

Review Article

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Emergence and Dissemination of *mcr*-Mediated Colistin Resistance in Food-Producing Animals

A. N. Radhika^{id}*, P. V. Kishor and Roshni

Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur - 680 651, Kerala Veterinary and Animal Sciences University, Kerala, India

*Corresponding author

ABSTRACT

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Colistin is a polypeptide cationic antibiotic with wide spectrum of activity against gram negative organisms especially bacteria of *Enterobacteriaceae* family. It was mainly used for multi-drug resistant gram-negative bacteria like *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Due to its potential Neuro- and Nephrotoxicity these were replaced by less toxic compounds like gentamicin, fosfomycin and sulphonamides etc. Emergence of multidrug resistant bacterial infections led to the reconsideration of colistin use in humans. Colistin is widely used in veterinary for therapeutic, prophylactic and metaphylactic use. China is the largest consumer and producer of colistin in the world and it is mainly used in pigs and poultry production. Overuse and misuse of colistin as animal growth promoters and underdosing for prolonged time has led to the emergence of transferable colistin resistance. Most common gut bacteria of animals commonly acquire mobile colistin resistant (*mcr*) genes like *E. coli*, *Salmonella*, *Klebsiella* etc., these genes can be transferred horizontally between bacteria, which pose a threat to public health worldwide. This article reviews the use of colistin resistance in human and veterinary medicine along with global distribution and impact of colistin resistance on public health.

Introduction

Antibiotic resistance has been a major public health problem worldwide. Polymixins were isolated for the first time by spore forming bacteria *Paenibacillus Polymixa subsp. colistinus* in Japan by Koyama and coworkers in the year 1947. These are bactericidal antimicrobial agents act by targeting Lipopolysaccharide A in the outer membrane of gram-negative bacteria thus being highly active against gram-negative bacteria. (Poirel *et al.*, 2017) Colistin is active against aerobic

gram-negative organisms causing nosocomial infections like *Acinetobacter baumannii*, *Klebsiella pneumoniae* and species of *Enterobacteriaceae* family. Colistin was previously used in bowel decontamination, infectious diarrhoea, Urinary tract infections, and several other infections in humans. Due to high toxicity of colistin it was replaced by less toxic compounds like carbenicillin and gentamicin. Until 2015, only chromosomal mediated colistin resistance was reported. Later in November 2015 (Liu *et al.*, 2019) first described the emergence of plasmid mediated colistin resistance in food,

environment and animals. Misuse of colistin in agricultural fields and for non-therapeutic use in animals has led to the emergence spread of antibiotic-resistant bacteria. Especially, plasmid mediated colistin resistance has proved to be potential threat for public health due to its horizontal transmission to humans and environment from animals through different members of *Enterobacteriaceae* family. Aquaculture also play a major role in transfer of plasmid mediated colistin resistance bacteria to humans. An intense selection pressure exerted by antibiotics plays important role in selection of antibiotic resistance bacteria which transmits between animals and humans (Al-Tawfiq *et al.*, 2017). The risk of acquiring resistant bacteria is more in Slaughterhouse workers, Veterinarians, and those who are in contact with farm workers. The antibiotic resistance can transfer from animals to humans through food chain either by contact or consumption of food products. Industrial scale incorporation of colistin in food animals feed as growth promoter has led to the emergence of colistin resistance (Poirel *et al.*, 2017). As a measure to combat antimicrobial resistance European union banned use of four class of growth promoters including colistin in the year 2006. Bacteria isolated from animals contain *mcr* genes more frequently than bacteria isolated from humans indicating the misuse of colistin in veterinary practice.

Use of colistin in human medicine

Colistin has been used for about 50 years in both Veterinary and human medicine. Polymixin B and E are commonly used in treatment of human infections caused by gram-negative bacteria. Since colistin is systemically toxic, it was commonly used for Topical administrations, ophthalmic use and systemic use was restricted to Cystic fibrosis (Li *et al.*, 2019). From the past two decades increased emergence of multidrug resistant gram negative bacteria has led to their reconsideration for treatment of multidrug resistant (MDR) gram negative bacteria. It is commonly used for Carbapenem resistant *Enterobacteriaceae* and *Acinetobacter spp.* for which other treatment options are limited. The treatment of infections caused by carbapenemase resistant bacteria is limited to tigecyclin and colistin. World health organisation (WHO) has listed colistin in critically important antibiotic for humans with highest priority (WHO, 2017). Colistin causes Nephrotoxicity ranging from 6-14% in some patients and 32 to 55% in others and to a lesser extent it causes neurotoxicity (Yahav, D. *et al.*, 2012).

Use of colistin in Veterinary medicine

In Veterinary medicine colistin is mainly used for prophylaxis, metaphylaxis and treatment of *Enterobacteriaceae* infection and for growth promotion (Abraham *et al.*, 2018). It is used for controlling diarrhoeal diseases in poultry and pig production. It is mainly used for the treatment of non-invasive susceptible *Enterobacteriaceae* in pigs, poultry, cattle and small ruminants (Abraham *et al.*, 2018). The main indication of colistin in poultry is treatment of mild colibacillosis. Colistin is used more commonly in veterinary medicine and agriculture than in humans so, animals play a major role as reservoirs for colistin-resistant bacteria (Abraham *et al.*, 2018). In Spain it is used in pigs during gestation, lactation period and metaphylactic purposes.

Mechanisms of colistin resistance

Colistin resistance occur by chromosomal mediated and plasmid mediated mechanisms. Until 2015, only chromosomal mediated resistance was reported. Liu *et al.*, first reported the emergence and spread of colistin resistance in china. Colistin is being positively charged highly active against gram – negative bacteria it binds to the Outer lipid membrane of bacteria which protects bacteria from penetration of hydrophobic and large antibiotics, leading to displacement of cations like calcium and magnesium from phosphate group of LPS, thus it disrupts outer membrane leading to cell death. Colistin and polymixin B differ by only one aminoacid at position 6 of peptide ring both compounds have similar mode of action and spectrum of activity (Luo *et al.*, 2020). Due to its mode of action colistin is active only against gram negative bacteria some of the gram negative cocci bacteria are inherently resistant to colistin e.g., *Proteus spp.*, *Burkholderia spp.*, *Serratia spp.*

Most Gram negative bacteria follow adaptive mechanisms to develop resistance. Commonly resistance to colistin occur by modification of outer membrane through change in LPS leading to reduction in negative charge, the other mechanisms involve overexpression of efflux-pump systems and overproduction of capsule polysaccharides. Colistin can also neutralize the biological properties of endotoxins in gram negative bacteria (Bailvaei *et al.*, 2015). Chromosomal mediated bacterial resistance involves the linkage of 4-amino-4 deoxy-l-arabinose (L- Ara4N) and phosphoethanolamine (pEtN) to the Lipid A group of LPS. The addition of

these groups is regulated by two component system PhoPQ- PmrAB and pmrC-pmrE genes. PhoPQ-PmrAB regulate synthesis of L- Ara4N and pEtN code for proteins responsible for addition of these components to LPS. In Enterobacteriaceae the PmrAB and PhoPQ two component system play an important role in the development of resistance by allowing bacteria to respond to environment by expression of genes. Mutations in the genes *mgrB*, *CrrB* in *Klebsiella spp.* and PmrAB genes in *Salmonella enterica* regulating PhoPQ and PmrAB genes will result in colistin resistance (Dalmolin *et al.*, 2018).

Novel plasmid mediated colistin resistance

In 2015, Chinese researchers identified the first mobile colistin resistance gene from an *E.coli* isolate (Abraham *et al.*, 2018). Mobile colistin resistant genes work by the action of phosphoethanolamine transferase, which acts by transferring glucosamine from Lipid A Where negative charge is reduced making colistin unable to bind (Gharaibeh *et al.*, 2019). There are 9 *mcr* determinants isolated from different food animals ranging from *mcr* - 1, -2, -3, -4, -5, -6, 7.1 and 8. Currently *mcr* genes are distributed worldwide. *mcr* -1, -3, 7.1 and -8 genes were first detected in China (Yang *et al.*, 2018, Yin *et al.*, 2017) four genes *mcr* -2, -4,-5 and -6 was reported in Europe (Carattol *et al.*, 2017, Borowiak *et al.*, 2017, AbuOun *et al.*, 2017, Xavier *et al.*, 2016) and *mcr* -9 gene was first reported in USA (Carroll *et al.*, 2019). *Mcr* -1 is the most prevalent gene worldwide. The discovery of *mcr* genes in migratory animals has pose a threat of global dissemination. Isolation of *mcr* genes from both unhealthy and healthy humans like travellers, indicate the spread of *mcr* genes. The first isolated plasmid mediated resistant *E. coli* was in Latin America (Catry *et al.*, 2015). Plasmid mediated colistin is highly prevalent in Aquaculture also and *mcr*-1 is the prevalent determinant. There is evidence of strong association between high prevalence and gut carriage of resistant bacteria. In aquaculture food chain IncHI2 plasmid carrying *mcr*-1 is highly prevalent (Shen *et al.*, 2019).

Global distribution

Global dissemination of *mcr* genes is an important public health threat and currently it can be detected in 57 countries. China and India has emerged as a hotspot for the antibiotic resistant bacteria with Kenya and Brazil

emerging as new hotspots. China is the world's highest user of colistin worldwide (Global polymixin industry report, 2015). Globally chicken and pig production accounts for 96% of colistin usage. The *mcr* genes has widely distributed throughout world within short span of time.

The distribution of *mcr* genes is most in Pathogenic *E. coli* about 53% among all bacteria and animals (53%) among all hosts. IncI2 are among the most reported plasmids (34%). The prevalence of *mcr* genes globally ranges from 0.1 – 9.3% (~4.6%). The prevalence is highest in environment (22%) followed by animals (11%), food (5.4%) and humans (2.5%). The *mcr* gene was first isolated in the *Escherichia coli* in china in the year 2011.The food chain significantly affect the transmission of antibiotic resistant bacteria around the world.

The global spread of *mcr* – 1 gene occurred thorough *Klebsiella*, *Enterobacter*, *E.coli* and *Salmonella spp* isolates of animal, human and environment origin. Human travel also facilitated the spread of *mcr* genes through enteric bacteria of travellers returning from Europe, Asia, and Africa where *mcr*- 1 is highly prevalent.

Use of colistin in low dose for prophylactic purpose contributes to the emergence of colistin resistance which can spread to humans, animals and environment directly or indirectly (Catry *et al.*, 2015). Studies show that *mcr* genes has been survived for more than three decades. With *mcr*-1 gene being the common gene. The *mcr* – 2 gene was first discovered in *E.coli* mostly in the last decade from the isolates of pig and calves in Belgium (Elbediwi *et al.*, 2019).

Since its discovery *mcr* – 1 has spread to more than 40 countries including five of seven continents. Wildlife has role in dissemination of *mcr* – 1 and it has been detected in migratory birds in South America, Asia and Europe. It has been observed that exchange of *mcr* bearing *E. coli* strains between companion animals and humans especially animals carrying *mcr*-1 *Enterobacteriaceae*. Colistin and carbapenem resistant *E. coli* carrying *mcr*-1 and *NDM*-5 caused a complicated urinary tract infection from USA in 2014 (Sun *et al.*, 2018). *E. coli* harbouring *mcr*-1 has been isolated from raw food samples like chicken meat, fish, mutton meat fruits and vegetables (Barlaam *et al.*, 2019).

Prevalence and transmission of resistance in bacteria common to humans and animals

E.coli; *E.coli* are the most frequent species among the *mcr* positive isolates (90%) among humans and animals. Prevalence of *E.coli* is significantly higher in humans and animals suggesting the possibility food- borne transmission to humans and animals. IncI2 plasmid is the most prevalent plasmid in *mcr* positive *E.coli* isolated from humans, animals, plants and animals (Shen *et al.*, 2016). The prevalence of colistin resistant *E. coli* is more in Asian countries compared to Europe.

Salmonella enterica: *S. enterica* are zoonotic pathogens which colonize in the intestine of animals capable of transferring from animals to humans directly or indirectly by consumption of food (Gallati *et al.*, 2013). Food producing animals especially poultry and swine are the primary reservoirs of *mcr* positive *S.enterica* strains (Luo *et al.*, 2020). *S. Enteritidis* were the predominant colistin resistant salmonella strains.

Klebsiella pneumonia: Increased use of colistin for multidrug resistant gram negative bacteria has led to the emergence of colistin resistance in *k. pneumonia* and the resistance has been increasing in many countries worldwide. The development of heteroresistance is high when compared to actual resistant rates. Emergence of colistin resistant has increased the total infections and infection related mortality especially in ICU patients.

Other Bacteria: high prevalence of *mcr-1* positive Enterobacteriaceae were reports from broiler production in china and also identified the presence of all novel *mcr* genes (*mcr-1* to *mcr-5*) in positive species (Abraham *et al.*, 2019).

Impact on public health

In some animal species use of colistin orally leads to poor bioavailability and absorption on GIT which further leads to emergence of resistant strains. These resistant strains are excreted in the faeces thus contaminating the environment and water bodies. The resistant strains may be transmitted to humans when these animal faeces is used as manure in countries where there is poor management with animal regulatory system. Samples collected from food animals and the isolation of *mcr* genes from commensale and pathogenic *E. coli* strains shows the indiscriminate use of colistin in food animals.

These resistant isolates can easily transmitted to humans via food chain posing a significant public health risk (Bitrus *et al.*, 2018). Colistin Resistance is highly transferable at the rate of 10^{-1} (Skov *et al.*, 2016). A hospital outbreak of *mcr* bearing *K. pneumonia* has been reported from the Guangzhou, China. Increased colistin resistance to Enterobacteriaceae has been reported from human and animal sources an increased resistance in *Klebsiella spp.* has been reported from Asian and Southern European countries (Menekşe *et al.*, 2019).

In EU colistin is the 5th most used antimicrobial in the food producing animals. Human nosocomial infection with colistin resistant strains particularly carbapenemase colistin resistant *K. pneumonia* with high mortality has been reported. Other antibiotics like aminopenicillins, trimethoprim, sulphonamides, tetracyclines and aminoglycosides can replace colistin based on countries and situation (Catry *et al.*, 2015). Higher prevalence of *mcr* genes in animals has raised an alarm about use of colistin in animals and spread of resistance to humans. In china there is high prevalence of animal origin *E.coli* isolates representing 20% of contaminated animals and the patient isolates of *Klebsiella pneumonia* is 0-7%. Similar to other antibiotics prior use of colistin itself is a risk factor for development of resistance. Studies on colistin resistant isolates show that colistin is the only independent risk factor for emergence of colistin resistance.

Mcr genes are mostly isolated from *E. coli* strains from raw pig meat and also from important food borne pathogens like *Camphylobacter* and *Salmonella*. In Australia isolation of clinical samples from aquaculture showed the common occurrence of colistin resistant *Aeromonas spp* with the prevalence of 55.5%.

In a study conducted by Huang *et al.*, the frequency of colistin resistance is high from pig farms and slaughter houses (24%) followed by chicken on farms (14%) and slaughter houses (9.5%). The resistance frequency is lowest in cow isolates (0.9%) (Huang *et al.*, 2017). Underdosing in patients with critical ill, Obesity, burns, renal failure, and also the use of colistin for Gastroenteral decontamination increases the threat of antibiotic resistance emergence (Catry *et al.*, 2015). In conclusion, Colistin is last resort antibiotic for treatment of multidrug resistant organisms and it has been listed as critically important antibiotic by World Health Organisation (WHO). Increased resistance to

colistin has created public health threat worldwide. The largest poultry meat producers like china and brazil use colistin more commonly in food producing animals for growth and treatment purpose which has resulted in high prevalence of colistin resistance among variety of Bacterial species. As a global One health measure European member states have decided to achieve 65% reduction in sale by 2020 (Skov *et al.*, 2016). Many researches advocate the use of one health strategy to stop antimicrobial resistance and the need to aware food suppliers at food supply chain to reduce colistin as growth promoter (Nusrat *et al.*, 2020). As announced by the Responsible use of Medicines in Agriculture alliance (RUMA) and China the use of critically important antibiotics like colistin in animals should be stopped (Al-Tawfiq *et al.*, 2017).

On 19th July 2019, Ministry of Health and family welfare, India has prohibited the manufacture, sale and distribution of the drug colistin and its formulations for food producing animals, poultry, aqua farming and animal feed supplements (Founou *et al.*, 2016). To reduce use of colistin use in aquaculture European Food Safety Authority (EFSA) has extended the Antimicrobial resistance monitoring to aquaculture (Shen *et al.*, 2020).

There is possible transmission of colistin resistant bacteria from aquaculture food chain, With *mcr-1* gene being highly prevalent. It is important to educate public and professionals regarding effects of misuse of antibiotics along with the emergence of spread of antibiotic resistance from animals to humans and environment.

It is also important to implement strict regulatory measures for the use of antibiotics in animal and human medicine with the use of technological advances. The measures proposed by WHO has to be implemented in the fight against emergence and spread of antimicrobial resistance

Author Contributions

A. N. Radhika: Investigation, formal analysis, writing—original draft. P. V. Kishor: Validation, methodology, writing—reviewing. Roshni:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the

current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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