FEDERATION OF VETERINARIANS OF EUROPE

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Reflection paper on the importance of polymyxin antibiotics in veterinary medicine

Summary

Polymyxins have been used for over 50 years in both humans and animals. Polymyxin B and polymyxin E (colistin) are the medically important active substances. In human medicine, it was used rarely due to its neuro- and nephrotoxicity until recently reclaimed as a last resort antibiotic to treat bacterial infections resistant to other antibiotics, particularly carbapenem-resistant Gram-negative bacteria. In veterinary medicine, both colistin and polymyxin B are authorised for animals in Europe. Colistin has been used mainly to treat infections caused by *Enterobacteriaceae* in farmed animals including cattle, pigs, poultry, small ruminants, rabbits and turkeys, mostly given orally as group treatment via premix, oral powder or oral solution. Colistin and polymyxin B are registered as well for topical administration such as eye and eardrops for companion animals.

Although it has been used extensively, **low resistance prevalence** developed as opposed to other antimicrobials. Since its discovery in 2015, the increasing prevalence of the plasmid-borne mobile colistin resistance gene *mcr* gained particular attention worldwide. Therefore, major efforts have been made in the European Union and **sales of colistin have decreased by more than 75% from 2011 to 2020** though the level of use differs greatly between Member States. Nonetheless, colistin remains **essential** in veterinary medicine and almost irreplaceable for intestinal **enterotoxemic *E. coli* infections in pigs and poultry** with the risk to become systemic. It is not recommended to replace the use of colistin with other antibiotics as those are less efficacious and/or classified with higher risk towards resistance and cross-resistance. In pigs, zinc oxide was used but is now discouraged due to its negative environmental impact and potentially co-selection of resistance genes. The best alternative is prevention of infections, which can be achieved through a wide choice of tools such as improving biosecurity and hygiene, appropriate nutrition, including feed restriction to prevent e.g. rabbit colibacilloses, breeding robust animals, regular veterinary visits to monitor animal health and welfare and to develop herd health plans as well as appling vaccination whenever possible.

Colistin should be used prudently, responsibly and uniquely for veterinary prescribed therapeutic treatments. Dosed appropriately, colistin acts bactericidal and resistance prevalence remained low over decades. It must be used after examination and diagnosis according to the indication and with respect to species-specific pharmacodynamics, pharmacokinetics and the lastest scientific evidence.

Main FVE recommendations:

- Recognise colistin as essential veterinary medicinal product
- Only use colistin in livestock after clinical examination, identification of the target bacteria and antimicrobial susceptibility testing (AST).
  - As a practical consequence, use of colistin becomes targeted and should be considered only for the treatment of clinical conditions when there are no alternative antibiotics available that could be clinically effective and/or classified as higher risk for (cross)-resistance.
  - If AST is not possible, treatment should be based on epidemiological information and recent knowledge of AST results of the target bacteria for the individual animal, at farm level or at local/regional level.
  - In addition, exemptions to the need of AST testing prior prescription are necessary, if
    - ► Sampling would be harmful to the animal (e.g., anaesthesia would be required)
    - ► Pathogens cannot be cultivated in routine culture systems
    - ► No scientifically proven AST method is available for the target pathogen
    - ► No alternative legal, safe and efficacious antimicrobial therapy is available
- Allow continued use of colistin under the cascade (off-label use) for all species and avoid classifying the polymyxin class under Art. 107, 6a of Reg. (EU) 2019/6.

Introduction: Polymyxin structure and its mechanism of action

Polymyxin antibiotics are nonribosomal, cyclic decapeptides, naturally occurring in the Gram-positive soil bacterium *Paenibacillus polymyxa* (1). They were discovered in 1947 in Japan as a secondary metabolite inhibiting the growth of other competing microorganisms (2). Five chemically distinguished compounds are known (polymyxins A, B, C, D, and E) of which polymyxin B and colistin (polymyxin E) are medically important ones. Structurally distinctive, this class acts bactericidal by disrupting the functional integrity of the cell membrane, leading to an escape of macromolecules and ions from the cell, and subsequently to cell damage and death. The initial binding target of polymyxins is the lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, where the "self-promoted uptake" pathway permits the uptake via electrostatic interactions. Thereafter, colistin attaches to the lipid A component of LPS, leading to a detergent-like mechanism of action that involves an increase in the permeability of the cell envelope followed by leakage of periplasmic and cytoplasmic contents, subsequent inner membrane lysis and ultimately cell death (3). In addition, colistin binds and neutralises free LPS, confering an important anti-endotoxin activity (4). However, the supramolecular interaction between colistin and free LPS may even be stronger than between colistin and the intact Gram-negative bacteria with colistin,

leading to a competition of binding, which may hamper the antibacterial effects of colistin Gram-negative bacteria but prevent endotoxemia (5).

However, Gram-negative bacteria with a LPS of lower binding affinity for polymyxins are intrinsically resistant due to modified phosphate groups, including some *Aeromonas* spp., *Brucella*, *Burkholderia cepacian, Campylobacter*, *Chromobacterium*, *Edwardsiella* spp. *Legionella*, *Morganella morganii, Neisseria* spp., *Proteus* spp., *Providencia* spp., *Serratia marcescens* and *Vibrio cholera* (6). Whereas colistin is not active against Gram-positive bacteria nor against anaerobes, polymyxin B exhibits a broader spectrum of activity, mostly against Gram-negative bacteria, but as well against Gram-positive bacteria including *Staphylococcus aureus* (7), *Streptococcus gordonii*, *Streptococcus agalactiae* (8), as well as against facultative anaerobic bacteria such as *Listeria monocytogenes* (9,10). The neuro- and nephrotoxicity of colistin restricted its use in human medicine until recently when colistin was reclaimed to treat multidrug-resistant (MDR) Gram-negative bacteria, particularly those caused by carbapenem-resistant *Enterobacteriaceae* (11). The horizontally acquired mobile colistin resistance mediated via conjugation of plasmids carrying the *mcr* gene and its variants are of major global concern (12).

Classification of polymyxins: essential both in human and veterinary medicine

Polymyxins are considered by both the human and animal health sectors as essential. The World Health Organization (WHO) categorises polymyxins as **Highest Priority Critically Important Antimicrobials** (HP-CIA) (13). The World Organisation for Animal Health (OIE) also classifies colistin as **Veterinary Critically Important Antimicrobials (V-CIA)** on its list of antimicrobial agents of veterinary importance (latest update 2019) (12-13).

In 2013, the European Medicines Agency's (EMA) Committee for Medicinal Products for Veterinary Use (CVMP) recommended the restriction of the indications for use of colistin to the 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 (15). However, following the discovery of the plasmid-borne *mcr-1* gene in 2015 (16), EMA updated its previous advice on the impact of and need for colistin use for human and animal health. Therefore, EMA recommended in 2016 (i) to **minimise sales to achieve a 65% reduction** in European Union-wide sales of colistin for use in animals; (ii) to reduce the use of colistin in animals at least to **a target level of 5 mg colistin/population correction unit (PCU)** on national level, and that (iii) this 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** (17). Accordingly, EMA updated its advice regarding the categorisation of antimicrobials, whereas Category A ("Avoid") includes antimicrobial classes not currently authorised in veterinary medicine in the European Union (EU). Colistin was then categorised in **Category B ("Restrict")** alongside with (fluoro-)quinolones, 3rd- and 4th-generation cephalosporins. Use of these antimicrobials in animals should be restricted to mitigate the risk to public health. They should be considered only for treatment of clinical conditions when there are no alternative antimicrobials in the lower categories C or D that could be effective. Especially for this category, use should be based on the results of antimicrobial susceptibility testing (AST), whenever possible. In April 2016, the CVMP recommended as well the withdrawal of

the marketing authorisations for all veterinary medicinal products for oral use containing colistin in combination with other antimicrobial substances, and the corresponding Commission Implementing Decision came into force in July 2016.

Indications for colistin in human and veterinary medicine

Initially, the use of colistin in human medicine was limited to ophthalmic and topical infections as well as to control lower airway bacterial infections with nebulised colistin owing to due to its neuro- and nephrotoxicity. Nowadays, polymyxins are reclaimed as last-resort antibiotics in human medicine to treat infections caused by MDR bacteria, particularly for the highly virulent nosocomial ESKAPE bacteria (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) (18). Yet, the use of colistin remains scarce in human medicine. In 2017 in the EU, the population-weighted mean consumption of polymyxins in humans was 0.06 mg per kg of estimated biomass, ranging from 0–0.2 (median 0.03) mg per kg (19).

In contrast, colistin has been used widely in veterinary medicine for decades, especially in pigs, poultry, and veal calves. Intestinal infections due to Gram-negative bacteria are the main indications in livestock. Most of the colistin applications in animals are for therapeutic oral group treatments as its use for **growth promotion is banned in the EU since 2006**. Colistin tablets are available for calves for the treatment of neonatal colibacillosis. Polymyxin B is on the list of substances essential for the treatment of Equidae for systemic treatment for endotoxemia (anti-toxigenic effect, not antibacterial as such) associated with severe colic and other gastrointestinal diseases (20). Both colistin and polymyxin B have been registered for topical administration such as eye and eardrops to individual veterinary patients (except polymyxin B for food-producing animals in the absence of MRLs). Colistin is also used to treat bacterial infections in aquatic animal species (21). Due to limited data on the pharmacokinetic/ pharmacodynamic properties of colistin and its prodrug colistimethate sodium, doses administered vary greatly between animal species, farm types and indications (22). In 2001, CVMP determined a positive benefit–risk balance for colistin when administered daily at 100,000 IU colistin/kg body weight for calves, lambs and pigs, and at 75,000 IU colistin/kg body weight daily in poultry for 3–5 consecutive days, yet only for gastrointestinal infections caused by non-invasive *E. coli*, which are susceptible to colistin (23). However, many practising veterinarians indicated in recent years the need to revise and harmonise the Summary of Product Characteristics (SPC), e.g., posology for appropriate dosage for either colistin or its prodrug activity and either in mg or IU.

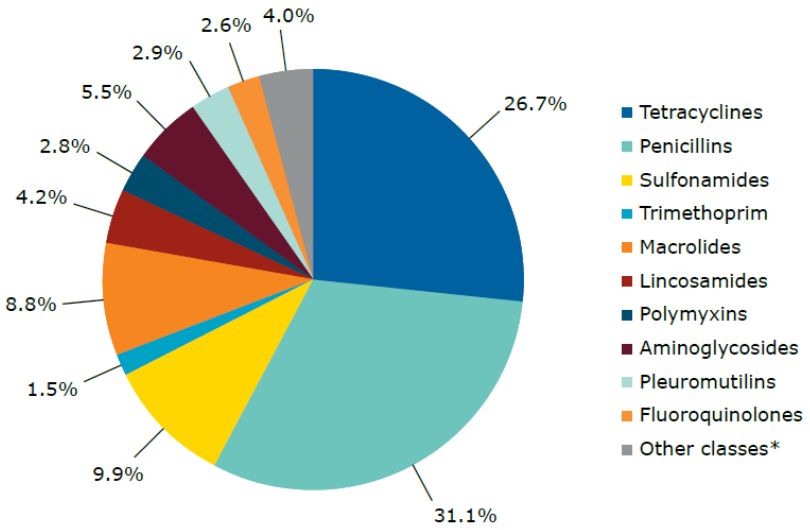
However, the unanimous acceptance of colistin in veterinary medicine was thoroughly questioned by the description of the plasmid-borne *mcr* gene in 2015 (16). Today, *mcr* genes were identified throughout the world, illustrating both its wide geographical distribution, its presence in humans and animals and the long-standing prevalence (24). A UEVP/FVE survey elucidated in 2017 the national considerations regarding colistin: A total of 85% (n=22/26) countries discussed the veterinary use of colistin, whereas 44% of the national veterinary associations (n=11/25) were involved in the development and discussion regarding the restriction of colistin. The vast majority (72%, n=18/25 countries) reclassified or considered to reclassify colistin under national guidelines as a CIA and 64% (n=16/25) set or discussed to set national targets for colistin use. Almost half of the countries (n=12/25), and in particular the Nordic countries, took other action regarding the use of colistin, e.g. only conditional use after AST.

Responsible and prudent use of colistin in veterinary medicine

Substantial efforts have been made by many EU Member States (MS) to reduce the overall use of antimicrobials in food-producing animals, including the creation of national usage and reduction targets, the measurement and benchmarking of prescribing and usage by veterinary practices and individual farms respectively, and through strategies to encourage antimicrobial stewardship (25). All EU MS have made an action plan on antimicrobial resistance (AMR). One of the aims is to reduce the use of HP-CIAs, this classification includes polymyxins.

Sales and use data of polymyxins in human and veterinary medicine: nearly 70% reduction in veterinary use, slightly increasing use for human patients

In Europe, colistin is almost exclusively used in food-producing animals and the EU/EEA population-weighted mean consumption of polymyxins in food-producing animals by far outweighed consumption in humans in 2017. This was even though sales of polymyxins in animals declined from 10.98 mg/PCU in 2011 to 2.58 mg/PCU in 2020, resulting in a drop of **76.5% between 2011 and 2020** (26). In 2020, polymyxins accounted for 2.8% of the total sales in 31 European countries in mg/PCU, whereas the sales of tetracyclines (26.7 %), penicillins (31.1 %) and sulphonamides (9.9 %) accounted for 67.7 % (Fig. 1). The vast majority of was sold as oral solution (57%), premix (22.5%) and oral powder (7.4%) (26).



*Others: Amphenicols, cephalosporins, other quinolones and other antibacterials (classified as such in the ATCvet system)

Figure 1. Sales of antimicrobial agents by antimicrobial class as percentage of the total sales for food-producing species in mg/PCU aggregated by 31 European countries for 2020. Reprinted from EMA (26).

However, there is a **wide variation between EU MS** in the extent of veterinary use of colistin with the highest consumption in Cyprus (15.9 mg/PCU), Portugal (11.7 mg/PCU) and Poland (9.1 mg/PCU), whereas Finland, Iceland and Norway reported no consumption of polymyxins in food-producing animals (Fig. 2). These variations were partly explained by differences in animal demographics, the selection of antimicrobial agents, dosage regimes, the type of data sources and veterinarians' prescribing habits. Yet, and based on the data available, the variation could not directly be linked to the

predominance of specific animal species, or a category or husbandry system in an individual MS (26). In 2020, Bulgaria, Cyprus, Germany, Hungary, Portugal and Poland, haven't reached the target level of 5mg/PCU regarding the sales yet whereas the remaining countries had sales below this level, including 16 countries with less than 1 mg/PCU (26). Estonia reduced sales of polymyxins remarkably and achieved in 2018 twice-lower level as the median in 31 European countries (27). There is little information available about how this decrease was achieved in various countries. Denmark put for example in place the VetStat "yellow card initiative" monitoring system which warns against the overuse of antimicrobials in pig farming for almost a decade (28).

Figure 2. Spatial distribution of sales of polymyxins for veterinary use in mg/PCU by countries for 2020. Reprinted from EMA (26).

Impact of colistin-resistant bacteria on veterinary and human health: *mcr-1* global concern

Various mechanisms of polymyxin resistance in Gram-negative bacteria have been described including chromosomal lipopolysaccharide modifications, efflux pumps, capsule formation and over-expression of membrane protein as well as the plasmid-borne resistance *mcr* genes (8,24). The underlying colistin resistance mechanism are complex. The main chromosomal mechanism of resistance is the modification of the bacterial outer membrane mainly mediated by upregulation the PhoP/PhoQ and PmrA/PmrB two-component regulatory system via the *pmrA/pmrB* and *phoQ* genes (29). Moreover, mutations that lead to the loss of the LPS, porin mutations and overexpression of efflux pump systems, overproduction of capsular polysaccharide (CPS) in some Gram-negative bacteria that hide the polymyxin binding sites and the release of CPS trapping polymyxins, as well as enzymatic inactivation of colistin have been described (30).

In late 2015, the plasmid-mediated *mcr-1* gene was first described in an *E. coli* strain isolated in China (16). MCR-1 is a transferase enzyme, which is membrane-anchored transmembrane portion linked to a zinc-binding catalytic that decreases the negative charge of the LPS and hence the binding affinity of colistin. This mediation renders the recipient strains resistant to polymyxins. Up to now, 22 new genetic variants of *mcr-*

1 have been identified in different countries, suggesting that the acquisition of *mcr-1* is most likely not a disadvantage to a host. Novel *mcr*-1 alleles have been reported, most recently the *mcr-10* (31). Though there is still a lack of information on the possibility of continuous evolution and origins of the *mcr* genes, it was suggested that *mcr-1* might have appeared earlier to its first description and most likely resulted from colistin use in food-producing animals already in the 1980s in China (32).

Antimicrobial Susceptibility Testing (AST) for polymyxins has its limitations

In the EU, harmonised AMR monitoring and reporting in certain zoonotic and commensal bacteria testing for colistin resistance became mandatory on the 1 January 2014. In 2020, the Commission Implementing Decision (EU) 2020/1729 came into force, providing the updated panel of antimicrobial substances to be included in AMR monitoring together with the EUCAST interpretative thresholds. Several chromogenic media are available to provide preliminary identification of colistin-resistant isolates and screening by routinly applied semiquantitative matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) is a promising tool to screen rapidly for colistin resistance (64). For colistin, accredited AST is defined as minimum inhibitory concentration (MIC) determination with broth microdilution (BMD) according to the ISO standard 20776-1 (33) and listed with a clinical breakpoint of >2 g/mL for *Salmonella* and *E. coli*. However, reliable AST for polymyxins is hampered due to their multi-component composition of commercially available polymyxin forms, their poor diffusion in agar due to their large molecular size, their cationic nature, and the development of heteroresistance (34,35). Hence, only BMD should be used for colistin MIC determination (36). Disk diffusion and gradient diffusion methods (E-test®) should be avoided as they do not consistently discriminate between susceptible and resistant isolates and available gradient tests may underestimate colistin MIC values (37). EUCAST published colistin breakpoints as well for Enterobacterales, *Pseudomonas,* and *Acinetobacter* (susceptible ≤2 µg/ml and resistant >2 µg/ml) (38). However, AST results for colistin are nowadays mostly reported with their MICs only as a consequence of recent data on polymyxin pharmacokinetics, pharmacodynamics, toxicity, and clinical outcomes, to emphasize that no MIC is associated with a high probability of treatment success for these antimicrobials (36). Phenotypical resistance is generally confirmed by molecular methods, including conventional PCR, loop-mediated isothermal amplification, as well combined with lateral flow biosensors, singleplex and multiplex real-time PCR, for which several high-performing commercial kits are available (65).

Resistance patterns in veterinary pathogens in Europe: resistance is uncommon and decreasing in some countries

The collected AMR data on Community level is harmonised with respect to sampling design, laboratory methodology, reporting and interpretation of resistance in indicator *E. coli* and *Salmonella* in livestock at slaughter and meat at retail. The latest EFSA report on AMR in zoonotic and indicator bacteria from humans, animals and food in 2018/2019 concluded that resistance to colistin was uncommon among *Salmonella* spp. and *E. coli* isolates recovered from food-producing animals (fattening pigs, calves, *Gallus gallus* and fattening turkeys) and carcases/meat derived from these animals, although moderate resistance was notably observed in certain *Salmonella* serovars due to their inherit resistance (39,40). Altogether 137 of the 13,598 isolates tested in 2018 and 2019 showed phenotypic resistance to colistin. Median levels of colistin resistance on MS level were 0% for pigs, broilers and turkeys and 0.6% in calves. Higher levels were however

reported in individual countries, up to 17.4% in turkeys, 4.7% in broilers, 3.4% in pigs and 2.4% in calves. Nonetheless, colistin was uncommon in the patterns of MDR *E.coli* isolates, at 1.5% in pigs, 1.6% in calves, 1.3% in broilers and 6.5% in turkeys.

Nonetheless, the **colistin resistance trends were statistically significant decreasing between 2014 and 2018** in *E. coli* isolates from broilers and turkeys at the MS-group level (Fig. 3). No trends in colistin resistance on MS-level were observed for pigs and calves. Statistical analyses of levels of complete susceptibility and the key outcome indicator of complete susceptibility revealed a progress towards lower levels of resistance in several countries and also at the EU MS-group level (41).

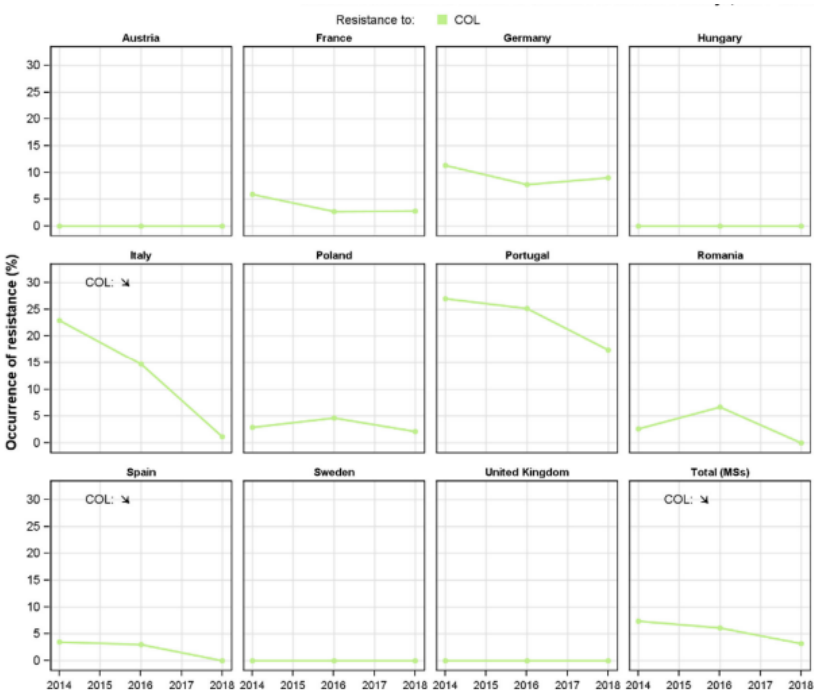


Figure 3. Temporal trends in resistance to colistin in indicator *E. coli* from fattening turkeys, 2014–2018 (11 MSs). Statistically significant increase (↑) or decrease (↓) indicated ($p \leq 0.05$). Reprinted from EFSA and ECDC, 2021 (41).

Studies showed that the consumption of polymyxins in food-producing animals overall, as well as specifically in poultry and pigs, was significantly associated with resistance to polymyxins in *E. coli* from food-producing animals for all years (single or combined years within 2014−2018 depending on the analysis) (19). However, colistin was eliminated after cessation of a therapeutic dose of 75,000 IU from chicken intestins within 4h and and though few isolated with a lower susceptibity persisted, the median MIC of *E. coli* isolates returned below baseline thereafter (62). This reinitiates the importance of prudent and responsible use of colistin in veterinary medicine.

Many countries made additional efforts and analysed as well isolates from clinical cases (Annexe 1). Remarkably low to very low levels of resistance were reported from all countries with decreasing or at least stable trends over the recent years. Only *Pasteurella multocida, Pseudomonas aeruginosa* and *Aeromonas* isolates showed higher resistance levels. German data on AST of *E. coli* from porcine clinical cases indicated that AMR reduction, particularly colistin resistance, followed the decreased colistin use over time (42). Pan-European AMR monitoring would be essential to analyse and evaluate in a harmonised manner the AMR patterns of colistin from veterinary clinical isolates.

Transfer of colistin resistance from veterinary to human pathogens: insufficient information available

Resistance plasmids can spread between bacteria of the same species, between different species and genera, within the primary host but as well can be transferred to secondary hosts, including humans (43,44). The marked differences of *mcr* prevalence in bacteria from animals and humans suggests an animal origin and subsequently spread to humans (16).

Currently, there is insufficient information available on the transmission of *mcr* genes to humans in regard of their prevalence in animals or how often the transfer of isolates with increased colistin MIC values from animals to human occur. Both analyses are hampered by different analytical methods and a lack of AST comparability. The transmission of *mcr-1* via the food production chain was studied in China, indicating that *mcr-1* could be transferred from bacteria in hatchery farms to retail meat products and further to human bacteria via the food chain, and that environmental vectors also played an important role (45). Whole-genome analyses provided evidence that *mcr-1* could be transferable between bacteria from animals and humans as well via the freshwater aquaculture supply chain (46). In Germany, isolates from slaughterhouses samples showed genotypically *mcr* prevalence in 26% (n=46/175), in 14% (n=25/176) from meat-processing plants, in 16% (n=16/100) from fresh food at retail and in 9% (n=5/54) from the urban environment in 2018/2019 whereas phenotypical resistance to colistin was shown in 7% of slaughterhouse isolates, in 1% from meat processing plants, in 6% from fresh food at retail and in none from the urban environment (47). The ban of colistin as feed additive in Chinese husbandry has led to a marked decrease in the *mcr-1* gene prevalence in porcine and human sources suggesting that preventive use in farmed animals created *mcr* reservoirs and highlights the importance of regulating the use of colistin for therapeutic use only (21).

Yet, there are no available studies demonstrating that the therapeutic use of colistin in veterinary medicine has resulted in transfer of colistin resistance from animals to humans. On European level, a comparison and analysis of human and veterinary isolates could unfortunately not be performed as data on polymyxin resistance in bacterial isolates from humans were not available (19). Colistin resistance in human clinical isolates is difficult to assess as colistin AST is generally not part of the initial routine panel for Enterobacterales, being performed instead at national level after referral of MDR isolates to a reference laboratory. In 2017, local EARS-Net participating laboratories did not test routinely for colistin susceptibility or used methods that are not recommended by EUCAST (48). Nevertheless, based on the limited current data, transmission of bacteria with higher colistin MICs between animals and humans cannot be excluded and a potential interference of the *mcr* prevalence in human and veterinary isolated can therefore not be ruled out (17). Previous investigations with corresponding isolates from humans and animals have shown that *mcr* genes occur with other resistance genes on the same plasmid, indicating the possibility of co-selecting *mcr* bearing bacteria (49,50).

The limited data available show however low levels of colistin resistance in human isolates (around 1%) (11,51). The lack of evidence of transmission of genotypically colistin resistant bacteria should preferably be addressed with in-depth epidemiological surveys including genotyping in veterinary medicine to provide a better understanding of the current situation. The harmonised AMR monitoring on community level provides information on the reservoirs of resistant bacteria that could potentially be transferred

between animals and between animals and humans and is relevant for both animal and public health. It is, therefore, crucial to continue the harmonised monitoring. However, isolates from clinical cases have added value to estimate a potential transfer from sick animals as zoonotic pathogens such as *E. coli* may as well be transferred from companion animals to humans and *vice versa* (52).

Legal, safe and efficacious alternatives are not available for veterinary medicine. EMA emphasised in 2016 that reduction of colistin use should not be compensated for by increasing the use of other types of antimicrobials (17). However, legal, safe and efficacious alternatives to colistin remains scarce for certain indications in animals, particularly *E. coli* infections in livestock. Though aminoglycosides are available as an oral powder and oral solution, their effectiveness is limited to the intestinal lumen as they are not resorbed. Therefore, septicaemic or enterotoxemic symptoms cannot sufficiently be alleviated by aminoglycosides. Penicillins have not yet proven to be clinically effective. Fluoroquinolones represent no alternative due to their broad-spectrum and therefore high categorisation unless the AST indicates these as the only remaining option. Sulphonamides and tetracyclines are no alternative due to their unfavourable resistance prevalence in veterinary isolates, though they are used in other countries which have withdrawn colistin for the treatment of livestock. In summary, colistin remains almost irreplaceable as treatment for intestinal infections with *E. coli* in pigs and poultry. Non-antibiotic medication such as zinc oxide was used frequently to prevent porcine intestinal infections but is now discouraged due to its unfavourable environmental impact and the potential un-quantifiable risk of colistin resistance co-selection, as it was reported for Methicillin-resistant *Staphylococcus aureus* (MRSA) (53). Non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin, ketoprofen and meloxicam may be used additionally to support the treatment of intestinal infections, though their clinical efficacy remains to be proven. The anti-endotoxin activity due to its strong LPS neutralising capacity remains however unmet through novel approaches such as nano-gold supramolecular traps are under development (5). In addition to improved rearing conditions and feeding, vaccination of piglets against *E. coli* strains has been shown as an effective alternative to control post-weaning diarrhoea at farm level (54). Both an oral live vaccine and an intramuscular toxoid vaccine are available, but their spectrum is limited to certain *E. coli* strains (*E. Coli* F4/F18 and STx2e producing *E. coli*, resp). In Estonia, a significant association between increased vaccination of piglets against *E. coli* and reduction of colistin use was seen (27). However, in most cases, successful disease prevention and decreased antimicrobial use will not be achieved by a single alternative measure. Instead, the reduction of colistin use should be advocated through additional measures such as improved farming conditions, biosecurity in between production cycles, and vaccination (17). On the European level, the effective implementation of different alternative measures in pig production, such as improvement of biosecurity, vaccination, improved feeding, and health care, resulted in a significant reduction in colistin use (55).

In human medicine, alternative non-polymyxin agents such as cefiderocol and cefepime-taniborbactam or cefepimezidebactam, which are both currently in clinical development with the potential to supply the complete spectrum of activity of polymyxins, are strongly preferred, but not accessible in all regions of the world (56,57). Innovative approaches include the use of broad-spectrum antibiotic adjuvants as well as plasmid-curing to reverse MDR and sensitise Gram-negative bacteria using CRISPR-Cas9 (58,59, 63).

Conclusion

Numerous methods can be employed to reduce the transfer of genetically colistin-resistant bacteria to humans, including preventive measures pre-harvest, improved biosecurity and animal health, and promising vaccinations. However, polymyxins remain an essential part of the antimicrobial therapy toolbox and are indispensable for specific treatments in veterinary medicine, particularly systemic *E. coli* infection in livestock. Harmonised AMR monitoring, including phenotypical and genotypic data sharing between countries as well as between human and veterinary medicine, will be essential to confidently determine the magnitude of transferred AMR bacteria, which is not assessed up to now.

These results suggest that from a 'One-health' perspective, there is potential to further develop responsible and prudent use of antimicrobials in animals and humans and thereby reduce AMR.

Responsible and prudent use of polymyxins in both human and veterinary medicine can effectively mitigate the risk of AMR. Current SPC and guidance for the responsible and prudent use of antimicrobials in veterinary medicine recommend the reservation of the use of polymyxins for treatment of clinical conditions in livestock (60,61).

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Annex 1. Colistin resistance patterns of veterinary clinical isolates according to national AMR surveillance systems

Programme	**FINRES-Vet**	**GERM-Vet**	**RESAPATH**	**FASFC monitoring**	**SWEDRES-Svarm**	**UK-VARSS**	**DANMAP**
Country	Finland	Germany	France	Belgium	Sweden	United Kingdom	Denmark
Year	2019	2018	2019	2019	2020	2019	2019
Sampling point	Clinical cases Slaughterhouse	Clinical cases Slaughterhouse	Clinical cases	Slaughterhouse Retail	Clinical cases Slaughterhouse	Clinical cases Slaughterhouse	Clinical cases Slaughterhouse Retail
AST method	Broth microdilution	Broth microdilution	Disk diffusion	Broth microdilution	Broth microdilution	Broth microdilution	Broth microdilution
AST interpretation	>2mg/L	>2mg/L	>2mg/L	>2mg/L	>2mg/L	>2mg/L	>2mg/L
E. coli	Porcine enteritis: 0% (CI 0.0-7.1) Porcine carcasses: 0% (CI 0.0-2.2) Avian colibacillosis: 0% (CI 0.0-12.5)	Porcine enteritis: 10% (25/255) Bovine enteritis: 0% (0/97) Bovine mastitis: 0% (0/224) Turkey colibacillosis: 1.5% (1/65) Laying hen colibacillosis: 0% (0/310) Broiler colibacillosis: 1% (1/98) Dog and cat enteritis: 0% (0/46) Dog and cat urogenital infections: 0% (0/85)	Piglet enteritis 1% Veal enteritis 1% Bovine mastitis 0% Turkey colibacillosis 0% Chicken colibacillosis 0%	Healthy veal calves, caecal content 2.3% Healthy calves (<1 year), rectal content 0% Fresh beef 0% Fattening pigs, caecal content 1.7% Fresh pork 0% Broiler chicken, caecal content 0.6% Fresh poultry meat 2.7%	Porcine enteritis and carcasses 0% Bovine mastitis 0% Broiler colibacillosis 0% Equine urogenital infections 0% Canine urogenital infections 0.2% Feline urogenital infections 0.4%	Porcine enteritis 0% Cattle, various indications 0% Pigs, various indications 1% Sheep, various indications 0% Chickens, various indications 0%	Porcine enteritis 0% Porcine caecal content 0% Fresh pork: 0% Bovine caecal content 0% Fresh beef: 0% Broiler caecal content: 0% Broiler meat: 0% Bovine mastitis: 0

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Salmonella enterica	Livestock carcasses 1.7% CI 0.3-8.9	Porcine enteritis 2% (1/47) Dog and cat enteritis 16% (5/31)	Not tested	Healthy broiler chicken (neck skin) 7.7% Poultry meat 5.4%	All animals 17.7%	Porcine carcasses 22%	Porcine enteritis 0% Porcine carcasses 0%
Mannheimia haemolytica	Not tested	Bovine respiratory diseases, incl. calves 0% (0/164) Small ruminant respiratory diseases 0% (0/51)	Not tested	Not tested	Not tested	Not tested	Not tested
Pasteurella multocida	Not tested	Bovine respiratory diseases 24% (36/149) Feline respiratory diseases 33% (12/36)	Not tested	Not tested	Not tested	Not tested	Not tested
Pseudomonas aeruginosa	Not tested	Poultry, various indications 40% (8/20)	Not tested	Not tested	Canine otitis[1] 0.6%	Not tested	Not tested
Aeromonas	Not tested	Fresh water fish, various indications 28% (9/32)	Not tested	Not tested	Not tested	Not tested	Not tested
Acinetobacter	Not tested	Bovine respiratory diseases 7% (2/29)	Not tested	Not tested	Not tested	Not tested	Not tested
Bibersteinia trehalosi	Not tested	Small ruminants, various indications 0% (0/30)	Not tested	Not tested	Not tested	Not tested	Not tested
Klebsiella pneumoniae	Not tested	Bovine mastitis 3% (3/97)	Not tested	Not tested	Bovine mastitis 4.4%	Not tested	Not tested

[1] For *P. aeruginosa,* a MIC of >4mg/L was considered as resistant