinary clinics of North America. Equine practice, Vol. 19 (3), pp.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, Vol. 56 (2), pp.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, Vol. 186 (2), pp.575-579.

Mulvey, M.R., L.F. Mataseje, J. Robertson, J. Nash, P. Boerlin, B. Toye, R. Irwin, and R.G. Melano, 2016. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet. Infectious diseases, Vol. 16 (3), pp.289-290.

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, Vol. 4 (3), pp.e00021-00013.

Nation, R.L., and J. Li, 2009. 'Colistin in the 21st century', Current opinion in infectious diseases, Vol. 22 (6), pp.535-543.

Nguyen, N.T., H.M. Nguyen, C.V. Nguyen, T.V. Nguyen, M.T. Nguyen, H.Q. Thai, M.H. Ho, G. Thwaites, H.T. Ngo, and S. Baker, 2016. 'The use of colistin and other critical antimicrobials on pig and chicken farms in southern Vietnam and their association with resistance in commensal Escherichia coli', Applied and environmental microbiology, Vol. pp.AEM. 00337-00316.

Nguyen, V.T. 2016. Prevalence and risk factors for faecal colonization with mcr-1 carrying bacteria in backyard poultry and humans in Vietnam - ePoster #PLB34 In 26th ECCMID (European Congress of Clinial Microbiology and Infectious Diseases). Amsterdam.

Nord, N.M., and P.D. Hoeprich, 1964. 'Polymyxin B and Colistin. A Critical Comparison', The New England journal of medicine, Vol. 270 pp.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, Vol. 16 (2), pp.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, Vol. 138 (3), pp.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, Vol. 57 (5), pp.2103-2108.

Official Journal of the European Union. 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/legal-content/EN/TXT/?qid=1416502774573&uri=CELEX:32013R0122.

Olaitan, A.O., S. Chabou, L. Okdah, S. Morand, and J.-M. Rolain, 2015. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet infectious diseases, Vol. 16 (2), pp.144-145.

Olaitan, A.O., S. Morand, and J.-M. Rolain, 2014. 'Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria', Frontiers in microbiology, Vol. 5 (643),

Onori, R., S. Gaiarsa, F. Comandatore, S. Pongolini, S. Brisse, A. Colombo, G. Cassani, P. Marone, P. Grossi, and G. Minoja, 2015. 'Tracking Nosocomial Klebsiella pneumoniae Infections and Outbreaks by Whole-Genome Analysis: Small-Scale Italian Scenario within a Single Hospital', Journal of clinical microbiology, Vol. 53 (9), pp.2861-2868.

Ozsurekci, Y., K. Aykac, A.B. Cengiz, C. Bayhan, B. Sancak, E.K. Oncel, A. Kara, and M. Ceyhan, 2016. 'Is colistin effective in the treatment of infections caused by multidrug-resistant (MDR) or extremely drug-resistant (XDR) gram-negative microorganisms in children?', Diagnostic microbiology and infectious disease, Vol.

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, Vol. 67 (4), pp.1027-1038.

Perrin-Guyomard, A., M. Bruneau, P. Houée, K. Deleurme, P. Legrandois, C. Poirier, C. Soumet, and P. Sanders, 2016. 'Prevalence of mcr-1 in commensal Escherichia coli from French livestock, 2007 to 2014', Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, Vol. 21 (6),

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

Petrillo, M., A. Angers-Loustau, and J. Kreysa, 2016. 'Possible genetic events producing colistin resistance gene mcr-1', The Lancet infectious diseases, Vol. 16 (2), pp.161-168.

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, Vol. 11 (2), pp.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, Vol. 53 (8), pp.3430-3436.

Pogue, J.M., D. Marchaim, D. Kaye, and K.S. Kaye, 2011. 'Revisiting "older" antimicrobials in the era of multidrug resistance', Pharmacotherapy, Vol. 31 (9), pp.912-921.

Poirel, L., N. Kieffer, N. Liassine, D. Thanh, and P. Nordmann, 2016. 'Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of Escherichia coli', The Lancet infectious diseases, Vol.

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, Vol. 62 (6), pp.1311-1318.

Prim, N., A. Rivera, J. Rodríguez-Navarro, M. Español, M. Turbau, P. Coll, and B. Mirelis, 2016. 'Characteristics of Escherichia coli isolates harbouring mcr-1 and epidemiological data of the patients, Barcelona, 2012–15 (n= 15)', Blood, Vol. 27 p.12.

Public Health Agency of Canada, 2016. 'Stakeholder Update February 2, 2016 - Issue: MCR-1 gene detected in Canada by PHAC researchers at the National Microbiology Laboratory', http://www.publichealth.gc.ca

Quesada, A., M. Ugarte-Ruiz, M.R. Iglesias, M.C. Porrero, R. Martínez, D. Florez-Cuadrado, M.J. Campos, M. García, S. Píriz, and J.L. Sáez, 2016. 'Detection of plasmid mediated colistin resistance (MCR-1) in Escherichia coli and Salmonella enterica isolated from poultry and swine in Spain', Research in veterinary science, Vol. 105 pp.134-135.

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, Vol. 56 (4), pp.2108-2113.

Rapoport, M., D. Faccone, F. Pasteran, P. Ceriana, E. Albornoz, A. Petroni, and A. Corso, 2016. 'mcr-1-mediated colistin resistance in human infections caused by Escherichia coli: First description in Latin America', Antimicrobial agents and chemotherapy, Vol.

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, Vol. 65 (12), pp.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, Vol. 41 (6), pp.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, Vol. 194 (12), pp.3173-3188.

Review on antimicrobial resistance (conference), 2015. 'Rapid diagnostics: stopping unnecessary use of antibiotics. Chaired by Jim O'Neill. October, 2015', http://amr-review.org/sites/default/files/Paper-Rapid-Diagnostics-Stopping-Unnecessary-Prescription-Low-Res.pdf

Rhouma, M., F. Beaudry, W. Thériault, N. Bergeron, S. Laurent-Lewandowski, J.M. Fairbrother, and A. Letellier, 2015. 'Gastric stability and oral bioavailability of colistin sulfate in pigs challenged or not with Escherichia coli O149: F4 (K88)', Research in veterinary science, Vol. 102 pp.173-181.

Richez, P., and D.G. Burch, 2016. 'Colistin in animals: a high risk for resistance selection in Europe?', Veterinary Record, Vol. 178 (4), pp.101-102.

Ruppé, E., E. Chatelier, N. Pons, A. Andremont, and S.D. Ehrlich, 2016. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet. Infectious diseases, Vol. 16 (3), p.290.

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', Journal of the American Medical Association, Vol. 207 (11), pp.2099-2101.

Schindler, M., and M.J. Osborn, 1979. 'Interaction of divalent cations and polymyxin B with lipopolysaccharide', Biochemistry, Vol. 18 (20), pp.4425-4430.

Schmithausen, R.M., S.R. Kellner, S.V. Schulze-Geisthoevel, S. Hack, S. Engelhart, I. Bodenstein, N. Al-Sabti, M. Reif, R. Fimmers, and B. Körber-Irrgang, 2015. 'Eradication of Methicillin-Resistant Staphylococcus aureus and of Enterobacteriaceae Expressing Extended-Spectrum Beta-Lactamases on a Model Pig Farm', Applied and environmental microbiology, Vol. 81 (21), pp.7633-7643.

Schorr, C., 2009. 'Performance improvement in the management of sepsis', Critical care clinics, Vol. 25 (4), pp.857-867.

SDa. 2015. Usage of antibiotics in livestock in the Netherlands in 2014. In http://www.autoriteitdiergeneesmiddelen.nl/Userfiles/pdf/SDa-rapporten/def-rapport-sda-het-gebruik-van-antibiotica-bij-landbouwhuisdieren-in-2014-incl-erratum.pdf.

Shen, Z., Y. Wang, Y. Shen, J. Shen, and C. Wu, 2016. 'Early emergence of mcr-1 in Escherichia coli from food-producing animals', The Lancet infectious diseases, Vol. 3 (16), p.293.

Skov, R.L., and D.L. Monnet, 2016. 'Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds', Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, Vol. 21 (9),

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, Vol. 23 (7), pp.1155-1162.

Stege, H., F. Bager, E. Jacobsen, and A. Thougaard, 2003. 'VETSTAT—the Danish system for surveillance of the veterinary use of drugs for production animals', Preventive veterinary medicine, Vol. 57 (3), pp.105-115.

Stoesser, N., A.J. Mathers, C.E. Moore, N.P. Day, and D.W. Crook, 2016. 'Colistin resistance gene mcr-1 and pHNSHP45 plasmid in human isolates of Escherichia coli and Klebsiella pneumoniae', The Lancet infectious diseases, Vol. 16 (2), pp.161-168.

Suzuki, S., M. Ohnishi, M. Kawanishi, M. Akiba, and M. Kuroda, 2016. 'Investigation of a plasmid genome database for colistin-resistance gene *mcr-1*', The Lancet infectious diseases, Vol.

Swedres-Svarm, 2014. 'Consumption of antibiotics and occurrence of antibiotic resistance in Sweden', http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/swedres_svarm2014.pdf

Tappenden, P., S. Harnan, L. Uttley, M. Mildred, C. Carroll, and A. Cantrell, 2013. 'Colistimethate sodium powder and tobramycin powder for inhalation for the treatment of chronic Pseudomonas aeruginosa lung infection in cystic fibrosis: systematic review and economic model', Health Technology Assessment, Vol. 17 (56),

Thanh, D.P., H.T. Tuyen, T.N.T. Nguyen, R.R. Wick, G. Thwaites, S. Baker, and K.E. Holt, 2016. 'Inducible colistin resistance via a disrupted plasmid-borne mcr-1 gene in a 2008 Vietnamese Shigella sonnei isolate', bioRxiv, Vol.

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, Vol. 74 (4), pp.251-263.

Tse, H., and K.-Y. Yuen, 2016. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet infectious diseases, Vol. 2 (16), pp.145-146.

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, Vol. 55 (7), pp.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, Vol. 41 (3), pp.292-293.

van Belkum, A., and W.M. Dunne, Jr., 2013. 'Next Generation Antimicrobial Susceptibility Testing', Journal of clinical microbiology, Vol. 51 (7), pp.2018-2024.

Van den Hof, J.A.R., D. Maes, S. Piepers, F. Boyen, F. Haesebrouck, W. Depondt, and J. Dewulf, submitted. 'Comparison of post weaning zinc oxide and colistin treatment in weaned piglets',

Veldman, K. 2016. The occurrence of the colistin resistance gene mcr-1 in Salmonella enterica and E. coli isolates from humans, livestock and retail meat in the Netherlands - Presentation #OLB02 In 26th ECCMID (European Congress of Clinial Microbiology and Infectious Diseases). Amsterdam.

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, Vol. 56 (3), pp.398-404.

Wasyl, D., A. Hoszowski, Z. M., and K. Szulowski, 2013. 'Antimicrobial resistance in commensal Escherichia coli isolated from animals at slaughter', Frontiers in microbiology, Vol. 4 (221),

Webb, H.E., S.A. Granier, M. Marault, Y. Millemann, H.C. den Bakker, K.K. Nightingale, M. Bugarel, S.A. Ison, H.M. Scott, and G.H. Loneragan, 2015. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet infectious diseases, Vol. 16 (2), pp.147-149.

Wright, M.S., A. Iovleva, M.R. Jacobs, R.A. Bonomo, and M.D. Adams, 2016. 'Genome dynamics of multidrug-resistant Acinetobacter baumannii during infection and treatment', Genome medicine, Vol. 8 (1), p.1.

Wright, M.S., Y. Suzuki, M.B. Jones, S.H. Marshall, S.D. Rudin, D. Van Duin, K. Kaye, M.R. Jacobs, R.A. Bonomo, and M.D. Adams, 2015. 'Genomic and transcriptomic analyses of colistin-resistant clinical isolates of Klebsiella pneumoniae reveal multiple pathways of resistance', Antimicrobial agents and chemotherapy, Vol. 59 (1), pp.536-543.

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, Vol. 899 pp.14-20.

Yao, X., Y. Doi, L. Zeng, L. Lv, and J.-H. Liu, 2016. 'Carbapenem-resistant and colistin-resistant Escherichia coli co-producing NDM-9 and MCR-1', The Lancet infectious diseases, Vol. 16 (3), pp.288-289.

Ye, H., Y. Li, Z. Li, R. Gao, H. Zhang, R. Wen, G.F. Gao, Q. Hu, and Y. Feng, 2016. 'Diversified mcr-1-Harbouring Plasmid Reservoirs Confer Resistance to Colistin in Human Gut Microbiota', mBio, Vol. 7 (2), pp.e00177-00116.

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, Vol. 17 (46),

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, Vol. 48 (6), pp.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, Vol. 68 (6), pp.1285-1296.

Zeng, K., Y. Doi, S. Patil, X. Huang, and G.-B. Tian, 2016. 'Emergence of plasmid-mediated mcr-1 gene in colistin-resistant Enterobacter aerogenes and Enterobacter cloacae', Antimicrobial agents and chemotherapy, Vol.

Zhang, R., Y. Huang, E.W.-c. Chan, H. Zhou, and S. Chen, 2016. 'Dissemination of the mcr-1 colistin resistance gene', The Lancet. Infectious diseases, Vol. 16 (3), p.291.

Zurfuh, K., L. Poirel, P. Nordmann, M. Nüesch-Inderbinen, H. Hächler, and R. Stephan, 2016. 'Occurrence of the Plasmid-Borne mcr-1 Colistin Resistance Gene in Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in River Water and Imported Vegetable Samples in Switzerland', Antimicrobial agents and chemotherapy, Vol. 60 (4), pp.2594-2595.