Review Article

Mechanisms in colistin-resistant superbugs transmissible from veterinary, livestock and animal food products to humans

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Abstract

In the era of antibiotic resistance, where multidrug-resistant (MDR), extensively drug resistant (XDR), and pan-drug resistant (PDR) Gram-negative infections are prevalent, it is crucial to identify the primary sources of antibiotic resistance, understand resistant mechanisms, and develop strategies to combat these mechanisms. The emergence of resistance to last-resort antibiotics like colistin has sparked a war between humanity and resistant bacteria, leaving humanity struggling to find effective countermeasures. Although colistin is used as a highly toxic antibiotic in infections that are not treated with routine antibiotics, its widespread use in animal breeding and veterinary medicine has contributed to the spread of colistin-resistant bacteria, plasmid-borne colistin resistance genes (*mcr*), and antibiotic residues in livestock and animal-derived foods. These sources can potentially transmit colistin resistance to humans through various routes. Therefore, managing the use of colistin in livestock and animal foods, implementing strict monitoring, and establishing guidelines for its proper use are essential to prevent the escalation of colistin resistance. This review article discusses the latest mechanisms of colistin antibiotic resistance, particularly biofilm production as a public health threat, the livestock and animal food sources of this resistance, and the routes of transmission to humans.

Key words: Animal foods, Colistin resistance, *mcr*, Multidrug-resistant, Veterinary medicine

Introduction

Today, the misuse and overuse of antibiotics, in both human and veterinary medicine, have contributed to the rapid emergence of superbug bacteria, including multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) infections, which has caught the scientific community and physicians off guard. The prospect of accepting resistance without treatment is deeply unsettling, and the possibility of returning to the pre-antibiotic era, where infections were more challenging to manage, is a looming concern that underscores the need to reassert antibiotic dominance over infections (Moghadam *et al*., 2020, 2021b, 2024). Infections with high antibiotic resistance not only pose a major therapeutic challenge in clinics and lead to prolonged hospitalizations, but also increase mortality and impose a significant burden on healthcare costs (Shahbandeh *et al*., 2020; Shariati *et al*., 2020; Moghadam *et al*., 2021a). Although doctors have banned the use of highly toxic antibiotics, like colistin in clinics for decades, uncontrolled infections with high levels of antibiotic resistance by MDR and XDR bacteria have forced doctors to use last-resort antibiotics with high toxicity (Taati Moghadam *et al*., 2016; Mousavi *et al*., 2021; Mohebi *et al*., 2023). One of the factors contributing to the antibiotic resistance crisis is the lack of new antimicrobial drug discovery and development in the last two decades. While more than 50 new antibiotic projects were implemented between 1980 and 2000, only

fewer than 15 projects have been implemented since then (Centres for Disease Control and Prevention, 2013; Moghadam *et al*., 2022). Antibiotics, unlike other drugs, lack economic justification, and doctors often permit patients to use them for a short period. Consequently, the incentives to invest in the production of new antibiotics are extremely low. Even if new antibiotics are developed, the initial return on investment will be small because they are initially prescribed at low rates to maintain their effectiveness. Thus, the struggle against bacterial infections with high antibiotic resistance is a war of inequality, and researchers and physicians are weakening day by day as the human antibiotic arsenal dwindles. The use of end-of-line antibiotics like colistin has become the first treatment option for MDR and XDR bacteria (Sadeghi Dosari *et al*., 2016; Kiaei *et al*., 2019; Chegini *et al*., 2020; Dousari and Satarzadeh, 2021; Rastegar *et al*., 2024a). Another crucial factor contributing to the emergence of antibiotic-resistant bacteria is the overuse of antibiotics in animal farming, which raises concerns about their spread in farms, larger environments, and wastewater (Xiong *et al*., 2018; Savin *et al*., 2022). Unfortunately, the excessive use of colistin in human and veterinary medicine over recent decades has led to the observation of resistance to this vital antibiotic in bacteria to which they were previously sensitive (Rhouma *et al*., 2016). The European Medicines Agency recommends that EU member states limit the sale of colistin for use in livestock to achieve a 65% reduction in its use. Additionally, colistin should be classified as a critical drug that is reserved for use only when other treatment options are unavailable (Hémonic *et al*., 2014). Among the various pressures, understanding the mechanisms of antibiotic resistance is crucial, as it enables an appropriate and accurate response to these resistances. Although unknown mechanisms have been proposed for colistin resistance, identifying current mechanisms and conducting further studies to identify new mechanisms will enhance the understanding of how to overcome colistin resistance and develop stronger, less toxic colistin derivatives (El-Sayed Ahmed *et al*., 2020). Previous studies have confirmed that livestock serves as a significant reservoir for plasmid-mediated colistin resistance, and highlighted the risks associated with meat consumption for the transmission of *mcr* genes to humans and across regions with varying levels of colistin use. Several prevalent sequence types (STs) associated with *mcr*, particularly ST1011, warrant further monitoring due to their representation of zoonotic bacteria circulating between different environments (Lu *et al*., 2023; Sismova *et al*., 2023). On the other hand, identifying transmission routes and sources of colistin resistance can facilitate effective management and the implementation of measures to prevent and inhibit resistance to this antibiotic.

Colistin and its mechanism

Colistin has been used as an antibiotic for several decades, with limited use due to the prevalence of side effects in patients as well as the introduction of new antibiotics. The widespread presence of bacteria with high antibiotic resistance, such as MDR and XDR, has led to the re-administration of colistin to treat infections caused by these resistant bacteria (Taati Moghadam *et al*., 2021; Rastegar *et al*., 2024b). Currently, colistin is used in clinical settings as a vital monotherapy antibiotic to combat infections caused by MDR and XDR bacteria. Despite its high toxicity, colistin remains useful due to its effectiveness against gram-negative antibiotic resistance (Poulikakos *et al*., 2014). Common side effects of intravenous colistin administration, observed in 6% to 58% of patients, include nephrotoxicity, which is significantly higher in patients with kidney disease compared with those with normal kidney function (Taati Moghadam *et al*., 2021). A significant factor contributing to the emergence of colistin-resistant bacteria is the widespread misuse of colistin in livestock globally, which can be transmitted to humans through contaminated food. Due to the substantial increase in colistin resistance, measures are being taken to manage and prevent its spread, particularly in developed countries, where its use in livestock is prohibited (García-Meniño *et al*., 2019). Colistimethate sodium and colistin sulfate are two types of colistin drugs available on the market, which are prescribed for treating antibiotic-resistant infections caused by Gram-negative bacteria. The amphiphilic nature of colistin allows it to interact with the lipid A of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, leading to the disruption of the outer membrane (Taati Moghadam *et al*., 2021). Colistin can kill Gram-negative bacteria through five distinct mechanisms (Fig. 1):

I. Anti-endotoxin activity: Colistin inhibits lipid A activity, thereby preventing endotoxin-induced shock caused by interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α).

II. Direct antibacterial activity via disruption of the outer membrane: Colistin binds to lipid A of LPS in the outer membrane, causing cell lysis and exhibiting direct antibacterial activity.

III. Respiratory enzyme inhibition: Colistin interferes with fundamental respiratory processes in bacteria, ultimately leading to bacterial death.

IV. Fenton reaction or hydroxyl radical death pathway: Colistin releases reactive oxygen species (ROS), causing DNA, lipids, and proteins damage, ultimately leading to bacterial death.

V. Vesicle-vesicle contact pathway: Colistin attaches to anionic phospholipid vesicles after transiting to the outer membrane, leading to the fusion of the inner leaflet of the outer membrane with the outer leaflet of the cytoplasmic membrane, resulting in a shortage of phospholipids and bacterial death (Moghadam *et al*., 2020).

Colistin is used in both veterinary and human medicine as a therapeutic agent, as well as in livestock to enhance body weight and feed intake through

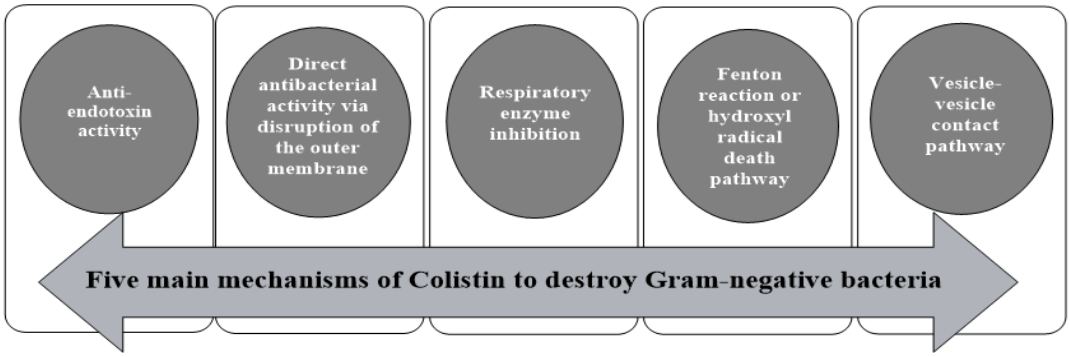

Fig. 1: The primary mechanisms of action of colistin collectively serve as a foundation for eliminating Gram-negative infectious bacteria in hospital settings

supplementation or as a growth promoter (Valiakos and Kapna, 2021). Although colistin remains an effective last-line antibiotic, the emergence of uncontrollable resistance poses a significant challenge for physicians. Unfortunately, colistin resistance has been reported in a wide range of Gram-negative bacteria, including *Stenotrophomonas* spp., *Morganella morganii*, *Klebsiella pneumoniae*, *Neisseria* spp., *Acinetobacter baumannii*, *Edwardsiella* spp., *Escherichia coli*, *Salmonella enterica serovar Typhimurium*, *Aeromonas* spp., *Pseudomonas aeruginosa*, *Enterobacter roggenkampii*, *Vibrio parahaemolyticus*, *Providencia* spp., *Serratia marcescens*, *Proteus* spp., *Vibrio cholera*, *Brucella*, *Legionella*, *Chromobacterium*, *Burkholderia cepacia*, and *Campylobacter* across various countries. This widespread resistance necessitates the development of accurate prevention strategies by thoroughly examining the mechanisms and identifying the sources of resistance (El-Sayed Ahmed *et al*., 2020; Taati Moghadam *et al*., 2021). Veterinary sales of colistin experienced a significant decline of 76.5% from 2011 to 2020; however, there is a scarcity of studies examining the real-world usage patterns of colistin across various sectors and countries in Europe. A survey conducted among veterinarians indicated that 51.9% had either stopped using colistin or had never used it, while 33.4% reported a reduction in their usage, 10.4% maintained their usage levels, and 2.7% increased their use. The primary reasons for colistin administration were gastrointestinal diseases in pigs, followed by septicemia in poultry. Overall, colistin is regarded as a crucial last-resort antibiotic for treating *E. coli* infections in pigs and poultry, particularly when no other legal, safe, and effective alternatives are available. Colistin is generally administered through various methods, with drinking water being the most common route (reported by 62.9% of veterinarians), especially in poultry, followed by incorporation into animal feed, and less frequently via intramuscular injection, primarily in cattle. Dosage recommendations differ by species; for example, the advised dosage for poultry is approximately 75,000 IU/kg, while for other livestock it is around 100,000 IU/kg (Kumar *et al*., 2020; Jansen *et al*., 2022).

Colistin resistance and mechanisms

Colistin resistance in livestock infections varies across different reports. For instance, one study found a prevalence of colistin resistance in *E. coli* from pigs at 24.3% at slaughter and 24.1% on farms in China. Another investigation indicated that the overall resistance among food animals was approximately 18.7%. Some reports suggest that colistin resistance rates can reach as high as 59% in *E. coli* isolates from livestock, particularly associated with colistin usage in poultry farming in Pakistan. Conversely, a study conducted in Switzerland detected no colistin resistance in *E. coli* isolates from livestock (Huang *et al*., 2017; Valiakos and Kapna, 2021). Colistin resistance in human infections varies across different studies. For example, a concerning prevalence of 84.3% for colistin-resistant *E. coli* was reported in Lebanon, underscoring significant public health risks linked to antibiotic use in agriculture and its effects on human health. In Nigeria, reports indicate that approximately 62.5% of *E. coli* isolates in humans were resistant to colistin. In contrast, the overall prevalence of colistin resistance among clinical isolates was found to be around 4.2%, suggesting lower resistance levels compared with some other regions. Additionally, the prevalence of *mcr*-mediated colistin resistance in healthy individuals was estimated at about 7.4%, which is relatively lower than the rates observed in livestock and certain high-prevalence countries (Valiakos and Kapna, 2021; Bastidas-Caldes *et al*., 2022). This section will focus on the latest mechanisms of colistin resistance reported in recent articles, with a particular emphasis on the transmission of plasmid-mediated resistance genes from livestock and animal products to humans. Colistin resistance in Gram-negative bacteria often arises from chromosomal mutations or the dissemination of transmissible plasmid genes (Fig. 2).

Chromosomal resistances

The chromosomal mechanisms of colistin resistance are remarkably diverse, encompassing a wide range of genetic alterations. This section will provide an exhaustive overview of these mechanisms, highlighting their various manifestations.

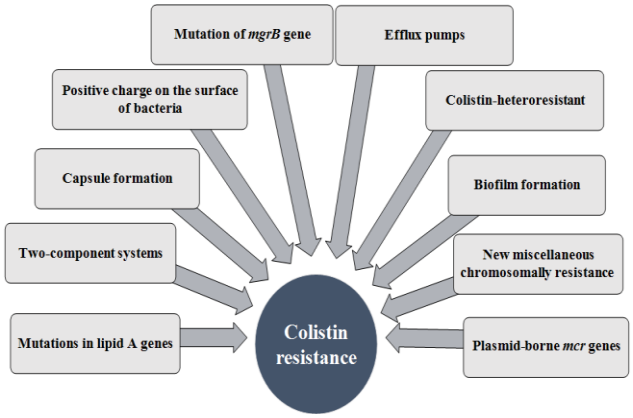

Fig. 2: A comprehensive overview of the latest mechanisms of resistance to colistin in Gram-negative bacteria reveals that the *mcr* mechanism is particularly significant in the dissemination of resistance and the transfer of this resistance from animals to humans

Mutations in lipid A genes

The lipid A synthesis genes, including *lpxA*, *lpxD*, *lpxO2*, and *lpxC*, are located on the chromosome of Gram-negative bacteria. Mutations in these genes can lead to defective lipopolysaccharide (LPS) synthesis, resulting in colistin resistance. The presence of the ISAba11 sequence in LPS-producing genes such as *lpxC* and *lpxA* can cause a loss of LPS-producing ability in Gram-negative bacteria, thereby conferring high resistance to colistin. This LPS deficiency in bacteria results in a reduced negative surface charge, which in turn reduces the affinity of colistin for the bacterial surface. Additionally, specific mutations in LPS genes, such as the *rfbJ* gene in group B *Salmonella* and the *rfbSE* gene in group D *Salmonella*, isolated from animal sources, can also contribute to increased colistin resistance (Moghadam *et al*., 2022).

Two-component systems

The PhoPQ and PmrAB two-component systems are crucial for intrinsic colistin resistance in Gram-negative bacteria, which are encoded on their chromosomes (Poirel *et al*., 2018). The PmrAB system consists of two components: a response regulator that responds to environmental stimuli and a histidine kinase that plays a crucial role in the function of the system. This two-component system senses the presence of ions such as Mg^{2+}, Al^{3+}, and Fe^{3+}, as well as different pH levels, to create distinct conditions (Mousavi *et al*., 2021). The PmrAB system influences the expression of lipid A genes, leading to colistin resistance, and also reduces the membrane entry of colistin by altering the outer membrane when mutations occur in the *pmrA* and *pmrB* genes (Mousavi *et al*., 2021). The PhoPQ system plays a significant role in enhancing bacterial virulence and colistin resistance by being activated by cationic antimicrobial peptides and sensing various environmental factors such as Mg^{2+} and Ca^{2+}, which can alter the LPS of Gram-negative bacteria (Cheung *et al*., 2008; Wi *et al*., 2017; Huang *et al*., 2020; Mirshekar *et al*., 2024). When bacteria are exposed to colistin in livestock (pigs, cattle, and chicken), selective pressure induces genetic mutations in *PmrA*, *PmrB*, *PhoP*, *PhoQ*, *MgrB*, and *PmrD*, leading to colistin resistance. Therefore, efforts to reduce colistin use in livestock should be prioritized to minimize the emergence of colistin-resistant bacteria (Delannoy *et al*., 2017; Kim *et al*., 2019). Nonsynonymous polymorphisms in the PmrAB two-component system of *S. enterica* and *E. coli* isolated from poultry eggs and swine faeces have been linked to colistin-resistant strains (Quesada *et al*., 2015).

Capsule formation

One of the distinctive features of Gram-negative bacteria is their ability to resist colistin through capsule possession. This is because the anionic interactions between bacterial capsule polysaccharides and polymyxin lead to colistin resistance. In contrast, certain bacterial components, such as the conjugative pilus expression (Cpx) and regulator of capsule synthesis (Rcs), regulate capsule formation and can induce efflux pumps like KpnEF and PhoPQ, thereby conferring colistin resistance. Specifically, Cpx activates KpnEF and Rcs activates PhoPQ, leading to the development of colistin resistance (Moghadam *et al*., 2022).

Positive charge on the surface of bacteria

It is noteworthy that bacteria can develop colistin resistance by modifying their surface LPS to create a positive charge through the expression of various compounds encoded by both chromosomal and plasmid genes. These compounds include galactosamine, produced by the chromosomally encoded *naxD* gene, 4-amino-4-deoxy-L-arabinose, mediated by the chromosomally encoded *arnBCADTEF-ugd* operon, and phosphoethanolamine, mediated by both chromosomally encoded *eptA* and plasmid-encoded *mcr* genes. This positive charge on the LPS surface reduces the affinity of colistin for binding to the bacteria, thereby conferring resistance. Additionally, the disruption of the outer membrane of Gram-negative bacteria can also contribute to colistin resistance (Mousavi *et al*., 2021).

Mutation of mgrB gene

The inactivation of the *mgrB* gene, a chromosomal gene in Gram-negative bacteria, is a common mechanism of colistin resistance. This occurs through the insertion of various insertion sequences, such as IS5-like, IS102, IS5 family, IS3-like, and ISKpn14, as well as missense or nonsense mutations in the gene (Mousavi *et al*., 2021). The *mgrB* gene normally acts to negatively regulate the PhoPQ two-component system, which in turn activates the *arnBCADTEF* operon. When the *mgrB* gene is inactivated, this negative feedback is lost, leading to increased expression of the *arnBCADTEF* operon and consequently, colistin resistance (Moghadam *et al*., 2022). This mechanism of *mgrB* gene inactivation has been widely observed in colistin-resistant Gram-negative bacteria isolated from various animal food sources and livestock, including laying hens, chickens, broilers, piglets, weaned pigs, fattening pigs, and sows, and its

prevalence has increased in recent years (Huang *et al*., 2017; Park *et al*., 2021).

Efflux pumps

Gram-negative bacteria can exhibit colistin resistance when they express resistance-nodulation-cell division (RND) family efflux pumps. These pumps are composed of four genes with distinct functions: *adeA*, *adeB*, *adeC*, and *adeR*. AdeA acts as a membrane fusion protein, *adeB* transports substrates from the cytoplasm or phospholipid bilayer to the extracellular environment, *adeC* functions as an outer membrane protein channel, and *adeR* serves as a regulator (Mousavi *et al*., 2021). In addition to RND efflux pumps, several other efflux pumps have been identified in Gram-negative bacteria, including sapABCDF, MexXY-OprM, CarO, kpnEF, acrAB-tolC, and *emrAB*. These pumps are thought to contribute to colistin resistance, although the exact mechanisms by which they do so remain unclear (Taati Moghadam *et al*., 2021).

Colistin-heteroresistant

Gram-negative bacteria exhibiting colistin heteroresistance have the potential to develop colistin resistance due to the presence of resistant subpopulations that coexist with susceptible populations. This intermediate condition, characterized by the presence of resistant subpopulations, can lead to unaccountable treatment failures. Colistin-resistant subpopulations are commonly detected in multidrug-resistant (MDR) Gram-negative bacteria, including *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*. Several mechanisms have been reported to contribute to colistin resistance in these subpopulations, including biofilm formation, activation of two-component regulatory systems such as PmrAB, PhoPQ, ParRS, CprRS, and ColRS, mutations in lipid A biosynthesis genes, overexpression of the *acrAB-tolC* efflux pump regulated by the *soxRS* system, and putrescine/YceI communication (Lin *et al*., 2019; El-Sayed Ahmed *et al*., 2020).

Biofilm formation

Biofilm formation is a survival strategy employed by bacteria to accumulate and form masses on various surfaces, thereby protecting themselves from environmental stressors. In biofilm conditions, the concentration of inhibitory antibiotics is higher compared with the planktonic state, increasing the likelihood of infection recurrence. Additionally, bacterial cells are shielded from immune responses and are challenging to remove in infections (Chegini *et al*., 2020). Research has shown that biofilm formation is more pronounced in colistin-resistant Gram-negative bacteria compared with avian pathogenic *E. coli* without biofilm. Colistin resistance can induce biofilm formation by enhancing the expression of *phoQ*, which is a key regulator of biofilm formation and quorum sensing (Klinger-Strobel *et al*., 2017; Park *et al*., 2021). This increased expression of biofilm-forming and quorum-sensing genes in colistin-resistant avian pathogenic *E. coli* is linked to changes in the *mgrB* gene, which is influenced by the dysfunctionality of the *phoPQ* two-component system. This dysfunctionality leads to colistin-induced resistance by increasing the expression of quorum-sensing genes and biofilm-forming genes (Stewart, 2002; Park *et al*., 2021). On the other hand, colistin resistance in Gram-negative bacteria resulting from mutations in lipid A biosynthesis genes, which lead to the loss of LPS, significantly impairs biological features such as biofilm formation. *In vitro* and *in vivo* studies have shown that LPS-deficient isolates exhibit decreased expression levels of biofilm-associated genes, resulting in reduced biofilm formation potential. Consequently, these isolates may not be able to use biofilm as a mechanism for colistin resistance due to the diminished rate of biofilm formation (Dafopoulou *et al*., 2015; Farshadzadeh *et al*., 2018; Azimi and Lari, 2019). In contrast, heterogeneous colistin-resistant sub-populations of *S. maltophilia* isolates have been found to exhibit increased biofilm formation potential (Martínez-Servat *et al*., 2018). Furthermore, it is noteworthy that antibiotic resistance gene transfer can occur more readily in biofilm conditions. Therefore, if a colistin-resistant bacterium carries the *mcr* genes, these genes can be easily transferred horizontally from one bacterium to another through plasmids, potentially contributing to the spread of colistin resistance (Azimi and Lari, 2019; Uruén *et al*., 2021).

New miscellaneous chromosomally resistance

Recent years have witnessed the emergence of novel mechanisms of colistin resistance specific to certain bacteria, collectively referred to as "miscellaneous chromosomally encoded resistance genes". For instance, the *lptD* gene is responsible for the production of fresh LPS in the bacterial outer membrane. If this gene is removed, the bacterium can become resistant to colistin due to the complete loss of LPS (Moghadam *et al*., 2022). Another miscellaneous mechanism of colistin resistance involves the detoxification of reactive oxygen species, which is mediated by genes such as *sodB* and *sodC* (Moghadam *et al*., 2022). *Burkholderia multivorans* has two genes, Bmul_2133 and Bmul_2134, responsible for the biosynthesis of hypopanoids. These genes are critical for stabilizing the penetration of the outer membrane and contribute to colistin resistance through a mechanism independent of LPS-binding activity (El-Sayed Ahmed *et al*., 2020). Additionally, the outer membrane protein OprH plays a role in colistin resistance. When its expression increases, it binds to the negatively charged LPS, leaving no space on the bacterial surface for polymyxin binding, thereby conferring resistance. In contrast, reduced expression of OprD, an outer membrane porin, provides the basis for polymyxin resistance in *P. aeruginosa* (El-Sayed Ahmed *et al*., 2020). The *lpxM* gene has been identified as a reducing polymyxin resistance gene in bacteria, responsible for lipid A acylation. If the *lpxM* gene is inactivated, 4-amino-4-deoxy-L-arabinose modifications do not occur, leading to colistin resistance (Mousavi *et*

al., 2021). Deletion mutations in the biotin synthesis locus are another new mechanism of polymyxin resistance. This locus plays a key role in lipid A production, and lower biotin levels result in decreased lipid A production since biotin is a main cofactor of lipid metabolism (Mousavi *et al*., 2021). The DedA family of membrane transporter proteins contributes to alterations in lipid A of *Burkholderia thailandensis* LPS, leading to colistin resistance (Panta *et al*., 2019). Lastly, the *vacJ* gene has been identified in Gram-negative bacteria, where a single mutation leads to the emergence of colistin-resistant bacteria (Mousavi *et al*., 2021).

Plasmid-borne *mcr* genes

Since 2015, there has been a significant and sustained emergence and spread of mcr in colistin-resistant clones of Enterobacterales among both humans and animals, particularly in developing countries. The transmission of *mcr* clones in hospitals and communities possess a significant risk for infection and potential outbreaks, both nationally and internationally (Biswas *et al*., 2024). The origin of *mcr* gene transfer to humans has been linked to livestock populations due to the nephrotoxicity and neurotoxicity of colistin, which is rarely administered in humans. The majority of mcr-1 carrying bacteria have been isolated from livestock populations, highlighting the potential for horizontal gene transfer from animals to humans (Poirel *et al*., 2017). The mobile genetic plasmid can carry *mcr* genes containing *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-8*, *mcr-9*, and *mcr-10*, which are responsible for colistin and polymyxin B resistance. These genes can spread colistin resistance among Gram-negative bacteria via horizontal transfer, posing a significant threat to public health (Moghadam *et al*., 2022). The *mcr* gene was initially detected on plasmid pHNSHP45 but has since been found on other plasmids such as IncF, IncY, IncP, IncI2, IncX4, IncHI2, and ColE10-like in various bacteria. This flexibility in plasmid hosts allows the *mcr* gene to spread widely among different bacterial species (Moghadam *et al*., 2022). Recent studies have identified new genetic variants of every *mcr* gene, which differ in one or more amino acids. For example, *mcr*-1 has 22 variants from *mcr*-1.1 to *mcr*-1.22, while *mcr*-2 has three variants including *mcr*-2.1, *mcr*-2.2, and *mcr*-2.3, which were detected in *E. coli* isolates from calves and piglets. Similarly, *mcr*-4 has genetic variants *mcr*-4.1 to *mcr*-4.6, which were reported in *E. coli* and *S. enterica* serovar Typhimurium from pigs. The *mcr*-5 gene has four genetic variants containing *mcr*-5.1 to *mcr*-5.4, which were isolated from *Salmonella* Paratyphi B in poultry. The *mcr*-8 gene has variants *mcr*-8.1 to *mcr*-8.4, which were detected in New Delhi metallo-β-lactamase harboring *K. pneumoniae* from both human clinical samples and food-producing animals (Xavier *et al*., 2016; AbuOun *et al*., 2017; Borowiak *et al*., 2017; Carattoli *et al*., 2017; Yin *et al*., 2017; Wang *et al*., 2018; Yang *et al*., 2018). The *mcr* genes have been rapidly distributed around the world, not only in human, animal, and traveler populations but also in foodstuffs and environmental samples. These genes have been isolated from various sources, including humans, living animals (e.g., pig, poultry, and cattle), the environment, and alimentary products (Valiakos and Kapna, 2021). The *mcr* genes produce products that bind phospho-ethanolamine residues to the lipid portion of LPS, leading to changes in LPS. This action causes LPS to react with low-affinity colistin. When the *mcr* plasmid integrates with its enzymatic activity in the bacterial membrane, a change in lipid A is observed, which ultimately results in changes in bacterial fitness, growth rate, and structural integrity of the outer membrane (Mousavi *et al*., 2021). *Neisseria* EptA is the most extensively studied lipid A–40-PEA transferase, classified within the 'YhjW/YjdB/YijP' alkaline phosphatase superfamily. This enzyme facilitates the transfer of phosphoethanolamine (PEA) from phosphatidylethanolamine (PE) to lipopolysaccharide (LPS)-lipid A, which ultimately contributes to intrinsic resistance against colistin. Both MCR-1 and MCR-2 are identified as lipid A–40-PEA transferases. A proposed model for the catalytic action of MCR-1 and MCR-2 suggests that these integral membrane enzymes mediate the transfer of PEA from the lipid donor substrate, PE, to Kdo2-lipid A, resulting in the formation of two products: PPEA-40-Kdo2-lipid A and diacylglycerol. Similar to *Neisseria* EptA, MCR-1 and MCR-2 may use a potential 'ping-pong' mechanism for enzymatic hydrolysis, consisting of two sequential half-reactions: (1) MCR-1 or MCR-2 hydrolyzes PE to generate diacylglycerol and PEA bound to the enzyme, and (2) it subsequently transfers the PEA group to Kdo2-lipid A, yielding the product PPEA-40-Kdo2-lipid A (Sun *et al*., 2018). Thus, we hypothesize that the transferable resistance to polymyxins arises from the role of MCR-1/2 in modifying LPS-lipid A with PEA. The PEA moiety is sourced from the physiological substrate PE. Consistent with this hypothesis, MALDI-TOF mass spectrometry analyses of purified bacterial LPS-lipid A confirm that MCR-1 and MCR-2 catalyzes the *in-vivo* transfer of PEA from PE to LPS-lipid A (Sun *et al*., 2018). A report has documented the presence of the rare *mcr*-1.26 gene in *E. coli* isolated from poultry, highlighting the temporal occurrence and high similarity of plasmids between poultry and human isolates. This suggests that poultry husbandry is the primary source of *mcr*-1.26 and indicates potential transmission between different environments and humans (Binsker *et al*., 2023). The prevalence of colistin-resistant *E. coli* in broiler chickens and their farming environments remains high, despite a decrease noted in previous studies following the ban on colistin as an animal feed additive. Among the *E. coli* isolates from cloacal swabs and farm environments, *mcr*-1 was identified as the dominant *mcr* gene. Additionally, the *mcr*-4 and *mcr*-5 genes were detected in fecal and feed samples, respectively. This study reports the prevalence of *mcr*-4, *mcr*-5, *mcr*-6, *mcr*-7, *mcr*-8, and *mcr*-9 genes in *E. coli* isolated from Malaysian broiler chickens and their farm environments. The findings suggest that MDR colistin-resistant *E. coli* strains

harboring virulence genes are present in broiler chickens and their farming environments, posing a significant risk of transmission to humans, animals, and the surrounding environment (Lemlem *et al*., 2023). IncHI2, IncI2, and IncX4 plasmids are the primary vectors facilitating the spread of *mcr*-1 from various geographical locations and sources, with the prevalence of Tn6330 potentially accelerating this transmission. The high occurrence of *mcr*-1-positive *E. coli* strains in pigs and pork indicates that these animals and their products are significant reservoirs for *mcr*-1-positive strains in humans, posing a potential public health threat. The horizontal transfer of *mcr*-1-bearing plasmids among diverse *E. coli* strains further underscores the importance of pigs and pork as critical sources of these resistant strains (Lu *et al*., 2023).

Colistin resistance in livestock impact on human health

Contaminated diet, water intake, and food of humans play a prominent role in the development of antibiotic-resistant Gram-negative bacteria, as have been shown that humans which consume sterile food, carry fewer drug-resistant bacteria. Therefore, it is the policy of different countries to properly monitor the use of antibiotics in veterinary medicine and human medicine in order to achieve acceptable results in reducing antimicrobial resistance in the future (Barlaam *et al*., 2019). Oral colistin is also characterized as a widely used antibiotic in livestock, with low bioavailability and gastrointestinal absorption. Subsequently, colistin-resistant bacteria, genes, and its degradation products are found in the treated livestock manure, which can be spread in the environment. Meanwhile, the lack of laws to control the release of animal wastewater containing antibiotics around the world, makes it difficult to prevent the spread of colistin resistance to humans through the environment (Fig. 3) (Rhouma *et al*., 2016).

Therefore, colistin-resistant bacteria are no exception to this rule, as different parts of the world have reported colistin-resistant bacteria or plasmids carrying this resistance in various sources of animal foods and their products. Although there were reports of colistin resistance in animal sources in the years before 2015 (Table 1), the first possible horizontal transmission of colistin resistance from animal to human was reported by Olaitan *et al*. (2015) in which six colistin resistance *E. coli* isolates were diagnosed in pigs' faeces transmitted to a boy (with no history of antibiotic therapy but fed pigs) but at the time could not identified the chromosomal mechanism of colistin resistance in the bacterium. In 2016, over 10.600 *E. coli* isolates in laying hens, broilers (caeca and carcass at slaughter and faeces at farm), chicken meat, turkey (caeca and carcass at slaughter and faeces at farm), turkey meat, beef cattle (faeces and colon content), beef, dairy products (bulk tank milk and cheese), veal calves (colon content at slaughter and faeces at farm), pig (colon content and fattening pigs at slaughter, and piglets, fattening pigs and faeces at farm), and pork from the years 2010-2015 were screened for colistin resistance. The results shown 505 isolates were resistant and only 402 isolates (79.8%) carried the *mcr*-1 gene that the mechanism of the rest of the colistin-resistant isolates was not associated with *mcr*-1 (Irrgang *et al*., 2016). Clonal spread of *mcr*-1 in IncHI2 plasmid-harboring *Salmonella* isolates were reported from food-producing animals (246 from avian and 30 from swine) in China, in 2016. Overall, 22

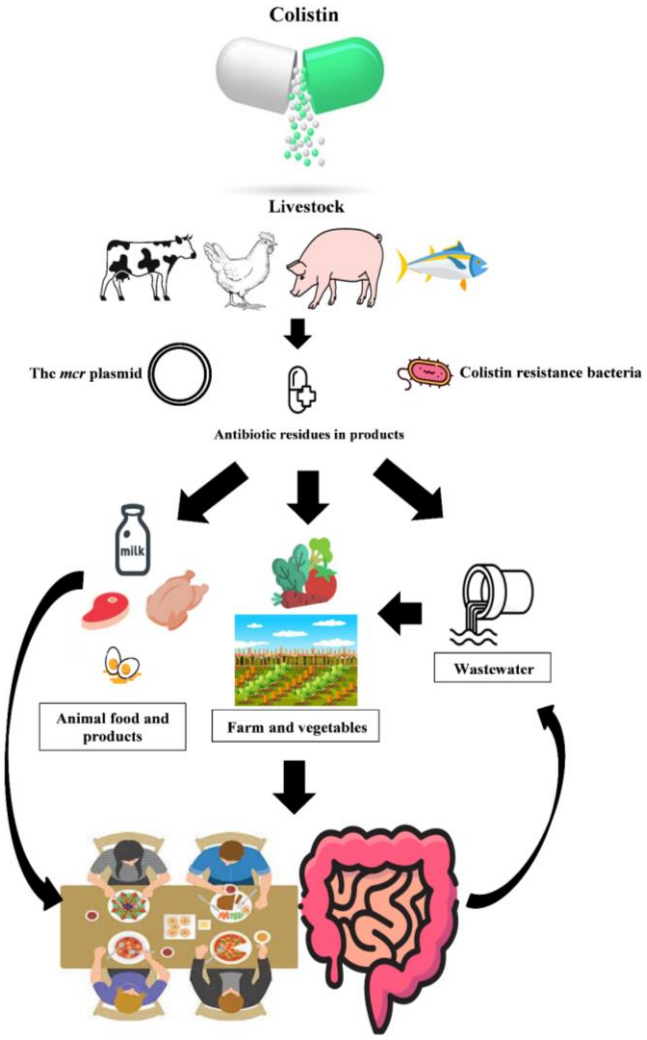

Fig. 3: When colistin administration is increased in veterinary medicine and food as an additive, it can lead to selective pressure on the gut microbiota of animals. Thus, colistin resistance bacteria, unprocessed colistin and *mcr*-carrying plasmids in the feces of these animals are released into the sewage and contaminate the aquatic environment. Humans can obtain colistin-resistant bacteria or *mcr* carriers when contamination reaches carcasses and meat during production, by handling, eating raw or undercooked meat, as well as on the farm. In this case, humans can develop clinical infections, and *mcr* can be transmitted from colistin-resistant bacteria to common bacteria in the gut microbiota, and then again, these resistant bacteria contaminate the aquatic environment through human feces. If water contaminated with colistin resistant bacteria and *mcr* genes is used in agriculture, contaminated vegetables are likely to reach consumers, as well as livestock drinking this contaminated fresh water and eating contaminated products

Table 1: Summary of studies on colistin resistance in livestock and animal food products as a hazardous source of superbug Gram-negative bacteria for public health

Sample	Animal source	Bacteria	Results	Reference
Meat	pork	*Campylobacter* spp.	Very high rate of colistin resistance (72.2%) was found in the bacteria	Ghimire *et al*. (2014)
Faecal sample	Pigs and human	*E. coli*	Although colistin resistance was detected, could not identify the chromosomal mechanism	Olaitan *et al*. (2015)
Meat, faeces, dairy products, and others	Broilers, hens, chicken, turkey, cattle, veal calves, and pig	*E. coli*	The results shown 505 isolates were resistant and only 402 isolates (79.8%) carried the *mcr*-1 gene	Irrgang *et al*. (2016)
Faecal sample	Avian and swine	*Salmonella enterica*	Overall, 22 isolates were resistant to colistin that only five isolates were *mcr*-1 positive and MDR	Li *et al*. (2016)
Lung and liver of chickens, lung, spleen and liver of pigs, milk of cows, and liver of ducks	Chickens, pigs, ducks, and cattle	*E. coli*	Although none of *E. coli* isolates were positive for the *mcr*-2 and *mcr*-3 genes, 2.7% (17/624) were positive for the *mcr*-1 gene	Yassin *et al*. (2017)
Food sample	Matrices from livestock and poultry meat products, aquatic products, milk and dairy products, egg products, fruit, vegetable condiments and others	*Salmonella*	They were identified seven isolates harbouring the *mcr*-1 gene	Hu *et al*. (2019)
Milk and faecal	Bovine and caprine	*E. coli*	The result shown *mcr*-1 gene and IncP- and IncFIB-type plasmids in 4 isolates were resistant to colistin	Hassen *et al*. (2019)
Faecal samples	Pigs, chicken, and cattle	*E. coli*	The *mcr*-1 and *mcr*-2 genes were identified in pigs, chickens and cattle	Zhang *et al*. (2019)
Meat, water, and environment	Broiler	*E. coli*	Prevalence of *mcr*-1 gene and colistin resistant *E. coli* were 10.55% and 11.76%, respectively	Palupi *et al*. (2019)
Faecal samples	Pigs	*E. coli*	Twenty-three colistin-resistant *E. coli* isolates carrying the *mcr*-1 gene were detected	Dandachi *et al*. (2019)
Meat and faecal samples	Pig, chicken, and cattle	*E. coli*	Colistin withdrawal policy and the reducing use of colistin in agriculture have had a remarkable impact on decreasing colistin resistance and *mcr*-1	Wang *et al*. (2020)
Milk	Bovine	*E. coli*	Two percent were colistin resistant and 19.7% harbored *mcr*-1-positive	Liu *et al*. (2020)
Foods	Raw milk, chicken drumstick, herby cheese, turkey wings, raw patty meat, and salted cheese	*E. coli*	Although 5 *mcr* genes were screened by performing PCR, none of the 4 colistin resistant isolates had the *mcr* genes	Güzel *et al*. (2020)
soil, solid manure, and feces	Broiler and pig	Direct DNA	Targeted genes were detected from 22.4% to 98.8% in broilers with higher contamination rates than pigs	Shi *et al*. (2021)
Meat	Chicken, pork, and beef	*Aeromonas* spp., *Yersinia* spp., *E. coli*, *Citrobacter* spp., *Klebsiella* spp., *Raoultella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Pantoea* spp., *Ewingella* spp., and *Kluyvera* spp.	The mcr-1 and mcr-3 were diagnosed in some of these bacteria	Odoi *et al*. (2021)
Milk	Cows	*E. coli*, *Aeromonas hydrophila*, *K. pneumoniae*, and *P. aeruginosa*	A total of 117 tested isolates, 61 (52.14%) were colistin resistant that 47	Tartor *et al*. (2021)

			harbored plasmid-borne *mcr* genes	
Meat	Beef	*E. coli*	Eight (3.8%) isolates were resistant to colistin and carried *mcr*-1 gene	Sabala *et al*. (2021)
Meat and faecal samples	Pigs and pork	*Salmonella* and *E. coli*	Colistin-resistance and *mcr*-1 gene was found in both *Salmonella* and *E. coli* isolates	Lay *et al*. (2021)
Milk, liver or heart blood, and faecal samples	Broilers, ostriches, cattle, sheep, pigeons, and dogs	*E. coli*	The researcher could not detect *mcr*-1 or *mcr*-2 positive *E. coli* isolates	Ilbeigi *et al*. (2021)
Retail chicken carcasses	Chicken	*E. coli* and *Citrobacter freundii*	Twenty chicken samples contaminated by *mcr*-1-positive isolates	Sadek *et al*. (2021)
Direct sampling	Chicken, pork, fish, and shrimp	*E. coli*	Colistin resistance were found in 46.0% (208/452) of retail food samples and *mcr* genes screening shown that in 65 (31.3%) of the 208 colistin resistance *E. coli* isolates	Le *et al*. (2021)
Meat	Poultry	*K. pneumoniae* and *E. coli*	They found high rates of chicken-meat batches (80%-100% – 4 months; 12% – the last month) with MDR *mcr*-1-positive	Ribeiro *et al*. (2021)
Direct sampling	Cooked/roast meat dishes, pasteurized milk, salads, and cold noodles/fried rice dishes	*E. coli*	Over 95% of *E. coli* isolates were MDR and four colistin-resistant *E. coli* were identified	Zhang *et al*. (2021)
Milk	Sheep and goat	*E. coli*	The *mcr*-1 harboring *E. coli* isolates were detected in 5.27% samples	Obaidat *et al*. (2022)
Faecal samples	livestock and poultry	*E. coli*	18.95% isolates were resistant to colistin which harbored *mcr*-1 genes	Shafiq *et al*. (2022)

Salmonella enterica were resistant to colistin that only five isolates were *mcr*-1 positive and MDR and belonged to ST34 *Salmonella enterica* serovar Typhimurium (Li *et al*., 2016). Yassin *et al*. (2017) were screened of *mcr*-1, *mcr*-2, and *mcr*-3 mediated colistin resistance in extraintestinal *E. coli* isolated from poultry and livestock in China. Although none of *E. coli* isolates were positive for the *mcr*-2 and *mcr*-3 genes, 2.7% (17/624) were positive for the *mcr*-1 gene (3.2%; 13/404 in chickens, 0.9%; 1/113 in pigs, 6.8%; 3/44 in ducks, and 0/63 in cattle) (Yassin *et al*., 2017). In Turkey, a study determined colistin resistance *E. coli* isolates in foods (raw milk, chicken drumstick, herby cheese, turkey wings, raw patty meat, and salted cheese) in 2011-2015. Although 5 *mcr* genes (*mcr-1* to *mcr-5*) were screened by performing PCR, none of the 4 colistin resistant isolates had *mcr* genes and was not identified *E. coli* isolates resistance mechanism (Güzel *et al*., 2020). Hassen *et al*. (2019) evaluated colistin resistance *mcr*-1 gene in CTX-M-1/CTX-M-15-producing *E. coli* isolates of bovine and caprine origins. Among 120 bovine faecal samples and 9 caprine raw milk samples, colistin resistance (MIC: 8-16 µg/ml) was detected in 4 isolates (3 faeces/1 milk) from bovine origin which were carried the *mcr*-1 gene and IncP- and IncFIB-type plasmids. It was interesting that the *mcr*-1 plasmid carrying isolates belonged to prominent international clones linked to MDR phenotype (Hassen *et al*., 2019). A study investigates colistin resistance *mcr*-1, extended-spectrum β-lactamase (ESBL) and carbapenemase genes in animal (broiler and pig) in environmental samples (soil, solid manure, and feces) in which all of them pose a threat to food safety and public health. Results revealed that targeted genes were detected from 22.4% to 98.8% in broilers with higher contamination rates than pigs, so broiler farm environments were declared as a main reservoir of *mcr*-1 genes (Shi *et al*., 2021). Lay *et al*. (2021) evaluated the prevalence of colistin resistance in *Salmonella* and *E. coli* from pigs and pork. Not only colistin-resistance rate in *Salmonella* (2.6%) was significantly lower *E. coli* than (10.4%), but also *mcr*-1 gene was lower in *Salmonella* (n=12) in comparison with *E. coli* (n=68) (Lay *et al*., 2021). Emergence of *mcr*-1 colistin resistance gene in Lebanese swine farms were reported by Dandachi *et al*. (2019). In total, 114 fecal samples, 23 colistin-resistant *E. coli* isolates carrying the *mcr*-1 gene were detected (Dandachi *et al*., 2019). Another study reported high abundance of MDR *E. coli* with identification of IncHI2/IncX4-plasmid harboring *mcr*-1in retail ready-to-eat foods (cooked/roast meat dishes, pasteurized milk, salads, and cold noodles/fried rice dishes) in China. Over 95% of *E. coli* isolates were MDR and four colistin-resistant *E. coli* were identified (Zhang *et al*., 2021). Hu *et al*. (2019) among 2555 *Salmonella* isolated from food samples (matrices from livestock and poultry meat products, aquatic products, milk and dairy products, egg products, fruit, vegetable condiments and others) in China between 2012 and 2016, were identified seven

isolates harbouring the *mcr*-1 gene. In 2019, the abundance of colistin resistance bacteria and *mcr*-1 and *mcr*-2 genes were measured in fecal samples of domestic animals (pigs, chicken and cattle). The prevalence of *mcr*-1 was higher than *mcr*-2 genes in colistin resistant *E. coli* isolates from pigs, chickens and cattle. Co-occurrence of *mcr*-1 and *mcr*-2 was detected 7.22% in chickens, in 20% in pigs, and 9.52% in cattle (Zhang *et al*., 2019). In another study among 249 *E. coli* isolates from bovine mastitic milk, 2% were colistin resistant and 19.7% harbored *mcr*-1-positive (Liu *et al*., 2020). In 2023, the prevalence of *mcr* gene was investigated as a colistin-resistant mobile gene in *E. coli* in sheep and goat dairy farms in Jordan. A total of 1158 milk samples, 34.5% of the isolates showed MDR and 61 (5.27%) samples infected with *E. coli* isolates harbored *mcr*-1 gene (Obaidat *et al*., 2022). In 2022, *E. coli* isolates were collected from 250 faecal samples collected from healthy food-producing livestock and poultry in Pakistan. A total of 153 *E. coli* isolates 84% were as MDR and 18.95% isolates were resistant to colistin which harbored *mcr*-1 genes (Shafiq *et al*., 2022). In Indonesia, samples were collected from small-scale poultry slaughterhouses (fresh meats and plucker swabs), flocks that use colistin sulfate (cloacal swabs, drinking water, and litters), small restaurants (cooked meats), and traditional markets (fresh meats) for discovery of a plasmid-mediated colistin resistance gene. The results showed the prevalence of *mcr*-1 gene and colistin resistant *E. coli* were 10.55% and 11.76%, respectively (Palupi *et al*., 2019). Ghimire *et al*. (2014) reported that pork meat was a source of *Campylobacter* spp. (*C. coli* 76% and C. *jejuni* 24%) with a very high rate of colistin resistance (72.2%). The mechanism of colistin resistance was not evaluated in this study (Ghimire *et al*., 2014). In 2021, research was screened colistin-resistant MDR and XDR Gram-negative bacteria from the milk of mastitic cows and raw unpasteurized milk in Egypt. A total of 117 tested isolates, 61 (52.14%) were colistin resistant that 47 harbored plasmid-borne *mcr* genes (*mcr*-1 in 31.91%, *mcr*-2 in 29.79%, *mcr*-3 in 34.04%, and each of *mcr*-4 and *mcr*-7 in 2.13% of *E. coli*, *Aeromonas hydrophila*, *K. pneumoniae*, and *P. aeruginosa* isolates) (Tartor *et al*., 2021). In 2021, Odoi *et al*. investigated the prevalence of *mcr* genes and colistin resistance in Gram-negative bacteria isolated among retail meats in Japan. Among 459 samples 99 isolates were colistin resistant including *Aeromonas* spp. (48/206, 23.3%), *Yersinia* spp. (5/112, 4.5%), *E. coli* (23/39, 59%), *Citrobacter* spp. (4/26, 15.4%), *Klebsiella* spp. (2/23, 8.7%), *Raoultella* spp. (2/16, 12.5%), *Enterobacter* spp. (7/14, 50%), *Pseudomonas* spp. (1/8, 12.5%), *Pantoea* spp. (5/7, 71.4%), *Ewingella* spp. (1/4, 25%), and *Kluyvera* spp. (1/2, 50%). The *mcr* gene was detected in 16 isolates: *mcr*-1 in 14 isolates of *E. coli* from 10 chicken samples, and *mcr*-3 in two isolates of *Aeromonas sobria* from pork and chicken samples (Odoi *et al*., 2021). Sabala *et al*. (2021) examined the prevalence of colistin-resistant in raw beef and ready-to-eat beef products in Egypt. Of 210 *E. coli* isolates, 8 (3.8%) were resistant to colistin and carried *mcr*-1 gene (Sabala *et al*., 2021). In 2021, 452 food samples containing chicken (n=116), pork (n=112), fish (n=112) and shrimp (n=112) were examined for colistin resistance *mcr* producing *E. coli*. Colistin resistance *E. coli* was found in 46.0% (208/452) of retail food samples, especially in 66.4% (77/116) in chicken, 55.4% (62/112) in pork, 42.0% (47/112) in fish, and 19.6% (22/112) in shrimp. The *mcr* genes screening shown that in 65 (31.3%) of the 208 colistin resistance *E. coli* isolates including *mcr*-1, *mcr*-3 and both *mcr*-1 and *mcr*-3 genes in 56/208 (26.9%), 1/208 (0.5%) and 8/208 (3.9%) isolates, respectively (Le *et al*., 2021). Sadek *et al*. (2021) occurrence of presence of *mcr*-1-positive Enterobacterales detected in retail raw chicken in Egypt. In the 345 retail chicken carcasses, 20 samples contaminated by *mcr*-1-positive isolates (*E. coli*, n=19; *Citrobacter freundii*, n=1). None of 20 isolates carried *mcr*-2- to *mcr*-10 (Sadek *et al*., 2021). In Iran, a molecular survey was preformed of *mcr*-1 and *mcr*-2 genes in *E. coli* isolates (sources between 2008 and 2016) of animal origin (broilers, ostriches, cattle, sheep, pigeons, and dogs). The researcher could not detect *mcr*-1 or *mcr*-2 positive *E. coli* isolates. They believed that pigs were the main source of plasmid resistance to colistin in previous studies and the lack of industrial pig breeding in Iran may be a probable cause for the bottom spread of *mcr*-1 and *mcr*-2 genes in food animals (Ilbeigi *et al*., 2021). However, Ribeiro *et al*. (2021) found high rates of chicken-meat batches (80%-100% – 4 months; 12% – the last month) with MDR *mcr*-1-positive *K. pneumoniae* and *E. coli*. Then, they proved that colistin voluntary withdrawal in Portuguese farms reflected in reducing presence of *mcr*-1-harboring bacteria from chicken meat and the risk of foodborne transmission to poultry-meat consumers (Ribeiro *et al*., 2021). Also, an epidemiology comparative study examined annual production and sales of colistin in agriculture from 2015 to 2019 as well as evaluated the prevalence of colistin-resistant *E coli* in pigs and chickens and *mcr*-1 in faeces from 118 animal farms (pig, chicken, and cattle) in China. Consequently, colistin withdrawal policy and the reducing use of colistin in agriculture have had a remarkable impact on decreasing colistin resistance and *mcr*-1 in animals and humans (Wang *et al*., 2020). According to the above reports, the spread of colistin resistance in livestock and animal foods around the world suggests that colistin withdrawal or restriction policy in animals could be a good management option to prevent public health hazards.

Conclusion

Antibiotic-resistant genes and bacteria pose a significant threat to public and animal health. The rapid loss of antibiotic efficacy requires coordinated action and the development of global policies. The discovery and spread of colistin-resistant superbugs and the *mcr* gene pose a serious threat to the latest antibiotic treatment options. The lack of specific pharmacokinetic data for colistin in livestock and the overuse of colistin in animal

farms exacerbates the problem. Therefore, implementing a colistin restriction policy, conducting accurate evaluations, and establishing an international monitoring system on the use of colistin in animal feed and farms are crucial to reducing bacterial resistance to this vital antibiotic. Effective identification and screening of animals and animal products regarding the mechanisms and presence of colistin resistance can significantly aid in addressing and implementing appropriate strategies to mitigate the spread of these resistances and their transmission to humans. Researchers can also help maintain the effectiveness of colistin by providing alternative competitive therapies that have less impact on the livestock digestive flora.

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Conflict of interest

The authors have no conflicts of interest to declare.

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