An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: A review

Mohammad H. Gharaibeh and Shoroq Q. Shatnawi

Abstract

Colistin, also known as polymyxin E, is an antimicrobial agent that is effective against a variety of Gram-negative bacilli, especially the Enterobacteriaceae family. Recently, the wide dissemination of colistin-resistance has brought strong attention to the scientific society because of its importance as the last resort for the treatment of carbapenem-resistant Enterobacteriaceae infections and its possible horizontal transmission. The mobilized colistin resistance (mcr) gene was identified as the gene responsible for unique colistin resistance. Indeed, despite many studies that have revealed a pan variation in the existence of this gene, not only for the mcr genes main group but also for its many subgroups, the problem is growing and worsening day after day. In this regard, this review paper is set to review the updated data that has been published up to the end of 2019 third quarter, especially when related to colistin resistance by the mcr genes. It will include the present status of colistin resistance worldwide, the mcr gene dissemination in different sectors, the discovery of the mcr variants, and the global plan to deal with the threat of antimicrobial resistance. In line with global awareness, and to stop antibiotic misuse and overuse, especially in agricultural animals, the study will further discuss in detail the latest alternatives to colistin use in animals, which may contribute to the elimination of inappropriate antibiotic use and to the help in preventing infections. This review will advance our understanding of colistin resistance, while supporting the efforts toward better stewardship, for the proper usage of antimicrobial drugs in humans, animals, and in the environment.

Keywords: animals, colistin alternatives, colistin resistance, Enterobacteriaceae, humans, mobilized colistin resistance-genes, one-health.

Introduction

Colistin is an antimicrobial agent that belongs to the polymyxin antibiotic class, which is produced by a Gram-positive bacterium known as *Paenibacillus polymyxa*. This class consists of five polymyxins, A, B, C, D, and E, where polymyxin E (colistin) and polymyxin B are used clinically [1]. The colistin class of antibiotics is one of the last antibiotics that are used to treat Enterobacteriaceae infections in humans, such as colistin sulfate (CS) for oral and topical use, and colistin methanesulfonate (CMS) sodium for parental use [2]. In addition, colistin is a popular drug in the animal field, not only to treat infections caused by Enterobacteriaceae but it is also used as a growth promoter and a protective agent [3]. The rules for colistin usage are highly different from one place to another. For example, while the USA government has prohibited the clinical use of colistin in animal production and in human treatment, due to its nephrotoxicity within the human body [4], China is considered to be the world’s highest users of colistin in agriculture [5]. In addition, Germany, Portugal, Italy, and Estonia have shown a higher colistin use than in other European countries [6]. In the past few years, several review articles have highlighted the growing problem of colistin resistance worldwide, especially with *Escherichia coli* in the human community, but when regarding animals and other pathogens, the information is still scarce, due to the weak monitoring of its use. In this present review, the study’s goal was to provide the latest information related to colistin resistance by the mobilized colistin resistance (mcr) genes, in humans, animals, and in various pathogens. This review provides important insights into: (i) Demonstrating the present status of colistin resistance, the mcr gene emergency, and the global plan to deal with the threat of antimicrobial resistance (AMR); (ii) discussing various colistin researches, not only in the field of humans and farm animals but also in the aquaculture sector, while, at the same time, demonstrating the relationship between these sectors in the dissemination of the plasmid resistance gene mcr; (iii) illustrating the mcr gene dissemination by trade and travel and the discovery of the mcr variants starting from mcr-1 up to mcr-9; and (iv) presenting suggested alternatives to colistin for the treatment of infectious diseases.
Colistin and its Resistance Emergency in Animals

The mcr genes are plasmid-borne genes that contribute to colistin resistance. To date, nine mcr variants have been described, as shown in Table-1, (mcr-1, -2, -3, -4, -5, -6, -7, -8, and -9). As mentioned earlier, many recent studies have shown the wide distribution of mcr-genes in animals. Rhouma et al. [3] indicated that the extensive use of colistin in production animals was recognized as the responsible agent for the emergence and dissemination of the plasmid-borne colistin resistance gene mcr-1. Recently, Liu et al. [7] showed evidence for the isolation of Gram-negative bacteria from resistant animals to colistin. Furthermore, many studies have detected the existence of the mcr-1 gene in different animal food species. For example, Meinersmann et al. [8] reported this gene in E. coli isolate of pig cecal contents from the USA. Also, Huang et al. [9] reported the mcr-1 gene from E. coli isolates of animal food origins (chickens and pigs) from China [9]. In addition, Barbieri et al. identified the mcr-1 gene in poultry samples from the year 2010. They examined 980 Avian pathogenic E. coli that was isolated from poultry and that was suffering from colibacillosis, and for the comparison analyses, they compared an additional 220 sets of non-infected avian fecal E. coli. The mcr-1 gene was reported in 12 isolates that were recovered from diseased production birds from China and Egypt. Remarkably, the date for the positive mcr-1 gene from the Egypt isolates was back in 2010. On the other hand, no mcr genes were reported in any of the healthy fecal isolates [10]. In other studies, the mcr-1 gene was identified in E. coli when it was isolated from diseased chickens and cows suffering from subsclinal mastitis in Egypt [10-12]. Barbieri et al. [10] suggested that the huge usage of colistin in animal agriculture, and its ready application as a therapeutic agent for colibacillosis and other infections in rabbits and calves, was responsible for the emergence of mcr-1 in Egypt [10]. The full mcr-1 gene isolates were sequenced and compared with the sequences currently described in NCBI [https://www.ncbi.nlm.nih.gov/nuccore/ku886144] and in Arcilla et al. [13]. Furthermore, the phenotypes and the genotypes of the mcr-1 positive isolates were determined as being colistin resistance and as extended-spectrum beta-lactam (ESBL). Earlier, Quesada et al. [14] proved that Spain detected mcr-1 in E. coli and in Salmonella enterica when isolated from farm animals. Interestingly, Hernández et al. [15] reported on a coexisting of mcr-1 with mcr-3 on the same IncHI2 plasmid which was reported in an E. coli strain cured of cow feces in a slaughterhouse in Spain. Haenni et al. [16] identified the presence of a special IncHI2/ST4 plasmid that was colocalizing mcr-1 and ESBL genes in French veal calve isolates of E. coli strains. A research study in China investigated the colistin spread in farm animals and revealed that mcr-1 and mcr-2 were detected in cattle, pig, and chicken origins of E. coli isolate, where mcr-1 was the higher percentage [17]. Moreover, in the same study, a cooccurrence of mcr-1 and mcr-2 was reported, but with a low ratio when in comparison to the mcr-1 and mcr-2 percentages [17]. Alba et al. [18] reported on mcr-1 and mcr-2 in E. coli from turkey origins in Italy. The cooccurrence of mcr genes was reported in Spain [19], where the mcr-1, mcr-4, and mcr-5 genes were found in multidrug-resistant (MDR) ST10 entero-toxicogenic and Shiga toxin-producing E. coli from swine with post-weaning diarrhea.

Mcr Gene Mechanisms and their Members

In 2016, the first report to show the emergence of the plasmid-mediated polymyxin resistance mechanism, mcr-1, in Enterobacteriaceae, was discovered in China [7]. The next step was to determine the mechanisms of plasmid-mediated colistin resistance and the mcr-1 gene. This was reported by Hinchliffe et al. [20] when they proved that the mcr-1 gene works to change the target of colistin through the action of the enzyme, phosphoethanolamine transferase, which transfers glucosamine from lipid A, while the negative charge of lipid A is reduced; consequently, colistin cannot connect. Interestingly, mcr-1 was not the only member of the mcr-genes, because a series of recent studies have indicated the mcr-gene presence is in humans and animals, and they have revealed nine different mcr genes. Four of them (mcr-1, 3, 7, 1, and 8) were first detected in China, and four genes (mcr-2, 4, 5, and 6) were reported in Europe. Finally, mcr-9 was reported in

Table-1: The first identification of the mcr genes by time and area.

| Gene   | Year | Country                  | Bacteria                | Sample origin | Length | References |
|--------|------|--------------------------|-------------------------|---------------|--------|------------|
| MCR-1  | 2016 | China                    | E. coli                 | Animal, Human | 1626 bp | [7]        |
| MCR-2  | 2016 | Belgium                  | E. coli                 | Animal        | 1617 bp | [28]       |
| MCR-3  | 2017 | China                    | E. coli                 | Animal        | 1626 bp | [24]       |
| MCR-4  | 2017 | Italy, Spain and Belgium | E. coli, Salmonella     | Animal        | 1626 bp | [25]       |
| MCR-5  | 2017 | Germany                  | Salmonella              | Animal, Food  | 1644 bp | [26]       |
| MCR-6  | 2017 | UK                       | M. pluranimalium        | Animal        | 1617 bp | [27]       |
| MCR-7.1| 2018 | China                    | K. pneumoniae           | Animal        | 1620 bp | [21]       |
| MCR-8  | 2018 | China                    | K. pneumoniae           | Animal        | ND      | [22]       |
| MCR-9  | 2019 | USA                      | S. Typhimurium          | Human         | ND      | [23]       |

E. coli=Escherichia coli, M. pluranimalium=Moraxella pluranimalium, K. pneumoniae=Klebsiella pneumonia, Salmonella Typhimurium=Salmonella Typhimurium

Available at www.veterinaryworld.org/Vol.12/November-2019/7.pdf
the USA, starting from 2016 up to 2019, as shown in Table-1 [7,21-28]. Of the recently detected genes, mcr-7.1 (1620bp) and mcr-8 have been reported in China in 2018, and both were hosted by Klebsiella pneumoniae from human and animal origins [21,22]. In addition, a recent study by Carroll et al. [23] identified mcr-9 in an MDR S. enterica serotype Typhimurium isolate, which was colistin-susceptible in the USA and demonstrated the phylogenetic tree connects between mcr-1 and mcr-9.

**The Discovery of mcr Variants**

Recently, some unknown selective pressure in the environmental section and in the animal field and human sectors was considered as being the responsible agents for the constant evolution of the mcr gene, which ended by producing the mcr variants, as suggested by Sun et al. [29]. However, within a short period of time, the mcr genes included several variants nearly all around the world, for instance, mcr-1 has 13 variants that are different by one amino acid from mcr-1 (mcr-1.1 to mcr-1.13) [30,31]. Recently, Wise et al. [32] collected 908 Enterobacteriaceae isolates worldwide, and the results revealed that while 22 isolates carried the basic mcr-1 gene, one of the isolates had the mcr-1 gene with a single amino acid variant mcr-1.1 and another isolate held mcr-1.5. The 13 mcr-1 subgroups have already been described in several countries, differing from mcr-1 by only one nucleotide. In Thailand, three isolates of mcr-3.1 and one isolate of mcr-3.2 were reported. Likewise, Ling et al. [33], in China conferred polymyxin resistance by the presence of mcr-3.2 in both Aeromonas salmonicida and E. coli. During the same year, mcr-3.7 was identified by Teo et al. [34], in Singapore, and it was not resistant to colistin or polymyxin B. This was while Chavda et al. [35] indicated mcr-4.3 in China from a human patient, but it did not show any colistin-resistance. Furthermore, Fernandes et al. [36] reported on a novel mcr-5.3 variant in South Africa from a horse that had not been previously treated by colistin. Hammerl et al. [37] also identified mcr-5.2 in Germany from swine fecal samples at farms and from cecal contents of swine at slaughter. Moreover, Wang et al. [38] investigated the presence of mcr-3.10 in a fecal sample from a duck in China. One study in Italy (2018) described the special diversity of six different mcr variants, with a high predominance of both mcr-1.1 and mcr-1.2 on conjugative IncX4 plasmids in Salmonella and in E. coli isolates from food-producing animals [18]. At the same time, mcr-1.13 was detected as a new variant within the chromosomes of E. coli from turkey and swine isolates. In addition, this study described mcr-3.2 and mcr-4.3 from cattle and mcr-4.2 from swine, where all of these variants were detected in E. coli [18]. Novel mcr-4.4 and mcr-4.5 variants were described in Spain, from pigs that were hosting five different isolates [19]. Finally, mcr-8 and mcr-8.4 were just identified by Wang et al. [39], in Raoultella ornithinolytica isolates, which are a member of the family from poultry samples in China.

**Mcr-gene Dissemination**

A few years ago, colistin resistance in Enterobacteriaceae was considered uncommon, but that fact was changed when Liu et al. reported on a novel plasmid colistin resistance gene, mcr-1, from animal-originated E. coli, and they then found multi-resistance plasmids from animals, humans, and retail meat in K. pneumoniae and E. coli [7]. Nowadays, this resistant gene, mcr-gene, is disseminated, all over the world, and it threatens the therapeutic effectiveness against multidrug-resistant bacteria. Worryingly, these kinds of pathogens are untreatable with regular antibiotics [40]. Moreover, by tracking the mcr gene discoveries in the world, one can notice that the first mcr (mcr-1) was identified in Asia in 2016 [7], followed by many studies identifying the existence of the mcr gene in different places, and reported on a worldwide dissemination, namely, in Asia, Europe, North America, Africa, and the Middle East [29-31,41]. In addition, since 2015 up until the present, a huge number of researches has been conducted into the emergence and dissemination of the mcr gene, and this has resulted in nine different mcr variants and many subvariants [22,42,43]. Here, in this study, examples of the dissemination of the mcr gene have been demonstrated in different continents and with different bacterial species. In fact, mcr-1 was first reported in China by Liu et al., but since then, this gene has been detected in several countries and from different sources, including natural and human-associated environments [44,45], food [46-48], animals [49,50], and humans [30,51], as shown in Table-2, and as modified from the Figure as published by Sun et al. [29], illustrating the pan mcr-1 distribution. In addition, mcr-1 was harboring in various species of the Enterobacteriaceae family, as has been indicated by many Epidemiological studies. Table-3 summarizes these bacterial species. Recently, several studies have shown how big the problem is in the Middle East. In August 2018, a study in Lebanon detected the existence of mcr-1 harbored by E. coli from swine fecal samples [52]. Another study in Lebanon by Hmede and Kassem investigated fresh fecal samples of broiler chickens and detected mcr-1 in E. coli [41]. Moreover, Eltai et al. [53] studied broiler chicken fecal samples in Qatar and they proved the presence of mcr-1 in E. coli isolates. Furthermore, the existence of the mcr-1 gene in colistin resistance Enterobacteriaceae in the Arabian Peninsula countries was reported by Sonnevend et al. [54] in four E. coli isolates of human origin; two isolates from Bahrain, one isolate from the Saudi Arabia and one isolate from the United Arab Emirates). In addition, mcr-3, as previously mentioned, was reported in China in 2017 from E. coli isolates [24]. In the same year, Litup et al. [55] also identified mcr-3 positive isolates in Denmark from
humans, harbored by Salmonella [55]. Nevertheless, Belayneh et al. [56] reported on mcr-3 in E. coli from healthy animals in South Korea. Moreover, mcr-5 was first reported in 2017 in Germany from poultry and animal-derived food products [57], one year later, it was proved to presence in China pigs, and showed a horizontal transmission of the resistant gene among Aeromonas hydrophila, while it existed widely among the Enterobacteriaceae, Pseudomonas, and Aeromonas species. The threat of mcr gene dissemination comes from its horizontal transmission. Initially, it was believed that the mcr genes were transmitted from domestic animals by their milk, meat, and eggs to humans, or by direct contact [14,58]. Conversely, Villa et al. [59] proved that mcr-1 could be transmitted to E. coli strains and colonized in different hosts, such as in humans and pets, all within the same place. However, Garcia et al. [19] showed that food-producing animals might have the ability to spread a cocktail of resistant mcr-genes, demonstrating an annoying threat to human health. On the other hand, the pan dissemination of mcr-1 has been connected with an elevation of human travel, as an explanation for the existence of the gene in enteric bacteria from European travelers returning to their home, before having visited countries with a high prevalence of mcr-1 in Asia, South America, and Africa [13,58]. Aquaculture is one of the most important food production practices worldwide. Some studies have focused on the aquaculture contribution to colistin resistance bacteria and dissemination. For example, Almeida et al. [60] reported on the isolation of Enterobacter cloacae from fish and shrimps having resistance to colistin, dependent on the minimal inhibitory concentration (MIC) results. Colistin resistance was reported in Salmonella aborty with novel mutations on the chromosomal pmrA and pmrB genes.

### Table-2: Global distribution of MCR-1 worldwide [29].

| Continent | Country     | Animal | Human | Environment |
|-----------|-------------|--------|-------|-------------|
| North America | USA       | ●      | ●     | ●           |
| South America | Canada     | ●      | ●     | ●           |
|             | Venezuela   | ●      | ●     |●           |
|             | Colombia    | ●      | ●     | ●           |
|             | Ecuador     | ●      | ●     | ●           |
|             | Brazil      | ●      | ●     | ●           |
|             | Bolivia     | ●      | ●     |●           |
| Asia       | China       | ●      | ●     |●           |
|             | India       | ●      | ●     |●           |
|             | Pakistan    | ●      | ●     |●           |
|             | Oman        | ●      | ●     |●           |
|             | Saudi Arabia| ●      | ●     |●           |
|             | Japan       | ●      | ●     |●           |
|             | Korea       | ●      | ●     |●           |
|             | Singapore   | ●      | ●     |●           |
|             | Malaysia    | ●      | ●     | ●           |
|             | Thailand    | ●      | ●     |●           |
|             | Vietnam     | ●      | ●     |●           |
|             | LAOS        | ●      | ●     |●           |
| Africa  | Egypt       | ●      | ●     |●           |
|          | Tunisia     | ●      | ●     |●           |
|          | Algeria     | ●      | ●     |●           |
|          | South Africa| ●      | ●     |●           |
| Europe  | Norway      | ●      | ●     |●           |
|          | Sedan       | ●      | ●     |●           |
|          | Estonia     |●      | ●     | ●           |
|          | Lithuania   |●      | ●     | ●           |
|          | Denmark     |●      | ●     | ●           |
|          | Germany     | ●      | ●     |●           |
|          | Spain       | ●      | ●     |●           |
|          | UK          | ●      | ●     |●           |
|          | France      | ●      | ●     |●           |
|          | Italy       | ●      | ●     |●           |
|          | Netherlands | ●      | ●     |●           |
|          | Portugal    | ●      | ●     |●           |
|          | Switzerland | ●      | ●     |●           |
|          | Belgium     |●      | ●     |●           |
| Australia |●      | ●     |●           |

### Table-3: Bacterial species and the relative mcr genes.

| Bacteria                  | MCR gene | Country                  | Origin | References |
|---------------------------|----------|--------------------------|--------|------------|
| E. coli                   |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia, Europe, Africa | Animal, Human | [7,14,25,28,37,38,67,68] |
| K. pneumonia              |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal, Human | [7,21,22,67] |
| Salmonella spp.           |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia, Europe, USA   | Animal, Food, Human | [23,25,26,66] |
| M. pluranimalium          |          | ● ● ● ● ● ● ● ● ● ● ● ● | Europe             | Animal          | [27] |
| E. aerogenes              |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Human          | [69] |
| E. cloacae                |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Human          | [69] |
| C. sakazakii              |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal          | [70] |
| S. sonnei                 |          | ● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Human          | [71] |
| Kluyvera spp.             |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Sewage         | [72] |
| Citrobacter spp.         |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia, South America | Food          | [73,74] |
| R. ornithinolytica        |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Food          | [46] |
| A. hydrophila             |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal          | [57] |
| A. caviae                 |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal          | [38] |
| P. mirabilis              |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal          | [38] |
| A. baumannii              |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Animal          | [75] |
| V. parahaemolyticus       |          |● ● ● ● ● ● ● ● ● ● ● ● | Asia       | Food          | [76] |

K. pneumoniae = Klebsiella pneumoniae, E. coli = Escherichia coli, M. pluranimalium = Moraxella pluranimalium, E. aerogenes = Enterobacter aerogenes, E. cloacae = Enterobacter cloacae, C. sakazakii = Cronobacter sakazakii, S. sonnei = Shigella sonnei, R. ornithinolytica = Raoultella ornithinolytica, A. hydrophila = Aeromonas hydrophila, A. caviae = Aeromonas caviae, P. mirabilis = Proteus mirabilis, A. baumannii = Acinetobacter baumannii, V. parahaemolyticus = Vibrio parahaemolyticus

Available at www.veterinaryworld.org/Vol.12/November-2019/7.pdf
Bacterial Resistance to Colistin

There are Enterobacteria isolates in humans, animals, and in the environment and this has been investigated and related to plasmid-mediated colistin that was encoded by the mcr-1 gene [65]. Table 3 represents the bacterial species and the specific types of mcr genes that are hosted [7,14,21,22,25-28,37,38,46,57,66-76]. Up-to-date, the presence of the mcr-1 gene has been proved in several bacterial species, for example, in E. coli, K. pneumoniae, S. enterica, Enterobacter aerogenes, E. cloacae, and more [77]. Besides, various types of bacteria have been identified to the harbor in more than one mcr gene; for example, Salmonella has been reported to host mcr-1, mcr-4, mcr-5, and mcr-9 [23,25,26,66]. Moreover, E. coli has been recognized as the superior host bacteria for the mcr-gene, by harboring mcr-1 up to mcr-5, with the chance for coexistence of more than one mcr gene [22,24-26,37]. Other studies have identified K. pneumoniae as a host for the new genes, such as mcr-7 and mcr-8, in addition to mcr-1 [7,21,22,67]. Up-to-date, Moraxella pluranimium, which was investigated by AbuOun et al. [27], was the only bacteria that hosted the mcr-6 gene from animal isolates in Europe.

The International Response to mcr Gene Dissemination

The emergence of the plasmid-mediated mcr gene has initiated the world’s awareness of the way of using colistin to treat the diseases caused by Enterobacteriaceae and, principally, to deal with the increasing applications of colistin, as a growth promoter in the veterinary field. The reduction in the overall use of colistin was the main idea for controlling the dissemination of the mcr genes internationally, as suggested by many researchers [78,79]. Recently, the World Health Organization added polymyxins as one of the critically important antimicrobial agents to be used in food-producing animals internationally [80]. In response to this, and to the predominant increase of common MDR bacterial infections in South Africa, they worked on the development of the “One Health-Based National Strategic Framework” for antibiotic resistance [81]. They also worked on the accomplishment plan [82], setting a program for the governance of stewardship for antibiotic use at different levels [83]. Moreover, The European Food Safety Authority (EFSA) applied the “One Health Approach,” due to the relationship of transferring bacteria from different sectors - humans, veterinary, and the environment. The EFSA also worked to increase their communication with food consumers because they can be part of the solution by changing attitudes and behaviors [84]. In June 2016, the European Medicines Agency developed a new concept on using colistin in European veterinary fields and they suggested that colistin should be carried out by the Antimicrobial Advice Ad Hoc Expert Group, which meant that colistin should be conserved for infections, where no other effective alternative drugs were available [85]. The American response took place in the US Centers for Disease Control and Prevention, by stating “the One Health concept recognizes that the health of humans is connected to the health of the animals and the environment” [84]. Robinson et al. [86], 2016 thought that the One Health Program depended on three main areas: Human health, animal fields, and environmental status. In 2016, the High Council for Public Health (HCSP) in France recommended the use of contact precautions with carriers of the mcr-1/mcr-2 Enterobacteriaceae strains [87]. The recommendations of the HCSP hold four critical points. First, plasmid-mediated colistin resistance should be investigated in all carbapenemase-producing Enterobacteriaceae (CPE) strains. Second, by applying the hygiene precautions into two types; either by applying a specific action to observe the emerging highly resistant bacteria besides those carrying the mcr-1 gene [88] or by applying the 2009 SF2H guidelines, namely, “cross-transmission: Contact precautions” [89]. Third, the proper action when plasmid-mediated colistin is detected is to report the resistant gene into the nosocomial infection reporting system; then, all strains carrying the mcr-1/mcr-2 gene, and not only for the CPEs should be sent to the National Reference Center for Antibiotic Resistance. Finally, the French epidemiological situation can be improved by an assessment of the prevalence of colistin resistance and the presence of the mcr-1 resistance gene among the Enterobacteriaceae strains originating from the community and from hospital laboratory data. The epidemiological surveys might also be regulated by a national working group made up of experts from HCSP, ONERBA, the National Reference Center for Antibiotic Resistance in Clermont-Ferrand, and the government agency of Santé publique France. All of the previous recommendations that were aimed to manage the dissemination threat of plasmid-mediated colistin resistance within the Enterobacteriaceae strains should be kept by...
updating any reports of emergency of any mcr genes in infectious cases [87]. Furthermore, the researchers in the literature spotted a light on re-evaluating the dosage regimens of colistin for the treatment of lung infections. Therefore, Lin et al. [90] aimed to develop a mechanism-based PK/PD model (MBM) that would determine the time course of the colistin concentrations in the epithelial lining fluid, the plasma, and the bacterial concentrations, post the administration of different dosage regimens of colistin in neutropenic infected mice. Furthermore, Lin et al. [90] compared the newly developed MBM and a previously developed population PK model of aerosolized CMS and they formed colistin in critically ill patients, to predict the efficacy of inhalational dosage regimens of colistin (as CMS) in humans. Researchers from Italy strongly recommended, not only to reduce the overall usage of colistin but also to decrease the use of other different types of antimicrobial agents at a primary production level. This was to reduce the effects of complex mechanisms behind the multidrug resistance and the coselection of critically highly important antimicrobials while staying in a “Consumer Protection” and a “One Health” perspective [18]. Interestingly, Thakur and Gray believed that researchers will never recognize the spread of the AMR challenge and will only tackle AMR effectively if they harmonize the surveillance between nations [91]. They also suggested that surveillance should be an international “One Health” effort to solve this critical threat to humans, animals, and environmental health because the world has already reached the top point or even passed the top era of this problem [91].

The Alternatives for Colistin

One of the most critical challenges to deal with infectious diseases nowadays is the limited treatment options due to two main points: First, the lack of development of new antimicrobial agents, and second, the persistent increase in global AMR [48,92]. Antibiotic resistance is believed to be a serious and growing global threat; specifically, the resistance to colistin is considered to be a great concern to the world community, due to the value of colistin, as it is the last choice available to treat multi-resistant Gram-negative bacteria [93]. By reducing the excessive use of antimicrobials, means to implement alternative measures, to limit the emergence and the spread of bacterial infections. Moreover, by increasing the awareness of people to stop the misuse of antibiotics and their overuse is critical. In this section, the strategies that are used to minimize antibiotic resistance will be reviewed, especially the antibiotic alternative options for reducing colistin resistance. One strategy has been to develop a better tolerated and more effective combination with the superior antimicrobial features of polymyxin, in addition to finding proper antibiotic combinations with colistin against polymyxin-non-susceptible Gram-negative bacterial infections. Vaara [94] reviewed four different programs that proved novel derivatives have better efficiency than the old polymyxins when applied to animal infection models; they were identified as Monash Cantab compound CA824, MicuRx compound 12, and compound NAB739 from northern antibiotics. These programs included three different programs that were superior to the known polymyxins in the rodent lung infection model with Pseudomonas aeruginosa and/or Acinetobacter baumannii. Interestingly, one of the programs showed a superior effect than did polymyxin B in mice infected by A. baumannii. The fourth program included compounds that were nearly ten-fold more effective in E. coli murine pyelonephritis than polymyxin B, which was compound NAB739 from northern antibiotics [94]. Moreover, to overcome the antibiotic resistance, a study by MacNair et al. [95] aimed to work in a new method using a combination of different antibiotics [95]. Briefly, they screened many Enterobacteriaceae that expressed mcr-1, against several antibiotics to decrease MIC in the presence of colistin. As a result, the greatest decline in MIC was reported by a combination of colistin and effective antibiotics against Gram-positive bacteria, such as rifampin and macrolides. These combinations were a successful treatment in two mouse models, against an mcr-1-positive K. pneumoniae infection [95]. These results are in agreement with those results as reported by Hu et al. [96], who proved the success of colistin combinations with azidothymidine, so as to treat antibiotic-resistant Enterobacteriaceae infections. On the other hand, there are many options available for reducing the use of colistin in animals by the use of non-antibiotic alternative products. There is a mounting interest in developing bacteriophage-based products for an administration to food animals as a new class of antimicrobial agents. Several studies have demonstrated that bacteriophages are “phages that are viruses capable of infecting bacteria,” in line with the ideas of antibiotic alternatives. For instance, Jeon et al. [97] recognized a novel A. baumannii lytic phage, the YMC 13/03/R2096 ABA BP (phage Bϕ-R2096), and the results strongly recommended that phage Bϕ-R2096 could be an alternative antibiotic agent to treat carbapenem-resistant A. baumannii infections [97]. Similar patterns of results were obtained by Prasanth Manohar et al., when they studied the isolation and the characterization of the bacteriophages that effected E. coli, K. pneumoniae, and the Enterobacter species. The bacteriophages were named as Escherichia virus myPSh2311, Klebsiella virus myPSh1235, and Enterobacter virus myPSh1140. These three phage cocktails were effective against mixed bacterial populations that were resistant to meropenem and colistin [98]. Others have shown that some feed additives, like guanidine acetic acid, could be used as antibiotic alternatives, and they would significantly affect carcass characteristics and the economic traits of broiler chickens [99]. Some authors
have also suggested the use of Artilysin® s, which are newly engineered enzyme-based experimental therapeutics that are effective against Gram-negative and Gram-positive pathogens. They could be used as bactericidal agents against all E. coli isolates that harbor the mcr-1 gene [100]. In addition, Art-175 could be a solution against bacteria and that may develop a pan drug resistance, due to its rapid and specific mode of action, by also decreasing the probability of inducing genetic resistance [101]. Recently, the main aim for a study by Otto et al. [102] was the non-antibiotic molecules in combination with polymyxin B.

Table-4: Alternative products to colistin modified from the PEW Report [104].

| Product type       | Definition                                                                 | Purpose of use | Animal species | References |
|--------------------|-----------------------------------------------------------------------------|----------------|----------------|------------|
| In-feed enzyme     | "Enzymes are biologically active proteins that break specific chemical bonds to release nutrients for further digestion and absorption" | •              | Swine, Chicken, Turkey | [105]     |
| Probiotics         | A definition approved by FAO/WHO (2001) states that "Probiotics are mono or mixed cultures of live organisms which, when administered in adequate amounts confer a health benefit to the host." | • • •          | Cattle, Swine, Chicken, Turkey | [106]     |
| Prebiotics         | A definition approved by (FAO, 2007) describes prebiotics as "non-viable feed components that confer a health benefit on the host associated with modulation of the microbiota" | •              | Calves, Swine, Chicken, Turkey | [107]     |
| Antimicrobial peptides | "AMPs are small molecular weight proteins with broad-spectrum antimicrobial activity against bacteria, viruses, and fungi" | • • •          | Cattle, Swine, Chicken | [108]     |
| Organic acids      | "Organic acids activity are short-chain acids (C1-C7) and are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing a hydroxyl group (usually on the α carbon) such as lactic, malic, tartaric, and citric acids" | • • •          | Cattle, Swine, Chicken, Turkey | [109]     |
| Phytochemicals     | "Phytogenic are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals‘ production performance, and improving the quality of food derived from those animals" | • • •          | Cattle, Swine, Chicken, Turkey | [110]     |
| Heavy metals       | "Heavy metals such as zinc and copper are naturally occurring and necessary as trace minerals in the diet but are extensively used in higher concentrations for growth promotion, and occasionally as therapy for enteric disease" | • • •          | Cattle, Swine, Chicken, Turkey | [111]     |
| Vaccines           | "Vaccines are substances used to mimic the development of naturally acquired immunity by inoculation of nonpathogenic but still immunogenic components of the pathogen in question, or closely related organisms" | •              | Cattle, Swine, Chicken, Turkey | [112]     |
| Immune modulators  | "The transfer of antibodies to elicit passive immune responses, are promising alternatives for disease prevention and potentially for treatment as well" | • • •          | Cattle, Swine, Chicken, Turkey | [104]     |
| Bacteriophages     | "Bacteriophages are viruses that infect and multiply in bacteria" | • • •          | Cattle, Swine, Chicken, Turkey | [113]     |
| Predatory bacteria | "Predatory bacteria such as the "MDR Gram-negative bacteria have emerged as a serious threat to human and animal health. Bdellovibrio spp. and Micavibrio spp. are Gram-negative bacteria that prey on other Gram-negative bacteria" | •              | In Experiment State | [114]     |
| Cas9               | "Cas9 and similar products work by reprogramming parts of the bacterial immune system (i.e., Cas9, a nuclease in the type II CRISPR system of bacteria) to selectively target specific parts of the bacterial genome (i.e., virulence factors), thereby selectively inactivating harmful bacteria that possess these virulence genes" | •              | In Experiment State | [104]     |

1=Growth promotion, 2=Disease prevention, 3=Disease treatment. AMPs=Antimicrobial peptides, MDR=Multidrug-resistant
This study demonstrated a potential efficacy of three antidepressants (amitriptyline, imipramine, and sertraline) and four antipsychotics (chlorpromazine, clonazepam, haloperidol, and levomepromazine), together with polymyxin B, against 20 tested Gram-negative bacteria that displayed various resistance mechanisms, including the carbapenemases. From these results, it was clear that only sertraline, chlorpromazine, and levomepromazine had a synergistic effect with polymyxin B against the *A. baumannii*, *E. coli*, and *K. pneumoniae* isolates. Among all of the non-antibiotics, only spironolactone, which had a good efficacy against the *E. coli* isolates, showed nontoxic levels of a minimum concentration for synergy with polymyxin B [102]. Furthermore, Cheng et al. identified three pairs of two-drug combinations that showed synergistic effects with two known antibiotics against the *A. baumannii* strain AB5075, including azithromycin/5-fluorouracil, CS/fluspirilene, and CS/Bay 11-7082 [103]. In this section, the study has focused on the existing alternatives to colistin use in animals. However, in the past two decades, many types of research have been focused on the development of alternatives to antibiotics in agricultural animals, such as probiotics, prebiotics, enzymes, peptides, phytochemicals, and heavy metals, such as copper and zinc. Vaccines, bacteriophages, and many other alternatives in Table-4 summarize the most important types [104-114], the possible times of application when used in the most important animals. Many of these alternatives have already been applied as an alternative to colistin, and the other options still need to be studied for the possibility of applying them as alternatives to colistin, or other antibiotics in the field.

**Conclusion**

Colistin resistance is a critical issue to deal with nowadays. Many studies have proved this resistance in several bacterial species and in different countries around the world. The *mcr* gene was identified as the responsible gene for unique colistin resistance because it is able to transmit horizontally from one bacterium to another and between animals, humans, and the environment. Most of the resistant bacteria were also featured as being MDR. In addition, the *mcr* variant genes were reported by many studies, and some of them showed resistance to colistin, while the others were susceptible. However, the scientific society has taken a response to reduce the negative effects of this resistance by applying some rules, such as forbidding the use of colistin, except for exceptional cases, and in the applied “One Health Approach.” Moreover, some researchers have launched a novel alternative to colistin, by the development of a new antibiotic, with better effects and with more tolerance than colistin, using antibiotic combinations with different antibiotics, or even with non-antibiotic particles. Overall, the antibiotic use of colistin must be reduced by establishing limits for its use. The current researchers hope that this review will help other researchers in building a better understanding of the colistin profile in different parts of the globe, such as in the emergence of its resistance and the proper actions to deal with this resistant problem. In addition, this study encourages them to work on papers about different detection methods for the colistin-resistant gene and titling the *mcr* genes in a well-set system.

**Authors’ Contributions**

MHG: Gave the idea and prepared the outlines. MHG and SQS: Designed the tables. MHG and SQS: Contributed equally to the drafting of the manuscript. MHG: Reviewed the final draft. Both authors have read and approved the final version.

**Acknowledgments**

The authors of the manuscript thank and acknowledge the Deanship of Research at Jordan University of Science and Technology for providing facilities and publication fee.

**Competing Interests**

The authors declare that they have no competing interests.

**Publisher’s Note**

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

**References**

1. Poirel, L., Jayol, A. and Nordmann, P. (2017) Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin. Microbiol. Rev.*, 30(2): 557-596.
2. Gurjar, M. (2015) Colistin for lung infection: An update. *J. Intensive Care*, 3(1): 3.
3. Rhouma, M., Beaudry, F. and Letellier, A. (2016) Resistance to colistin: What is the fate for this antibiotic in pig production? *Int. J. Antimicrob. Agents*, 48(2): 119-126.
4. Olaitan, A.O. and Li, J. (2016) Emergence of polymyxin resistance in Gram-negative bacteria. *Int. J. Antimicrob. Agents*, 48(6): 581.
5. QY Research Medical Research Centre. The Global Polymyxin Industry Report 2015. Available from: http://www.qyresearch.com. Retrieved on 20-10-2015.
6. Irrgang, A., Roschanski, N., Tenhagen, B.A., Grobbel, M., Skladnikiewicz-Ziemer, T., Thomas, K. and Kaesbohrer, A. (2016) Prevalence of *mcr*-1 in *E. coli* from livestock and food in Germany, 2010-2015. *PLoS One*, 11(7): e0159863.
7. Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J. and Yu, L.F. (2016) Emergence of plasmid-mediated colistin resistance mechanism *mcr*-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect. Dis.*, 16(2): 161-168.
8. Meinersmann, R.J., Ladely, S.R., Plumbee, J.R., Cook, K.L. and Thacker, E. (2017) Prevalence of *mcr*-1 in the cecal contents of food animals in the United States. *Antimicrob. Agents Chemother.*, 61(2): e02344-16.
9. Huang, X., Yu, L., Chen, X., Zhi, C., Yao, X., Liu, Y. and Liu, J.H. (2017) High prevalence of colistin resistance and *mcr*-1 gene in *Escherichia coli* isolated from food animals in China. *Front. Microbiol.*, 8(4): 562.
10. Barbieri, N.L., Nielsen, D.W., Wannemuehler, Y., Cavender, T., Hussein, A., Yan, S.G. and Logue, C.M.
(2017) Mcr-1 identified in avian pathogenic Escherichia coli (APEC). PLoS One, 12(3): e0172997.

11. Gao, R., Hu, Y., Li, Z., Sun, J., Wang, Q., Lin, J. and Zhu, B. (2016) Dissemination and mechanism for the MCR-1 colistin resistance. PLoS Pathog., 12(11): e1005957.

12. Khalifa, H.O., Ahmed, A.M., Orehby, A.F., Eid, A.M., Shimamoto, T. and Shimamoto, T. (2016) Characterisation of the plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli isolated from animals in Egypt. Int. J. Antimicrob. Agents, 47(5): 413.

13. Arcilla, M.S., van Hattem, J.M., Matamoros, S., Melles, D.C., Penders, J., de Jong, M.D. and Schultzz, C. (2016) Dissemination of the mcr-1 colistin resistance gene. Lancet Infect. Dis., 16(2): 147-149.

14. Quezas, A., Ugarte-Ruiz, M., Iglesias, M.R., Porroro, M.C., Martínez, R., Flores-Cuadra, D. and Domínguez-L, (2016) Detection of plasmid mediated colistin resistance (MCR-1) in Escherichia coli and Salmonella enterica isolated from poultry and swine in Spain. Res. Vet. Sci., 105(1): 134-135.

15. Hernández, M., Iglesias, M.R., Rodríguez-Lázaro, D., Gallardo,A.,Quijada,N.M.,Miguela-Villoldo,P,andSáez,L. (2017) Co-occurrence of colistin-resistance genes mcr-1 and mcr-3 among multidrug-resistant Escherichia coli isolated from cattle, Spain, September 2015. Eurosurveillance, 22(31): 30586.

16. Haenni, M., Poirel, L., Kieffer, N., Châtère, P., Saraz, E., Métayer, V. and Madec, J.Y. (2016) Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids. Lancet Infect. Dis., 16(3): 281-282.

17. Zhang, X., Zhang, B., Guo, Y., Wang, J., Zhao, P., Liu, J. and He, K. (2019) Colistin resistance prevalence in Escherichia coli from domestic animals in intensive breeding farms of Jiangsu province. Int. J. Food Microbiol., 291(16): 87-90.

18. Alba, P., Leekitcharoephon, P., Franco, A., Feltrin, F., Ianzano, A., Caprioli, A. and Battisti, A. (2018) Molecular epidemiology of mcr-encoded colistin resistance in Enterobacteriaceae from food-producing animals in Italy revealed through the EU harmonized antimicrobial resistance monitoring. Front. Microbiol., 9(12): 1217.

19. Garcia, V., Garcia-Menifo, I., Mora, A., Flament-Simon, S.C., Diaz-Imenez, D., Blanco, J.E. and Blanco, J. (2018) Co-occurrence of mcr-1, mcr-4 and mcr-5 genes in multidrug-resistant ST10 enterotoxogenic and shiga toxin-producing Escherichia coli in Spain (2006-2017). Int. J. Antimicrob. Agents, 52(1): 104-108.

20. Hinchcliffe, P., Yang, Q.E., Portal, E., Young, T., Li, H., Tookoe, C.L. and Tansawai, U. (2017) Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1. Sci. Rep., 7(6): 39392.

21. Yang, Y.Q., Li, Y.X., Lei, C.W., Zhang, A.Y. and Wang, H.N. (2018) Novel plasmid-mediated colistin resistance gene mcr-7.1 in Klebsiella pneumoniae. J. Antimicrob. Chemother., 73(7): 1791-1795.

22. Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S. and Wang, Y. (2018) Emergence of a novel mobile colistin resistance gene mcr-9 in multidrug-resistant Proteus mirabilis. Emerg. Microbes Infect., 7(12): 1.

23. Carroll, L.M., Gaballa, A., Guldemann, C., Sullivan, G., Henderson, L.O. and Wiedmann, M. (2019) Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible Salmonella enterica serotype typhimurium isolate. mBio, 10(3): e00853-e00919.

24. Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z. and Wang, Y. (2017) Novel plasmid-mediated colistin resistance gene mcr-8 in Salmonella enterica serotype Typhimurium. J. Antimicrob. Chemother., 83(3): e00543-e00617.

25. Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A. and Magiistrati, C.F. (2017) Novel plasmid-mediated colistin resistance gene mcr-4 in Salmonella and Enterobacteriaceae. Veterinary World, 22(31): 30589.
bacteria in Germany. *Lancet Infect. Dis.*, 16(3): 282-283.

41. Hmede, Z., & Kassem, I. I. (2018). The colistin resistance gene, *mcr-1*, is Prevalent in Commensal *E. coli* Isolated from Lebanese Pre-harvest Poultry. *Antimicrob. Agents Chemother.*, 62(11): e01304.

42. Garcia-Graells, C., De Keersmaecker, S.C., Vanneste, K., Pochet, B., Vermeersch, K., Roosens, N. and Botteldoorn, N. (2018) Detection of plasmid-mediated colistin resistance, *mcr-1* and *mcr-2* genes, in *Salmonella* spp. isolated from food at retail in Belgium from 2012 to 2015. *Foodborne Pathog. Dis.*, 15(2): 114-117.

43. Quan, J., Li, X., Chen, Y., Jiang, Y., Zhou, Z., Zhang, H. and Yu, Y. (2017) Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: A multicentre longitudinal study. *Lancet Infect. Dis.*, 17(4): 400-410.

44. Trung, N.V., Matamoros, S., Carrique-Mas, J.J., Nghia, N.O., Abdfarag, E.A., Al-Romaihi, H., Wehedy, E., Hmede, Z., & Kassem, I. I. (2018). The colistin resistance gene *mcr-1* in *E. coli* isolated from pigs in Great Britain from 2013 to 2015. *Microb. Drug Resist.*, 25(2): 233-240.

45. Guang, Y., Chen, L., Ma, L., Zhang, X., Wang, J., Li, X., Sun, J., Ding, Y., Li, X., and Liu, J. (2018) Detection of plasmid-mediated colistin resistance, *mcr-1* genes, in *Salmonella* spp. isolated from poultry in China. *Microbiome*, 5(1): 70.

46. Antunes, P., Campos, J., Mourão, J., Pereira, J., Novais, C. and Peixe, L. (2018) Cropwater is a major source of colistin-resistant strains carrying *mcr-1* gene from pigs in the same animal. *Int. J. Antimicrob. Agents*, 51(5): 674-675.

47. Nhung, N.T., Chieu, T.T.B. and Hardon, A. (2017) Zoonotic infection with *Salmonella* from Vietnamese food-producing animals in South Korea. *Int. J. Infect. Dis.*, 66(1): 100-106.

48. Li, X.P., Fang, L.X., Song, J.Q., Xia, J., Huo, W., Fang, J.T., Zhang, H. and Peixe, L. (2018) Inflow water is a major source of *Salmonella* and *E. coli* resistance genes, in *Salmonella* isolates from domestic animals and humans in a household, carry colistin resistance gene *mcr-1* in Ecuador. *bioRxiv*, 10(1): 350587.

49. Monte, D.F., Fernandes, M.R., Ceredeia, L., Esposito, F., Galvão, J.A., Franco, B.D. and Landgraf, M. (2017) Chicken meat as a reservoir of colistin-resistant *Escherichia coli* strains carrying *mcr-1* genes in South America. *Antimicrob. Agents Chemother.*, 61(5): e02718-e02816.

50. Wang, Q., Sun, J., Li, J., Ding, Y., Li, X.P., Lin, J. and Peng, Y. (2017) Expanding landscapes of the diversified *mcr-1*-bearing plasmid reservoirs. *Microbiome*, 5(1): 70.

51. Dugnett, N.A., Sayers, E., AbuOun, M., Ellis, R.J., Nunez-Garcia, J., Randall, L. and Brenna, C. (2017) Occurrence and characterization of *mcr-1*-harbouring *Escherichia coli* isolated from pigs in Great Britain from 2013 to 2015. *J. Antimicrob. Chemother.*, 72(3): 691-695.

52. Zhang, X.F., Doi, Y., Huang, X., Li, H.Y., Zhong, L.L., Zeng, K.J. and Tian, G.B. (2016) Possible transmission of *mcr-1*-harbouring *Escherichia coli* between companion animals and human. *Emerg. Infect. Dis.*, 22(9): 1679.

53. Dandachi, I., Fayad, E., El-Bazzal, B., Daoud, Z. and Rolain, J.M. (2018) Prevalence of extended-spectrum beta-lactamase-producing gram-negative bacilli and emergence of *mcr-1* colistin resistance gene in Lebanese swine farms. *Microb. Drug Resist.*, 25(2): 233-240.

54. Eltai, N.A.O., Abdalrahim, H., Wehedy, E., Mahmoud, M.H., Alawad, O.K. and Yassine, H.M. (2017) Antibiotic resistance profile of commensal *Escherichia coli* isolated from broiler chickens in Qatar. *J. Food Prot.*, 81(2): 302-307.

55. Sonnevend, A., Ghazawi, A., Alqahtani, M., Shibli, A., Jamal, W., Hashmei, R. and Pal, T. (2016) Plasmid-mediated colistin resistance in *Escherichia coli* from the Arabian Peninsula. *Int. J. Infect. Dis.*, 50(1): 85-90.

56. Litup, E., Kil, K., Hammerum, A.M., Roer, L., Nielsen, E.M. and Torpdahl, M. (2017) Plasmid-borne colistin resistance gene *mcr-3* in *Salmonella* isolates from human infections, Denmark, 2009-17. *Euro. Surveill.*, 22(31): 30587.
from hospital sewage carrying the mcr-1 colistin resistance gene. Antimicrob. Agents Chemother., 60(12): 7498-7501.

73. Sennati, S., Di Pilato, V., Riccobono, E., Di Maggio, T., Villagran, A.L., Pallecchi, L. and Giani, T. (2017) Citrobacter braakii carrying plasmid-borne mcr-1 colistin resistance gene from ready-to-eat food from a market in the Chaco region of Bolivia. J. Antimicrob. Chemother., 72(7): 2127-2129.

74. Li, X.P., Xiong, L.X., Jiang, P., Sun, J. and Liu, X.P. (2017) Emergence of the colistin resistance gene mcr-1 in Citrobacter freundii. Int. J. Antimicrob. Agents, 49(6): 786.

75. Ma, F., Chen, C., Zheng, X., Liu, Y., Chen, H., Zhong, L. and Yang, Y. (2019) Identification of a novel plasmid carrying mcr-4.3 in an Acinetobacter baumannii strain in China. Antimicrob. Agents Chemother., 63(6): e00135-e00219.

76. Lei, T., Zhang, J., Jiang, F., He, M., Zeng, H., Chen, M. and Wu, Q. (2019) First detection of the plasmid-mediated colistin resistance gene mcr-1 in virulent Vibrio parahaemolyticus. Int. J. Food Microbiol., 308(2): 108290.

77. Beceiro, A., Moreno, A., Fernández, N., Vallejo, J.A., Aranda, J., Adler, B. and Bou, G. (2014) Biological cost of different mechanisms of colistin resistance and their impact on virulence in Acinetobacter baumannii. Antimicrob. Agents Chemother., 58(1): 518-526.

78. Harbarth, S., Balkhy, H.H., Goossens, H., Jarlier, V., Kluytmans, J., Laxminarayan, R. and Pittet, D. (2015) Antimicrobial resistance: One world, one fight! Antimicrob. Resist. Infect. Control., 4(1): 49.

79. Litzbauer, M. (2012) Grid integration of electric vehicles considering the mobility needs. World Electr. Veh. J., 5(3): 629-634.

80. World Health Organization. (2017) Critically Important Antimicrobials for Human Medicine: Ranking of Antimicrobial Agents for Risk Management of Antimicrobial Resistance Due to Non-human Use. World Health Organization, Geneva.

81. National Department of Health. (2014). Antimicrobial Resistance National Strategy Framework, 2014-2024. National Department of Health, South Africa.

82. Mendelson, M. and Matsoso, M.P. (2015) The South African antimicrobial resistance strategy framework. AMR Control, 5(1): 54-61.

83. South African National Department of Health. Guidelines on Implementation of the Antimicrobial Strategy in South Africa. One Health Approach and Governance. Available from: http://www.health.gov.za/index.php/antimicrobial-resistance?download=2194:antimicrobial-stewardship-guidelines-governance-june2017. Retrieved on 04-10-2017.

84. Kaplan, D. (2014) Encyclopedia of Food and Agricultural Ethics. Vol. 16171624. Springer, Dordrecht.

85. European Medicines Agency. Updated Advice on the use of Colistin Products in Animals within the European Union: Development of Resistance and Possible Impact on Human and animal Health. Draft. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guide-line_2016/05/WC500207233.pdf. Retrieved on 10-11-2017.

86. Robinson, T.F., Bu, D.P., Carrique-Mas, J., Fève, E.M., Gilbert, M.L., Gilbride, D. and Laxminarayan, R. (2016) Antibiotic resistance is the quintessential one health issue. Trans. Royal Soc. Trop. Med. Hyg., 110(7): 377-380.

87. Lepelletier, D., Bonnet, R., Pléiat, P., Nicolas-Chanoine, M.H., Berger-Carbonne, A., Chidiac, C. and Grandbastien, B. (2018) Emergence of plasmid-mediated colistin resistance (mcr-1) among Enterobacteriaceae strains: Laboratory detection of resistance and measures to control its dissemination. Med. Microbiol., 48(4): 250-255.

88. Ardouin, S.M., Quiroga, M.P., Ramírez, M.S., Merker, A.K., Errecalde, L., Di Martino, A. and Centron, D. (2012) Transposons and integrons in colistin-resistant clones of Klebsiella pneumoniae and Acinetobacter baumannii with epidemic or sporadic behaviour. J. Med. Microbiol., 61(10): 1417-1420.

89. Hayakawa, K., Marchaim, D., Divine, G.W., Pogue, J.M., Kumar, S., Lephart, P. and Kaye, K.S. (2012) Growing prevalence of Providencia stuartii associated with the increased usage of colistin at a tertiary health care center. Int. J. Infect. Dis., 16(9): e646-e648.

90. Lin, Y.W., Zhou, Q.T., Han, M.L., Onufrak, N.J., Chen, K., Wang, J. and Li, J. (2018) Mechanism-based pharmacokinetic/pharmacodynamic modeling of aerosolized colistin in a mouse lung infection model. Antimicrob. Agents Chemother., 62(3): e01965-e02017.

91. Thakur, S. and Gray, G.C. (2019) The mandate for a global “one health” approach to antimicrobial resistance surveillance. Am. J. Trop. Med. Hyg., 100(2): 227.

92. Torpdahl, M., Hasman, H., Litrup, E., Skov, R.L., Nielsen, E.M. and Hammerum, A.M. (2017) Detection of mcr-1 encoding plasmid-mediated colistin-resistant Salmonella isolates from human infection in Denmark. Int. J. Antimicrob. Agents, 2(49): 261-262.

93. Teo, J.W., Chew, K.L. and Lin, R.T. (2016) Transmissible colistin resistance encoded by mcr-1 detected in clinical Enterobacteriaceae isolates in Singapore. Emerg. Microb. Infect., 5(1): 1-12.

94. Vaara, M. (2018) New polymyxin derivatives that display improved efficacy in animal infection models as compared to polymyxin B and colistin. Med. Res. Rev., 38(5): 1661-1673.

95. MacNair, C.R., Stokes, J.M., Carfæe, L.A., Fiebig-Comyn, A.A., Coombes, B.K., Mulve, M.R. and Brown, E.D. (2018) Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. Nat. Commun., 9(1): 458.

96. Hu, Y., Liu, Y. and Coates, A. (2019) Azithromycin produces synergistic activity in combination with colistin against antibiotic-resistant Enterobacteriaceae. Antimicrob. Agents Chemother., 63(1): e01630-e01718.

97. Jeon, J., Park, J.H. and Yong, D. (2019) Efficacy of bacteriophage treatment against carbapenem-resistant Acinetobacter baumannii in Galleria mellonella larvae and a mouse model of acute pneumonia. BMC Microbiol., 19(1): 70.

98. Manohar, P., Lundborg, C.S., Tamhankar, A.J. and Nachimuthu, R. (2019) Therapeutic characterization and efficacy of bacteriophage cocktails infecting Escherichia coli, Klebsiella pneumoniae and Enterobacter species. Front. Microbiol., 10(3): 574.

99. El-Faham, A.I., Ali, N.G. and Abdelaziz, M.A.M. (2018) Assessment of some feed additives as anti-biotic alternatives, in relation to carcass characteristics and economic traits of broiler chickens. Egypt. Poult. Sci. J., 38(2): 709-723.

100. Gerstmans, H., Rodriguez-Rubio, L., Lavigne, R. and Briers, Y. (2016) From endolysins to Artilysin® s: Novel enzyme-based approaches to kill drug-resistant bacteria. Biochem. Soc. Trans., 44(1): 123-128.

101. Schirmeier, E., Zimmermann, P., Hofmann, V., Beibl, M., Gerstmans, H., Maervoet, V.E. and Briers, Y. (2018) Inhibitory and bactericidal effect of Artilysin® Arti-175 against colistin-resistant mcr-1-positive Escherichia coli isolates. Int. J. Antimicrob. Agents, 51(3): 528-529.

102. Otto, R.G., van Gorp, E., Kloezen, W., Meletiadis, J., van den Berg, S. and Mouton, J.W. (2019) An alternative strategy for combination therapy: Interactions between polymyxin B and non-antibiotics. Int. J. Antimicrob. Agents, 53(1): 34-39.

103. Cheng, Y.S., Sun, W., Xu, M., Shen, M., Khrawesh, M., Sciotti, R.J. and Zheng, W. (2018) Repurposing screen identifies unconventional drugs with activity against multi-drug resistant Acinetobacter baumannii. Front. Cell. Infect. Microbiol., 8(1): 438.

104. Alternatives to Antibiotics in Animal Agriculture. The Pew Charitable Trusts. Available from: https://www.pewtrusts.org/en/research-and-analysis/reports/2017/07/
alternatives-to-antibiotics-in-animal-agriculture. Retrieved on 30-07-2019.

105. Thacker, P.A. (2013) Alternatives to antibiotics as growth promoters for use in swine production: A review. *J. Anim. Sci. Biotechnol.*, 4(1): 35.

106. FAO/WHO. (2001) Probiotics in Food Health and Nutritional Properties and Guidelines for Evaluation FAO Food and Nutrition Paper, FAO/WHO. Available from: http://www.fao.org/3/a-a0512e.pdf. Retrieved on 06-08-2019.

107. Pineiro, M., Asp, N.G., Reid, G., Macfarlane, S., Morelli, L., Brunser, O. and Tuohy, K. (2008) FAO Technical meeting on prebiotics. *J. Clin. Gastroenterol.*, 42(3): S156-S159.

108. Izadpanah, A. and Gallo, R.L. (2005) Antimicrobial peptides. *J. Am. Acad. Dermatol.*, 52(3): 381-390.

109. Dibner, J.J. and Buttin, P. (2002) Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poult. Res.*, 11(4): 453-463.

110. Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. (2008) Use of phytoecogenic products as feed additives for swine and poultry. *J. Anim. Sci.*, 86(Suppl 14): E140-E148.

111. Wales, A. and Davies, R. (2015) Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics*, 4(4): 567-604.

112. Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.P. and Jungersen, G. (2007) Current status of veterinary vaccines. *Clin. Microbiol. Rev.*, 20(3): 489-510.

113. Joerger, R.D. (2003) Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. *Poult. Sci.*, 82(4): 640-647.

114. Kadouri, D.E., To, K., Shanks, R.M. and Doi, Y. (2013) Predatory bacteria: A potential ally against multidrug-resistant Gram-negative pathogens. *PLoS One*, 8(5): e63397.

**********