AUCTORES
Globalize your Research

International Journal of Clinical Case Reports and Reviews
Nashida Ahmed *

Open Access

Review Article

Vegetables and Fruit as a Reservoir of Mobilized Colistin Resistance (MCR) Positive Gram-Negative Bacteria

Nashida Ahmed

Department of Public Health, North South University, Bashundhara, Dhaka, Bangladesh and School of public Health Nanjing medical university Nanjing 211166, China.

***Corresponding Author:** Nashida Ahmed, Department of Public Health, North South University, Bashundhara, Dhaka, Bangladesh and School of public Health Nanjing medical university Nanjing 211166, China.

Received Date: December 19, 2024 | **Accepted Date:** December 26, 2024 | **Published Date:** January 15, 2025

Citation: Nashida Ahmed, (2025), Vegetables and Fruit as a Reservoir of Mobilized colistin resistance (mcr) positive Gram-negative bacteria, *International Journal of Clinical Case Reports and Reviews*, 22(3); **DOI:10.31579/2690-4861/659**

Abstract:

It has been widely acknowledged that "antimicrobial resistance" which makes antibiotic treatment of bacterial disease ineffective, is one of the most urgent health risks that the world is currently facing. Food chain is one of the major pathways of spread of this antibiotic resistance which is a great public health challenge. Colistin, an orally active polypeptide antibiotic has excellent activity against various Gram-negative bacteria and last-resort therapy used against infections caused by these bacteria. In November 2015, a novel plasmid-mediated colistin resistance gene, mcr-1, was identified in E. coli isolates. So far, the mcr gene has been identified in gram negative bacteria, most of them are E. coli that are isolated in food animals, environments, different types of fruits and vegetables and humans. The aim of this review is to provide a brief summary of the currently available scientific literatures on the fruits and vegetables as a carrier of mcr genes in gram negative bacteria with an emphasis on its Geographical distribution and Molecular identification.

Key words: antimicrobial resistance; antibiotic treatment; plasmid-mediated; mcr-1; E. coli isolates

1. Introduction

Fruits and vegetables add a variety in our diets which is also a great source of nutrition. Monotonous diet is unhealthy both for human and planet because it can end up with loss of eco diversity. Our diet should include at least 400 g of fruits and vegetables per day for proper wellbeing and freedom from disease recommended by World Health Organization (WHO). In 2017, around 3.9 million deaths troll were attributed worldwide for not eating enough fruit and vegetables (WHO, 2019). Data suggest that diet containing inadequate fruits and vegetables can leads to mortality around 14 percent from gastro-intestinal cancer, about 11 percent from ischemic heart disease, and about 9 percent from stroke [1]. Considering this theory, consumption of fresh produce has become a part of healthy diets and its popularity also increasing to global population [2] Sometime fruits and vegetable consumed raw as a form of salad which can leads to increasing number of foodborne disease outbreaks if proper hygiene is not maintained. [3]. Moreover, microbial contamination in organic food could also act as a storage of antibiotic resistance genes which could leads to its transmission to human body, after its consumption. This could lead a great public health threat [2-4]. In vegetation harvesting antibiotic could also be used as chemical tools for prevention of growth of microrganismo [5]. Use of night soil on crop fields could be another source of antibiotic resistance bacteria [6]. It is also assumed that crops may contaminated, through several routes, including dirty irrigation water or inappropriate post cultivate hygienic practices of the worker related to transportation, storage method and environment, processing and preparation[7,8].

Upon ingestion of antibiotic-resistant bacteria through contaminated food, it can exchange the resistant gene with normal human or animal gut flora while passing through the intestine. It could be a significant public health emergency which can leads to an additional dispersion of antibiotic-resistant bacteria in to the atmosphere [2]. In this way fecal waste can come in contact with the raw food stuff during cultivation in the form of wastewater irrigation and the use of organic fertilizer and a cycle of bacterial pathogens contamination could continue throughout its production to supply[9,10].When researcher studied this contamination pathway for common pathogens, it was not been assessed the contamination pathway of fresh produce with antibiotic resistant bacteria and their suspectable genes. Indeed, such information is beneficial for humanity which can reduce human exposure to ARB/ ARG through fruits and vegetables [11]. It is documented that diarrheagenic E. coli strains in cucumber, lettuce and spinach can cause diarrhoea and other gastronomic

diseases [12]. Other study also identified fruits associated with enterotoxigenic E. coli positive for heat-stable enterotoxin-1 gene astA, a causative agent for diarrheal infections [13].

The emergence of carbapenem- and colistin-resistant Gram-negative Bacteria (GNB) is of great public health concern. These bacterial have been reported vastly among aquatic biome, anthropoid environment and food commodities all over the world [14-16], but we still know a very little about fresh fruits and vegetables as a carrier of carbapenem and colistin resistance genes in Gram-negative bacteria [17]. We have some evidence of drug resistance in GNB isolates on vegetables indicating most of these bacteria are environmental species with a few faecal Enterobacterales species [2,18].

Colistin antibiotic is one of the last resorts in treating infections caused by multi-drug-resistant Gram-negative bacteria in humans. Drug resistance in bacteria could be chromosomal or plasmid mediated which can develop when a drug is being overly used. Colistin recognized as last resort among different classes of antibiotics. Researcher found that colistin resistance in Enterobacteriaceae could be plasmid-borne mobile colistin resistance genes(mcr). In the recent past, the plasmid transferred resistance to colistin was attributed to the mcr gene. Mcr-1 is the most predominant among all the mcr type [19]. So far 10 mcr homologs have been identified [20]In Pigs and chickens in China where mcr-1 was first identified. 21[21]Mcr gene most commonly found in ecoli bacteria [22,23]The other mcr variants have been reported in a limited number of species[24] In Klebsiella, mcr-8 and mcr-9 have been reported. The mcr-8 gene was initially identified in the K. pneumoniae plasmid pKP91 of swine origin [25] and mcr-9 was detected in silico from a clinical colistin susceptible Salmonella enterica serotype Typhimurium strain in 2010 in United States[26]The expression of mcr-9 normally requires exposure to colistin and a qseBC system next to mcr-9 for expression induction [27]. Lastly the mcr variant mcr-10 was identified, which was originally detected in the clinical Enterobacter roggenkampii strain 090065 in China [20].

Mcr gene-producing Gram-negative bacteria isolated from fresh vegetables and fruit also have been reported in several countries around the world [19]. Therefore, in this review we tried to understand the prevailing situation on dissemination of mcr gene-producing Gram-negative bacteria and their genetic characteristics and from fresh vegetables and fruits all around the world.

What is Antimicrobial Resistance?

Microbes constitutes of 70 % of total biomass of the earth's biosphere and they play a vital role in the sustainability of the environment. Microbial cells have the natural ability to adapt themselves upon harmful compounds since the very begging on the planet [28], Microbial genetic transfer to the next generation ensures the transfer of acquired characteristics which is pioneer to the evolution of stable genetic determinants. This gene transfer characteristics in microorganism also the leading cause for microbial diversity [29]. When bacteria can grow and adapt with the environment in the presence of antibiotic that signify its resistance against that particular antibiotic [30]. Their survival ensures their adaptability and their adaptability ensure an emerging threat to the living world. [31,32]. By gene mutation this resistance in bacteria can develop naturally [33]. However, commercialization of antibiotics in medicine, agriculture, food industries are expediting this process. Antimicrobial-resistant microbes are present in living environment (in water, soil, and air) [34-36]. It can spread through environment to animal, animal to person or person to person and again person or animal to environment.

Over 7 million mortality per year caused by antimicrobial resistance alone, and this mortality can be around 10 million by the year 2050, accounting for about 100 trillion USD worldwide [32,37]. It is estimated that around 2 million population in USA infected with bacteria per year that are resistant to first line antimicrobial drugs, accounting 20 billion US dollars. Similar scenario has also been seen in European Union where approximate 900,000 disability-adjusted lives and more than 30,000 death was identified each year [38]. This similar situation of antibiotic resistance is also affecting the BRIC countries, i.e., Brazil, Russia, India, and China[38], [39] During the period 2010–2015, the consumption of antibiotics (DDD/1000 individuals/ day) increased in China (89 %), Tunisia (69%) and India (13 %) respectively [40]. Sometimes it become difficult to estimate the true burden of bacterial resistance in some specific geography due to scarce data and surveillance that makes AMR tackling difficult. Researchers usually estimate the AMR effects by evaluating mortality and morbidity rate, hospital stay length and health-care costs for selected pathogen-drug combination in specific geographical region[3,41-45]. Worldwide low- and middle-income countries are more vulnerable to AMR burden because food safety and security are not maintained in these regions and lacking in access to health and sanitary water supply[46] Most of the developing countries in the world still lacks in quality health care facilities with poor sanitary conditions and a higher incidence of communicable and non-communicable diseases. Unrestricted use of antibiotics is the principal cause of dissemination of antibacterial resistance in these region [36,47,48]. The local temperature of a specific geographical area also influences the abundance and distribution of Antibacterial resistance gene in the environment. It was found in a study in USA that an increase in population density and local temperature was consistent with an increase number of pathogens like E. coli, klebsiella pneumoniae and staphylococcus aureus [40,49]. World Health Organization (WHO) has a expressed the urgency of taking action against antimicrobial resistance by stating, “no action today, no cure tomorrow” and "post-antibiotic era" may soon become reality of the 21st century [38]. WHO has also proposed a global action plan to combat antimicrobial resistance [50,51]. Microbial community developed a progressive resistance to commonly used antibiotics at present leading to condition to reduction in beneficial treatment for infectious diseases [52,53].

Antimicrobial resistance: a global crisis

AMR is a widespread challenge for public health that initially derives from antibiotics and antibiotic resistance genes, but it can also be linked to improper local sanitation, environmental pollution and other factors [54]. Though antibiotics are used primarily for control of infection both human and animal, sometime it used as growth promoter in poultry and livestock. Also, non metabosied or residual antibiotic can be released by human and animal excreta into the environment [55]. These factors contribute to genetic selection pressure for the emergence of multidrug-resistant bacterial infections in the community. Salmonella spp. and Campylobacter spp. are the major pathogens in this category. In addition, difference in resistance mechanisms in bacterial isolate from human and animal are still not very clear. The study on mechanism of resistant microbes should be broad ranged. It should not only include the evolution of resistance at the molecular level within a given organism but also its

transmission mechanisms and pathways between organisms, and dissemination between humans and animal hosts including soil and water.

Approximately 80% of marketed antibiotics in the USA are used either as growth supplements or to control animal infections. The global map of antibiotic use estimated the use of 63 151 tonnes of antibiotics in livestock in 2010 [56]. This situation would pose a worldwide economic burden of approximately US$120 trillion (US$3 trillion per annum), which is roughly equal to the total current annual healthcare budget of the USA. However Medically important antimicrobials are banned in USA but bacitracin and carbadox, which are classified as medically important by the World Health Organization, are still used as growth promoters in pigs [57]. Recent trends of antibiotic usage will lead to the death of approximately 444 million people before 2100, and birth rates will also rapidly decline by 2050 [58].

Regions	Mortality
Asia	4,730,000
Africa	4,150,000
Europe	390,000
Latin America	392,000
North America	317,000
Oceania	22,000

Table 1: Mortality Rates by 2050 Due to AMR in Different Regions[40]

Source: https://amr-review.org/sites/default/files/AMR Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf. Accessed September 17, 2019. Creative Commons Attribution 4.0 International Public License (https://creativecommons.org/licenses/by/4.0/legalcode) [37].

Introduction to Colistin:

Colistin antibiotics belong the polymyxin family and produced by *Paenibacillus polymyxa var. colistinus* which is bactericidal with significant concentration work against gram-negative bacteria, discovered in1940s [59,60]. The polymyxin family contains polymyxins E, D, C, B, and A. but only colistin (polymyxin E and polymyxin B) have been clinically used in treatment of both the veterinarian and human. Colistin antibiotics consist of a mix of the polymyxin E1 and E2 (containing bactericidal penta-cationic lipo-peptides). In humans, this antibiotic is mostly used to treat diseases caused by the pan or multidrug-resistant microorganisms [61]. Colistin is usually given by parenteral route as an inactive prodrug form namely sodium salt of colistin methanesulfonate [62].

Mode of action of colistin.

Colistin is a narrow spectrum antibacterial drug against gram-negative bacteria [63]. The mode of colistin action has not been completely discovered yet, we only know the colistin binds to the lipopolysaccharides (LPS) and phosphorlipids of the outer membrane of Gram-negative bacteria and causes disruption of the cell membrane followed by cell death. Gram-positive bacteria, all cocci, or anaerobes lack the outer membrane so colistin has no activity against them though it is an active antibiotic against a broad diversity of Gram-negative bacteria [64]. Lipid A, of the cell membrane of the bacteria plays a significant role in the cell permeability action and Colistin specifically targets lipid A. This takes place via an electrostatic interaction between positively charged diaminobutyric acid residues of colistin and negatively charged phosphate groups of lipid A [65]. Because of this interaction Colistin displaces divalent cations (Ca2þ and Mg2þ), causing an alteration in the LPS structure which forms destabilized areas, through which colistin crosses the outer membrane and eventually damages the structure of the phospholipid bilayer of the inner membrane of the bacterial cell. This whole process leads to inner membrane lysis and cell death [66].

Mechanisms underlying colistin resistance

Chromosomal point mutation thought to be the only cause of acquired bacterial colistin resistance by the researcher earlier. Nonresistance bacteria can also develop acquired colistin resistance, which could be due to changing in LPS structure or capsular polysaccharides receptor that act as a binding site for colistin antibiotic and this could be happened by transformation of bacterial cell surface [67]. But Plasmid mediated mcr genes of some aquatic, zoonotic and human environmental bacteria could also cause resistance to colistin according to the recent studies [68]. When mcr-1 gene reside together with other resistant genes for example MBL, ESBL or NDM gene in bacteria, it can result as colistin resistance and as some life threatening infectious disease treatment could become difficult for this resistance it could be a great challenge to public health and health care provider as colistin is one of the last line of antibiotics [62].

There are two main mechanism works behind how gram-negative bacteria develop colistin resistance. Number one is inactivation of lipid-A biosynthesis pathway and alteration in outer membrane LPS. Number two mechanism is pmrAB two component system[26]Dissemination of antibiotic resistance in gram negative bacteria usually take place through integrons, through which exogenous gene expression and active capture take place. Bacteria tend to grow in challenging conditions by these genetic elements which causes increase in strength by achieving more genetic material from other species. Thus, by natural transformation integrons increases bacterial distribution and resistance through metamorphosis [26,69].

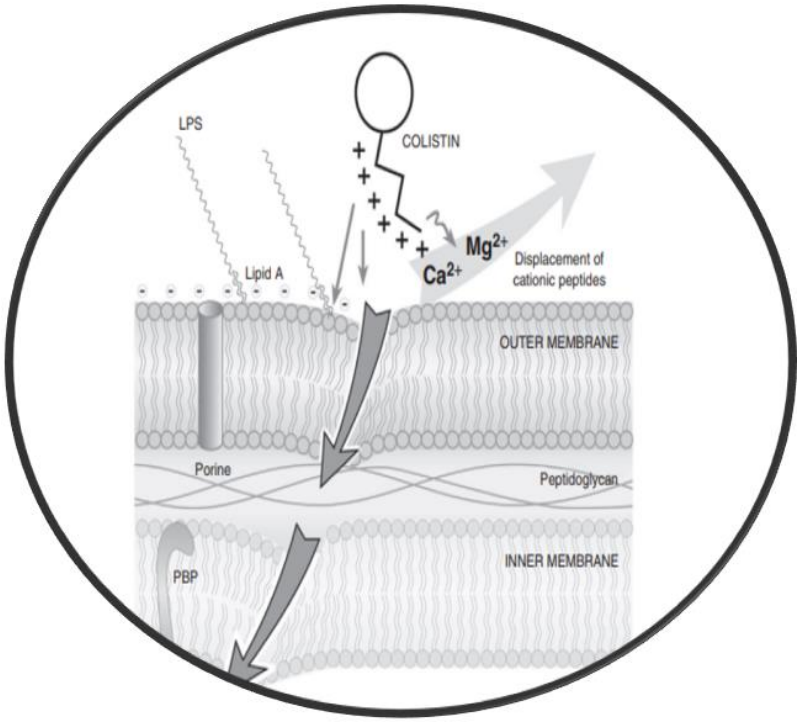

Figure 1. Action of colistin on bacterial membrane. The cationic cyclic decapeptide structure of colistin binds with the anionic LPS molecules by displacing calcium and magnesium from the outer cell membrane of Gram-negative bacteria, leading to permeability changes in the cell envelope and leakage of cell contents. By binding to the lipid, A portion of LPS, colistin also has an anti-endotoxin activity. Disruption of the membranes should promote permeability for more conventional anti-pseudomonals. LPS: lipopolysaccharides; PBP: penicillin-binding protein. (From Martis et al.) [70]

Colistin resistance in Enterobacteriaceae:

The expression of the L-Ara4N and PEtn transferase is regulated by the two-component regulatory system PmrA/PmrB, in E. coli and Salmonella enterica by sensing environmental properties, for example PH, Fe3+ and Mg2+ level. Alteration of expression of a set of genes involved in modification of lipid A caused by the presence of polymyxins.[71], [72]. The mechanisms of resistance to cationic antimicrobial peptides of Salmonella typhimurium regulates through two-component systems PhoP–PhoQ and PmrA–PmrB [73]. The PmrA–PmrB regulon encode the Polymyxin resistance and its products modify the LPS core and lipid A regions with ethanolamine and add amino arabinose to the 4 phosphate of lipid A [74]. Mutations in the pmrA locus of S. typhimurium confer an increase in resistance to polymyxins. As pmrA mutants survive better in human neutrophils, it suggests that this locus plays a role in virulence [75]. Polymyxin-resistant mutants of S. typhimurium and E. coli have a higher substitution of the ester-linked phosphate group in the lipid A portion of the LPS by 4-amino-4-deoxy-L-arabinose and show larger amounts of 2-aminoethanol esterifying phosphates in the core oligosaccharide [76] The 4-aminoarabinose substitution is almost stoichiometric in strains of Proteus mirabilis, Chromobacterium violaceum, and Burkholderia cepacian that exhibit innate resistance to polymyxins [76]. It has been suggested that the mgrB alteration can be a common mechanism of colistin resistance in KPC-producing K. pneumoniae (KPC-KP) in the clinical setting [77]. Comparative genomic analysis of a pair of sequential KPC-KP isolates from the same patient including a colistin-susceptible isolate (KKBO-1) and a colistin-resistant isolate (KKBO-4) selected after colistin exposure revealed that insertional inactivation of the mgrB gene, encoding a negative regulator of the PhoQ/PhoP signaling system, which is a genetic mechanism for acquired colistin resistance [78]. A recent study confirmed the MgrB regulatory role in K. pneumoniae and was in agreement with the known association between upregulation of the PhoQ/PhoP system and activation of the pmr HFIJKLM operon, which eventually leads to resistance to polymyxins by modification of the lipopolysaccharide target [79].

Mobilized colistin resistance (mcr)

Total, nine mobilized colistin resistance genes referred to as mcr-1 to mcr-9 was identified up to 2019 [26] and the mcr-10 gene was identified in 2020. All ten mcr genes are mostly identical to each other [20]. The mcr genes encode phosphoethanolamine transferase enzymes that bind a phosphoethanolamine (PEtN) moiety to the lipid A of Gram-negative bacteria's outer membrane, which causes reduction to its net negative charge allowing colistin resistance. mcr-1 gene is the most predominant type of mcr genes and its transfer of colistin resistance by plasmid has been ascribed. mcr-1 is a phosphatidyl-ethanolamine transferase; it works by lowering the binding affinity of colistin to its target site by modulating the lipid A residues of the LPS [80]. The mcr-1 gene modify the target of colistin and then stimulating transmission of PEA into glucosamine saccharide of the lipid A in the outer membrane of bacteria and that's how it provides resistance [21], by lowering in the net-negative charge of the lipid A head group and lower binding affinity to colistin. The mcr-1 catalytic mechanism (other PEA-transferase enzymes of bacteria) still not fully understood [81]. The catalytic domain of the enzyme mcr-1 resembles the zinc metalloproteins. The phosphoethanolamine is inhibited by metal chelating agents like the dipicolinic acid (DA) and ethylene diamine tetra-acetic acid (EDTA) [82,83] as shown in Fig. 2a and b [84].The natural occurrence of mcr-1 has been limited to five

species of bacteria. K. pneumonia, E. coli, Salmonella enterica, E. cloacae, and E. aerogenes and experimentally being transmitted to P. aeruginosa by conjugation. The host reservoirs that have possibility of carrying the mcr1-harbouring enterobacteria, could be a variety of livestock/poultry (dogs, cattle, chickens and pigs) or it could be human. Alarmingly, coexistence with other multi-drug resistance genes, have been observed in mcr-1 gene that increases the possibilities pan-drug resistance et al. reported the presence of mcr-1 gene in different parts of the world [85].

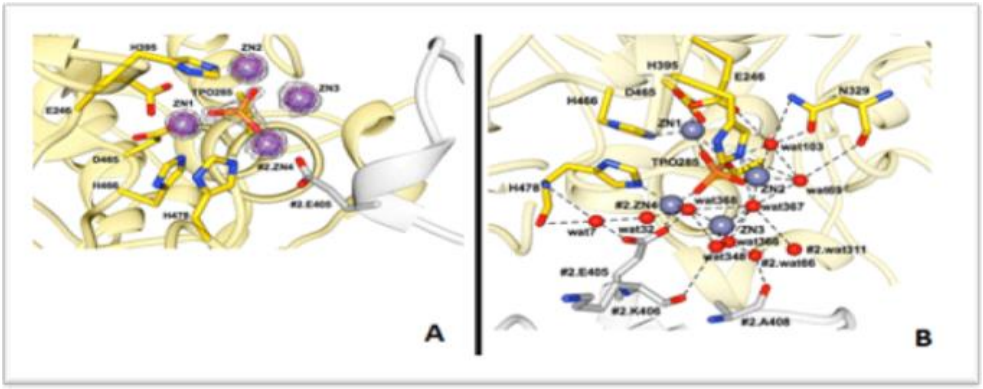

Figure 2: The catalytic domain structure of the mcr-1 enzyme[84] A. the structure of the active-site of phosphothreonine with associated zinc ions. B. Represent ions of zinc which specified in the mcr-1 active site. Dissemination and spread of mcr-1 gene.

Geographical distribution of mcr-1 and Emergence of Colistin-Resistant Escherichia coli in Food Chains:

Plasmid-borne resistance to polymyxins has been reported for few different enterobacterial species mainly among E. coli isolates and rarely for Salmonella enterica, Enterobacter spp., and K. pneumoniae. mcr-1-producing isolates in other species, such as Cronobacter sakazakii [86] and Kluyvera ascorbate [87] also had been reported. According to current literature, the distribution of mcr-1 is worldwide, covering all continents [88]. The mcr-1-producing E. coli isolates have been identified in several animals and animal food products, including chickens and chicken meat, pigs and piglets, cattle, calves, and turkeys, so it is speculated that the original source of the gene, or at least of its mobilization and emergence, might be the animal world but it also been found in human. The corresponding samples were collected from different parts of the world, not only in Asian countries (Cambodia, China, Japan, Laos, Malaysia, Taiwan, Singapore, and Vietnam) but also from Europe (Belgium, Denmark, France, Germany, Portugal, Italy, the Netherlands, Spain, Sweden, Switzerland, and the UK), the Americas (Argentina, Brazil, and Canada), and Africa (Algeria, Egypt, South Africa, and Tunisia). The emergence of mcr-positive isolates animals, is not a recent event. A retrospective Chinese study identified positive isolates recovered from chickens during the 1980s [89], and they were discovered as early as 2005 in veal calves in France [90]. So, it could be possible there has been some silent dissemination of that resistance mechanism throughout the last few decades, and the current situation shows an ongoing further dissemination rather than an emerging phenomenon.

It has been demonstrated clearly AMR E. coli transmission via food animals and food products [91]. But because of lack of sufficient data, it is not possible to make a complete risk assessment on the role of food in the transfer of the human infections caused by E. coli mcr-1 [14]. It is a great challenge for both food operators (farmers, livestock transporters, slaughterhouse workers, food handlers, etc.) and consumers the presence of E. coli mcr-1 in bred animals and foods. Different studies have documented dissemination of E. coli carrying the mcr-1 gene in healthy food producing animals in several countries [92,93]. But still we don't have enough studies on the prevalence of this organism in food and moreover certain food products, for example, the milk sector has been poorly investigated, and the prevalence of E. coli mcr-1 in fishery products is totally absent [14].

Literature Search Strategy and Data Collection

The dissemination of mcr-producing Gram-negative bacteria in fresh produce is a major public health threat, since they are a very suitable pathway for the spread of antibiotic-resistant bacteria from farm to fork. Until December 2024, out of thirteen (13) molecular studies, ten (10) studies have revealed the isolation of Gram-negative bacteria producing mcr genes on fresh vegetables and fruits. They have been used and are accessible through the PubMed database using the "mcr", "fresh vegetables", "vegetables" and "fruits".

Vegetables and Fruit Isolates with the mcr Gene

The transferable plasmid-mediated colistin resistant mcr-1 gene carrying bacteria are of great public health concern.[94-96]. Up to now, thirteen studies have reported mcr-producing Gram-negative bacteria, especially isolates of Enterobacteriaceae species, from fresh produce that mostly originated from China (Table 2).

The mcr-1 gene was first reported in 2014 in Switzerland,60 ESBL-producing members of Enterobacteriaceae isolated from 42 imported vegetable samples (11 from the Dominican Republic, 13 from India, 11 from Thailand, and 8 from Vietnam) were screened by PCR for the presence of the mcr-1 gene and the mcr-1 gene was detected in 2 out of 60 vegetable strains (products from Thailand and Vietnam)[97]and sequencing of the amplicons showed a 100% identity with the published mcr-1 sequence. The colistin resistance was transferable by transformation experiments into Escherichia coli DH5-alpha. All strains were Escherichia coli and belonged to different multilocus sequence types (MLSTs), harbored different blaESBL genes, and showed a multi resistance phenotype [97]. The diversity of ESBL genes and MLSTs identified among mcr-1-positive isolates suggests that the mcr-1 gene might be carried on different plasmids. This study also showed that international trade of fresh vegetable could be a possible route of transmission of colistin-resistant Enterobacteriaceae [98]. In 2015 a study

reported PMCR-encoding gene mcr-1 detected in an E. coli isolated from a lettuce sample in Portugal and evaluation its genetic relation with the other reported mcr-1-producing E. coli isolated from fresh produce was done. Colistin MIC of the vegetable E. coli isolate revealed a non-wild-type phenotype to colistin. This isolate was also resistant to other antibiotic classes, such as penicillin, quinolones, aminoglycosides, and phenicol, consistent with a multidrug resistant phenotype. The transferability of the mcr-1 gene was achieved, with the transconjugant TcINSali25 (mcr-1) exhibiting the respective resistance to colistin [99]. Another study in Guangzhou, China was held from June to November 2016. A total of 133 fruit surface samples were collected from retail fruits market. Mcr-1-harboring E. coli GB110 and K. pneumoniae GB015 were identified from apple and orange samples, respectively. It was found that E. coli GB110 was susceptible to all tested agents except colistin and polymyxin B; and K. pneumoniae GB015 was resistant to colistin, polymyxin B and ampicillin [100]. Mcr-1 was successfully transferred to streptomycin-resistant E. coli C600 through conjugation in both isolates, suggesting that mcr-1 was located on transferable plasmids and mcr-1 was located on ~62.9 kb IncFIA and ~204.2 kb IncHI1 plasmids for the transconjugants of E. coli GB110 and K. pneumoniae GB015, respectively. MLST analysis of E. coli GB110 showed that it belonged to sequence type 189 (165 cplx), which was reported from poultry retail meat mediating the spread of extended-spectrum β-lactamase (ESBL) genes in Colombia [101] while K. pneumoniae GB015 belonged to ST442. ST442 and ST11, the most common carbapenem-resistant clones in China, are considered to be the progenitors of K. pneumoniae ST258, which is widespread worldwide as the most successful multidrug-resistant clone of K. pneumoniae [102]. Another study published in 2019 was conducted a surveillance of the prevalence of mcr in fresh vegetables from 23 cities of 9 provinces in China and analyzed the characteristics of mcr bearing plasmids. A total of 528 fresh vegetable belonging to 18 types were analyzed between May 2017 to April 2018. Among them 19 fresh vegetable samples belonging to 10 types carried mcr-1 gene. From the 19 samples, 24 mcr -1 positive isolates were retrieved and 23 were identified as *E. coli* and isolate CTX145B was *Enterobacter cloacae* [103]. MLST subtyping identified 16 types along with a new ST type not previously registered in the *ecoli* MLST database, and most prevalent sequence types were ST744 and ST224.All 24-isolate showed multi drug resistance, but were susceptible to meropenem and tigecycline[103]. Another study between May 2017 and December 2018., were identified two isolates from leaf rape and spinach, carried both blaNDM-5/9 and mcr-1 recovered from fresh vegetables in China and the characteristics of resistance plasmids were also analyzed. In total, 712 fresh vegetable samples were collected, from 29 cities or districts of 10 provinces in China. sequence analysis showed that both isolates were E. coli.[104]. Both isolates showed resistance to all beta-lactams, tetracyclines, fluoroquinolones, fosfomycin and colistin tested, which were therapeutic agents in clinics in many countries[105].Notably, both isolates remained susceptible to amikacin and tigecycline, similar to the E. coli isolates producing both NDM and mcr-1 from humans in China. MLST analysis showed that isolates 690 and 701 belonged to ST156 and ST2847, respectively [106].

Another study was performed to investigate the prevalence and antimicrobial susceptibility of mcr harboring colistin-resistant Enterobacteriaceae from retail vegetables and food animals in South Korea during a national surveillance project in 2018. For this study, 1324 fresh vegetables from across the country were obtained from farmers' markets. The mcr-1 gene was detected in Escherichia coli isolates from (1/1324) of vegetables, (2/34) of chickens, (4/59) of pigs, and (0/57) of cattle. Other mcr genes were not detected. All seven of the mcr-1- positive isolates showed multidrug resistance and co-produced β-lactamases. Multilocus sequence typing analysis revealed five known E. coli sequence types (STs), including ST10 in the vegetable sample This is the first report of the mcr-1 gene in vegetable samples in South Korea [107]. In 2019 one study was conducted to screen for extended spectrum cephalosporin-, carbapenem- and colistin-resistant Gram-negative bacteria in fresh vegetables in Algeria. Out of 400 vegetable samples, the mcr-1 gene was detected in two isolates from two coriander samples (2.99%; n = 2). However, no other mcr gene was identified. The PCR results showed that the transconjugant TCL17 harbored the blaOXA-48 gene, and TCL38 carried the mcr-1-encoding gene. Multi-locus sequence typing analysis revealed three E. coli sequence types, including ST2298 in OXA-48-producing isolate, ST216 and the epidemic clone ST101 in mcr-1-positive strains[108]. In June 2019, in Hangzhou of Zhejiang Province, China another study revealed carbapenem-resistant P. mirabilis in five of the eight vegetable samples[109]The five P. mirabilis isolates were from one tomato, two lettuce and two cucumber samples. All P. mirabilis isolates carried blaNDM, while blaNDM-5 was found in all E. coli isolates. Worryingly, except M15061H, the remaining four E. coli isolates co-harbored mcr-1 and blaNDM-5. The four E. coli isolates carrying both mcr-1 and blaNDM were also resistant to colistin to fluoroquinolones. MLST analysis showed that all four E. coli isolates harboring both mcr-1 and blaNDM-5 from three types of vegetables in two markets belonged to the ST6050 type. The mcr-1 in the four ST6050 E. coli isolates could not be transferred into the recipient E. coli C600, although the conjugation experiment was performed three times. [109] Two studies published in Malaysia in July 2020 and June 2023. The study published on July 2020, was conducted to determine the occurrence of colistin-resistant E. coli raw chicken meat and bean sprouts. Results showed that of the E. coli isolated from raw chicken meat were positive for the colistin resistance encoding gene, mcr-1, whereas all the E. coli isolates from bean sprouts were negative for colistin resistance encoding genes [110], and second study which published on June 2023, was conducted to determine the prevalence, antibiotic susceptibility profile and phylogroups of colistin resistant E. coli isolated from poultry farm, chicken meat and vegetables samples from markets in Kelantan, Malaysia. Isolates harbored multiple mcr-genes (mcr-1, mcr-3, mcr-6, mcr-8 and mcr-9) from chicken origin only, while no mcr was detected in vegetables [111]. Another study on detection of chromosomal and plasmid-mediated mechanisms of colistin resistance in Escherichia coli and Klebsiella pneumoniae from Indian food sample was carried out on raw food samples, including poultry meat, fish, mutton meat, fruit and vegetables. In this study, food samples were collected from 22 sources (14 shops and 8 households) in Chennai, a major metropolitan city in India, in the period October–November 2017. From the 51 positive samples, 71 bacterial isolates were identified, including 11 E. coli, 29 Klebsiella spp., 17 Enterobacter spp., 2 Citrobacter spp. and 12 Pseudomonas spp. Sixteen samples had more than one Col-R isolates. Of the 71 isolates screened, 3 E. coli (one mutton and two poultry meat samples) were found to harbour mcr-1 gene. No mcr gene was detected in vegetables and fruits samples [112]. Another study showed NTS carrying the mcr-1 gene was isolated from lettuce, beef and pork products in various foods at a frequency of 1.07% (3/280) in 2017, and from goose eggs and field snails at a frequency of 0.69% (4/579) in 2018 (data not published) [113]. The other study was carried out in Molina, Chile. Four sampling efforts were performed between May 2019

and January 2020. A total of 478 vegetable samples were collected. Colistin resistance gene mcr-1 and ESBL coding genes found in isolates obtained from vegetables and water. The gene mcr-1 was found in 2/155 isolates; both isolates corresponded to E. coli that were isolated during the summer season, from two distinct beet samples from the same market [114]. One study in central Arkansas in the United States between September-December 2020 carried out on 88 vegetable samples. In this study the combination of microbiology, genomics and metagenomics approaches to assess antibiotic resistance in retail vegetables in the United States. ESBL-producing *E. hormaechei* and *S. fonticola*, and AmpC-producing *E. hormaechei* were isolated from retail vegetables. Multidrug-resistant ESBL-producing *E. hormaechei* carrying *mcr-9* resistance gene was isolated for the first time in vegetables [115].

Considering that colistin is a last-resource antibiotic used for the treatment of infections caused by multidrug resistant bacteria, the detection of a mobile colistin resistance gene in a raw vegetable and fruits constitutes a serious and unprecedented public health concern.

Vegetable/fruit type	mcr gene	Isolation period	Species	Isolation Number	Country	Other antibiotic resistance Genes	Sequence type	Plasmid type	Reference
1.Coriander	mcr-1	between March and December 2019	E. coli	1	Algeria	ND	ST216 and ST101	ND	[108]
2.a. Apple	mcr-1	from June to November 2016	E. coli	1	China	aadA2, aadA1, floR, cmlA1, sul2, sul3, tetA, tetM, dfrA12, mdfA	ST189	ncHI1, IncFIA	[100]
2.b. Orange	mcr-1		K. pneumoniae	1		blaSHV-110, mcr-1, qnrS1, oqxA, oqxB, fosA6, sul1, tetA, dfrA1	ST442	IncHI1, IncFIB	
3.Lettuce	mcr-1	2018	E. coli	1	South Korea	TEM-1, CTX-M-55	ST10	ND	[106]
4a. leaf rape	mcr-1	May 2017 and Dec 2018	E. coli	1	China	blaNDM-5, fosA3	ST156	IncX4	[104]
4b. Spinach	mcr-1		E. coli	1		blaNDM-9, fosA3	ST2847	IncI2	
5.Lettuce	mcr-1	2015	E. coli	1	Portugal	aadA1, aac (3)-Iv, aph(4)-Ia, aph(6)-Ia, aph(6)-Id, blaTEM-1B, mcr-1.1, sul2, tet(A), floR-type	ST1716	IncHI2/ST4	[99]
6.a. Tomato	mcr-1	June 2019	E. coli	1	China	blaNDM-5	ST6050	IncHI2	[109]
6.b. Cucumber				1					
6.c. Cucumber				1					
6.d. Lettuce				1					
7a. Cha-om	mcr-1	2014	E. coli	1	Switzerland	blaCTX-M-55	ST167	ND	[97]
7.b. Basil Leaves	mcr-1	2014	E. coli	1		blaCTX-M-65	ST4683	ND	
8.a. Cucumber	mcr-1	between May 2017 and April 2018	E. coli	1	China	ND	ST744	X4	[103]
8.b. Curly endive	mcr-1		E. coli	1		ND	ST13	X4	
8.c. Pak choi	mcr-1		E. coli	1		ND	ST648	I2	
8.d. Tomato	mcr-1		E. coli	1		ND	ST713	X4	
8.e. Leaf rape	mcr-1		E. coli	1		ND	ST744	X4	

8.f. Carrot	mcr-1		E. coli	1		ND	ST5539	X4	
8.g. Romaine lettuce	mcr-1		E. coli	1		ND	ST10	X4	
8.h. Green Pepper	mcr-1		E. cloacae	1		ND	ST5873	X4	
8.i. Cucumber	mcr-1		E. coli	1		ND	ST744	X4	
8.j. Cucumber	mcr-1		E. coli	1		ND	ST1115	I2	
8.k. Cucumber	mcr-1		E. coli	1		ND	ST744	I2	
8.l. Carrot	mcr-1		E. coli	1		ND	ST13	X4	
8.m. Tomato	mcr-1		E. coli	1		ND	STUT	I2	
8.n. Spinach	mcr-1		E. coli	1		ND	ST2253	I2	
8.o. Romaine lettuce	mcr-1		E. coli	1		ND	ST2705	HI2	
8.p. Leaf lettuce	mcr-1		E. coli	1		ND	ST6397	I2	
8.q. Pak choi	mcr-1		E. coli	1		ND	ST1196	I2	
9.Beet	mcr-1	2019-2020	E. coli	2	Chile	blaTEM, blaCTX-M		ND	[114]
10.a. Bean sprouts	mcr-9	2020	Enterobacter hormaechei		US				
10.b. Organic parsley	mcr-9					blaSHV66	S11-1, S17-1, S45-4	ND	[115]
10.c. Organic baby spinach	mcr-9								

Table 2. Mcr genes reported in Gram-negative bacteria isolates from vegetables and fruit worldwide.

Conclusions:

This review provides an understanding on fresh vegetables and fruits could be a major vehicle of transmission of multidrug resistant Gram-negative bacteria. Colistin has been widely used in animal production industry to enhance productivity and control diseases & these in turn led to the emergence of colistin resistance. This drug was almost stopped use to treat the human infection due to its potential side effects. But due to multidrug-resistant Gram-negative bacteria and the lack of potent new antibiotics to treat infections caused by these pathogens the use of colistin emerged as the drug of choice in clinical use for human. Fresh fruits and vegetables might have been underestimated as a vehicle of Gram-negative bacteria in spreading colistin resistant mcr-1 gene. Sustained surveillance of resistance in foodborne pathogens in the food chain, especially fresh vegetables and fruits, is urgent for preventing the transmission of mcr-producing Enterobacteriaceae to ensure the health of food consumers. Further investigations are required for monitoring such organisms in fresh vegetables and fruits to ensure food safety in all over the world and emphasizes the necessity of paying close attention to these products as a future public health issue.

References:

1. A. Afshin et al., (2017). Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study, *The Lancet*, vol. 393, no. 10184, pp. 1958–1972, May 2019
2. A. H. A. M. van Hoek, C. Veenman, W. M. van Overbeek, G. Lynch, A. M. de Roda Husman, (2015). Prevalence and characterization of ESBL- and AmpC-producing Enterobacteriaceae on retail vegetables, *Int J Food Microbiol*, vol. 204, pp. 1–8, jul.
3. P. Xylia, G. Botsaris, A. Chrysargyris, P. Skandamis, and N. Tzortzakis, (2019). Variation of microbial load and biochemical activity of ready-to-eat salads in Cyprus as affected by vegetable type, season, and producer, *Food Microbiol*, vol. 83, pp. 200–210, oct.
4. B.-T. Liu and F.-J. Song, (2019). Emergence of two Escherichia coli strains co-harboring mcr-1 and blaNDM in fresh vegetables from China , *Infect Drug Resist*, vol. Volume 12, pp. 2627–2635, Aug.
5. V. O. STOCKWELL and B. DUFFY, (2012). Use of antibiotics in plant agriculture, Revue Scientifique et Technique de l'OIE, vol. 31, no. 1, pp. 199–210, Apr.
6. M. Zalewska, A. Błażejewska, A. Czapko, and M. Popowska, (2021). Antibiotics and Antibiotic Resistance Genes in Animal Manure – Consequences of Its Application in Agriculture, *Front Microbiol*, vol. 12, Mar.
7. C. S. Hölzel, J. L. Tetens, and K. Schwaiger, (2018). Unraveling the Role of Vegetables in Spreading Antimicrobial-Resistant Bacteria: A Need for Quantitative Risk Assessment, *Foodborne Pathog Dis*, vol. 15, no. 11, pp. 671–688, nov.
8. C. N. Berger et al., (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens, *Environ Microbiol*, vol. 12, no. 9, pp. 2385–2397, Sep.
9. J. C. Chee-Sanford et al., (2009). Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genes following Land Application of Manure Waste, *J Environ Qual*, vol. 38, no. 3, pp. 1086–1108, May.

10. Y. He et al., (2020). Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment, *NPJ Clean Water*, vol. 3, no. 1, p. 4, Feb.
11. Y. Jung, H. Jang, and K. R. Matthews, (2014). Effect of the food production chain from farm practices to vegetable processing on outbreak incidence, *Microb Biotechnol*, vol. 7, no. 6, pp. 517–527, Nov.
12. L. M. Carroll, A. Gaballa, C. Guldimann, G. Sullivan, L. O. Henderson, (2019). Identification of Novel Mobilized Colistin Resistance Gene mcr-9 in a Multidrug-Resistant, Colistin-Susceptible Salmonella enterica Serotype Typhimurium Isolate, *mBio*, vol. 10, no. 3, jun.
13. R. Gao et al., (2016). Dissemination and Mechanism for the MCR-1 Colistin Resistance, *PLoS Pathog*, vol. 12, no. 11, p. e1005957, Nov.
14. I. Caniaux, A. van Belkum, G. Zambardi, L. Poirel, and M. F. Gros, (2017). MCR: modern colistin resistance, *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 36, no. 3, pp. 415–420, Mar.
15. B.-T. Liu, X.-Y. Zhang, S.-W. Wan, J.-J. Hao, R.-D. Jiang, (2018). Characteristics of Carbapenem-Resistant Enterobacteriaceae in Ready-to-Eat Vegetables in China, *Front Microbiol*, vol. 9, jun.
16. M. U. Anyanwu, I. F. Jaja, and O. C. Nwobi, (2020). Occurrence and Characteristics of Mobile Colistin Resistance (mcr) Gene-Containing Isolates from the Environment: A Review, *Int J Environ Res Public Health*, vol. 17, no. 3, p. 1028, Feb.
17. W. Chelaghma, L. Loucif, M. Bendahou, and J.-M. Rolain, (2021). Vegetables and Fruit as a Reservoir of β-Lactam and Colistin-Resistant Gram-Negative Bacteria: A Review., *Microorganisms*, vol. 9, no. 12, Dec.
18. J. Luo et al., (2017). Emergence of mcr-1 in Raoultella ornithinolytica and Escherichia coli Isolates from Retail Vegetables in China, *Antimicrob Agents Chemother*, vol. 61, no. 10, oct.
19. A. Touati et al., (2017). First detection of Klebsiella pneumoniae producing OXA-48 in fresh vegetables from Béjaïa city, *Algeria*, *J Glob Antimicrob Resist*, vol. 9, pp. 17–18, jun.
20. C. Wang, Y. Feng, L. Liu, L. Wei, M. Kang, (2020). Identification of novel mobile colistin resistance gene mcr-10, *Emerg Microbes Infect*, vol. 9, no. 1, pp. 508–516, Jan.
21. Y.-Y. Liu et al., (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*, vol. 16, no. 2, pp. 161–168, Feb.
22. Y. Shen et al., (2018). Anthropogenic and environmental factors associated with high incidence of mcr-1 carriage in humans across China, *Nat Microbiol*, vol. 3, no. 9, pp. 1054–1062, Jul.
23. C. M. Sia et al., (2020). The characterization of mobile colistin resistance (mcr) genes among 33 000 Salmonella enterica genomes from routine public health surveillance in England, *Microb Genom*, vol. 6, no. 2, Feb.
24. N. Phetburom et al., (2021). Klebsiella pneumoniae Complex Harboring mcr-1, mcr-7, and mcr-8 Isolates from Slaughtered Pigs in Thailand, *Microorganisms*, vol. 9, no. 12, p. 2436, Nov.
25. B. Wu et al., (2020). Heterogeneity and Diversity of mcr-8 Genetic Context in Chicken-Associated Klebsiella pneumoniae, *Antimicrob Agents Chemother*, vol. 65, no. 1, Dec.
26. L. M. Carroll, A. Gaballa, C. Guldimann, G. Sullivan, L. O. Henderson, and M. Wiedmann, (2019). Identification of Novel Mobilized Colistin Resistance Gene mcr-9 in a Multidrug-Resistant, Colistin-Susceptible Salmonella enterica Serotype Typhimurium Isolate, *mBio*, vol. 10, no. 3, jun.
27. N. Macesic et al., (2021). Silent spread of mobile colistin resistance gene mcr-9.1 on IncHI2 'superplasmids' in clinical carbapenem-resistant Enterobacterales, *Clinical Microbiology and Infection*, vol. 27, no. 12, pp. 1856.e7-1856.e13, Dec.
28. F. Lebreton, A. L. Manson, J. T. Saavedra, T. J. Straub, A. M. Earl, and M. S. Gilmore, (2017). Tracing the Enterococci from Paleozoic Origins to the Hospital, *Cell*, vol. 169, no. 5, pp. 849-861.e13, May.
29. J. Srivastava, H. Chandra, N. Singh, and S. J. S. Kalra, (2016). Understanding the Development of Environmental Resistance Among Microbes: A Review, Clean (Weinh), vol. 44, no. 7, pp. 901–908, Jul.
30. M. Boolchandani, A. W. D'Souza, and G. Dantas, (2019). Sequencing-based methods and resources to study antimicrobial resistance, *Nat Rev Genet*, Mar.
31. E. M. Wellington et al., (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria, *Lancet Infect Dis*, vol. 13, no. 2, pp. 155–165, Feb.
32. U. Hofer, (2019). The cost of antimicrobial resistance, *Nat Rev Microbiol*, vol. 17, no. 1, pp. 3–3, Jan.
33. R. Chait, K. Vetsigian, and R. Kishony, (2012). What counters antibiotic resistance in nature? *Nat Chem Biol*, vol. 8, no. 1, pp. 2–5, Jan.
34. W. Cheng, H. Chen, C. Su, and S. Yan, (2013). Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China, *Environ Int*, vol. 61, pp. 1–7, Nov.
35. M. Cycoń, A. Mrozik, and Z. Piotrowska-Seget, (2019). Antibiotics in the Soil Environment—Degradation and Their Impact on Microbial Activity and Diversity, *Front Microbiol*, vol. 10, Mar.
36. K. B. Pouwels, A. Chatterjee, B. S. Cooper, and J. V Robotham, (2019). Antibiotic resistance, stewardship, and consumption, *Lancet Planet Health*, vol. 3, no. 2, p. e66, Feb.
37. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations the Review on Antimicrobial Resistance Chaired by Jim O'Neill, 2016.
38. S. Reardon, (2014). Antibiotic resistance sweeping developing world, *Nature*, vol. 509, no. 7499, pp. 141–142, May.
39. R. C. Founou, L. L. Founou, and S. Y. Essack, (2017). Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis, *PLoS One*, vol. 12, no. 12, p. e0189621, Dec.
40. A. S. Oberoi, Y. Jia, H. Zhang, S. K. Khanal, and H. Lu, (2019). Insights into the Fate and Removal of Antibiotics in Engineered Biological Treatment Systems: A Critical Review, Environ Sci Technol, vol. 53, no. 13, pp. 7234–7264, Jul.

41. N. R. Naylor et al., (2018). Estimating the burden of antimicrobial resistance: a systematic literature review, *Antimicrob Resist Infect Control*, vol. 7, no. 1, p. 58, Apr.
42. A. Cassini et al., (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis, *Lancet Infect Dis*, vol. 19, no. 1, pp. 56–66, Jan.
43. C. Lim et al., (2016). Epidemiology and burden of multidrug-resistant bacterial infection in a developing country, *Elife*, vol. 5, Sep.
44. E. Temkin, N. Fallach, J. Almagor, B. P. Gladstone, E. Tacconelli, and Y. Carmeli, (2018). Estimating the number of infections caused by antibiotic-resistant Escherichia coli and Klebsiella pneumoniae in 2014: a modelling study, *Lancet Glob Health*, vol. 6, no. 9, pp. e969–e979, Sep.
45. R. Laxminarayan et al., (2013). Antibiotic resistance—the need for global solutions, *Lancet Infect Dis*, vol. 13, no. 12, pp. 1057–1098, Dec.
46. H. Waseem et al., (2019). Assessment of knowledge and attitude trends towards antimicrobial resistance (AMR) among the community members, pharmacists/pharmacy owners and physicians in district Sialkot, Pakistan, *Antimicrob Resist Infect Control*, vol. 8, no. 1, p. 67, Dec.
47. S. Yadav and A. Kapley, (2019). Exploration of activated sludge resistome using metagenomics, *Science of The Total Environment*, vol. 692, pp. 1155–1164, Nov.
48. J. M. A. Blair, (2018). A climate for antibiotic resistance, *Nat Clim Chang*, vol. 8, no. 6, pp. 460–461, jun.
49. D. MacFadden, S. McGough, D. Fisman, M. Santillana, and J. Brownstein, (2017). Antibiotic Resistance Increases with Local Temperature, *Open Forum Infect Dis*, vol. 4, no. suppl_1, pp. S179–S179.
50. Organization WH. Global Action Plan on Antimicrobial Resistance. 2015. Geneva, Switzerland: 2015.
51. S. Hernando-Amado, T. M. Coque, F. Baquero, and J. L. Martínez, (2019). Defining and combating antibiotic resistance from One Health and Global Health perspectives, *Nat Microbiol*, vol. 4, no. 9, pp. 1432–1442, Aug.
52. T. U. Berendonk et al., (2015). Tackling antibiotic resistance: the environmental framework, *Nat Rev Microbiol*, vol. 13, no. 5, pp. 310–317, May.
53. T. S. Crofts, A. J. Gasparrini, and G. Dantas, (2017). Next-generation approaches to understand and combat the antibiotic resistome, *Nat Rev Microbiol*, vol. 15, no. 7, pp. 422–434, Jul.
54. G. S. Tillotson and S. H. Zinner, (2017). Burden of antimicrobial resistance in an era of decreasing susceptibility, *Expert Rev Anti Infect Ther*, vol. 15, no. 7, pp. 663–676, Jul.
55. J. Davies and D. Davies, (2010). Origins and Evolution of Antibiotic Resistance, *Microbiology and Molecular Biology Reviews*, vol. 74, no. 3, pp. 417–433, Sep.
56. T. P. Van Boeckel et al., (2015). Global trends in antimicrobial use in food animals, *Proceedings of the National Academy of Sciences*, vol. 112, no. 18, pp. 5649–5654, May.
57. J. B. Arsand et al., (2020). Presence of antibiotic resistance genes and its association with antibiotic occurrence in Dilúvio River in southern Brazil, *Science of The Total Environment*, vol. 738, p. 139781, oct.
58. C. Lee Ventola, (2015). The Antibiotic Resistance Crisis Part 1: Causes and Threats.
59. M. E. Falagas, S. K. Kasiakou, and L. D. Saravolatz, (2005). Colistin: The Revival of Polymyxins for the Management of Multidrug-Resistant Gram-Negative Bacterial Infections, *Clinical Infectious Diseases*, vol. 40, no. 9, pp. 1333–1341, May.
60. D. R. Storm, K. S. Rosenthal, and P. E. Swanson, (1977). Polymyxin and Related Peptide Antibiotics, *Annu Rev Biochem*, vol. 46, no. 1, pp. 723–763, jun.
61. K. A. T. A. T. K. Koyama Y, (1950). A new antibiotic 'colistin' produced by spore-forming soil bacteria, *J Antibiot*;3:457–458.
62. D. R. Storm, K. S. Rosenthal, and P. E. Swanson, (1977). Polymyxin and Related Peptide Antibiotics, *Annu Rev Biochem*, vol. 46, no. 1, pp. 723–763, jun.
63. A.-P. Magiorakos et al., (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, *Clinical Microbiology and Infection*, vol. 18, no. 3, pp. 268–281, Mar.
64. M. E. Falagas, S. K. Kasiakou, and L. D. Saravolatz, Colistin: The Revival of Polymyxins for the Management of Multidrug-Resistant Gram-Negative Bacterial Infections, *Clinical Infectious Diseases*, vol. 40, no. 9, pp. 1333–1341, May 2005.
65. Z. Yu, W. Qin, J. Lin, S. Fang, and J. Qiu, (2015). Antibacterial Mechanisms of Polymyxin and Bacterial Resistance, *Biomed Res Int*, vol. 2015, pp. 1–11.
66. C. Wanty et al., (2013). The Structure of the Neisserial Lipooligosaccharide Phosphoethanolamine Transferase A (LptA) Required for Resistance to Polymyxin, *J Mol Biol*, vol. 425, no. 18, pp. 3389–3402, Sep.
67. T. Velkov, P. E. Thompson, R. L. Nation, and J. Li, (2010). Structure−Activity Relationships of Polymyxin Antibiotics, *J Med Chem*, vol. 53, no. 5, pp. 1898–1916, Mar.
68. F. F. Andrade, D. Silva, A. Rodrigues, and C. Pina-Vaz, (2020). Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges, *Microorganisms*, vol. 8, no. 11, p. 1716, Nov.
69. A. O. Olaitan, S. Morand, and J.-M. Rolain, (2014). Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria, *Front Microbiol*, vol. 5, Nov.
70. J. Osei Sekyere, (2019). Mcr colistin resistance gene: a systematic review of current diagnostics and detection methods, *Microbiologyopen*, vol. 8, no. 4, Apr.
71. N. Martis, S. Leroy, and V. Blanc, (2014). Colistin in multi-drug resistant Pseudomonas aeruginosa blood-stream infections, *Journal of Infection*, vol. 69, no. 1, pp. 1–12, Jul.
72. M. D. Adams et al., (2009). Resistance to Colistin in Acinetobacter baumannii Associated with Mutations in the Pmr AB Two-Component System, *Antimicrob Agents Chemother*, vol. 53, no. 9, pp. 3628–3634, Sep.
73. J. H. Moffatt et al., (2010). Colistin Resistance in Acinetobacter baumannii Is Mediated by Complete Loss of Lipopolysaccharide Production, *Antimicrob Agents Chemother*, vol. 54, no. 12, pp. 4971–4977, Dec.
74. J. S. Gunn et al., (1998). PmrA–PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and

polymyxin resistance, *Mol Microbiol*, vol. 27, no. 6, pp. 1171–1182, Mar.

75. K. L. Roland, L. E. Martin, C. R. Esther, and J. K. Spitznagel, (1993). Spontaneous pmrA mutants of Salmonella typhimurium LT2 define a new two-component regulatory system with a possible role in virulence, *J Bacteriol*, vol. 175, no. 13, pp. 4154–4164, Jul.
76. E. A. Groisman, J. Kayser, and F. C. Soncini, (1997). Regulation of polymyxin resistance and adaptation to low-Mg2+ environments, *J Bacteriol*, vol. 179, no. 22, pp. 7040–7045, Nov.
77. A. Cannatelli et al., (2014). MgrB Inactivation Is a Common Mechanism of Colistin Resistance in KPC-Producing Klebsiella pneumoniae of Clinical Origin, *Antimicrob Agents Chemother*, vol. 58, no. 10, pp. 5696–5703, oct.
78. A. Cannatelli et al., (2013). In Vivo Emergence of Colistin Resistance in Klebsiella pneumoniae Producing KPC-Type Carbapenemases Mediated by Insertional Inactivation of the PhoQ/PhoP mgrB Regulator, *Antimicrob Agents Chemother*, vol. 57, no. 11, pp. 5521–5526, Nov.
79. A. S. Bray et al., (2022). MgrB-Dependent Colistin Resistance in Klebsiella pneumoniae Is Associated with an Increase in Host-to-Host Transmission, *mBio*, vol. 13, no. 2, Apr.
80. M. Moosavian and N. Emam, (2019). The first report of emerging mobilized colistin-resistance (mcr) genes and ERIC-PCR typing in Escherichia coli and Klebsiella pneumoniae clinical isolates in southwest Iran , *Infect Drug Resist*, vol. Volume 12, pp. 1001–1010, Apr.
81. H. Nikaido, (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited, *Microbiology and Molecular Biology Reviews*, vol. 67, no. 4, pp. 593–656, Dec.
82. M. Coppi et al., (2018). A simple phenotypic method for screening of MCR-1-mediated colistin resistance, *Clinical Microbiology and Infection*, vol. 24, no. 2, pp. 201.e1-201.e3, Feb.
83. F. Esposito et al., (2017). Detection of Colistin-Resistant MCR-1-Positive Escherichia coli by Use of Assays Based on Inhibition by EDTA and Zeta Potential, *J Clin Microbiol*, vol. 55, no. 12, pp. 3454–3465, Dec.
84. V. Stojanoski, B. Sankaran, B. V. V. Prasad, L. Poirel, P. Nordmann, and T. Palzkill, (2016). Structure of the catalytic domain of the colistin resistance enzyme MCR-1, *BMC Biol*, vol. 14, no. 1, p. 81, Dec.
85. H. Ye et al., (2016). Diversified mcr-1 -Harbouring Plasmid Reservoirs Confer Resistance to Colistin in Human Gut Microbiota, *mBio*, vol. 7, no. 2, May.
86. B.-T. Liu, F.-J. Song, M. Zou, Z.-H. Hao, and H. Shan, (2017). Emergence of Colistin Resistance Gene mcr-1 in Cronobacter sakazakii Producing NDM-9 and in Escherichia coli from the Same Animal, *Antimicrob Agents Chemother*, vol. 61, no. 2, Feb.
87. F. Zhao and Z. Zong, (2016). Kluyvera ascorbata Strain from Hospital Sewage Carrying the mcr-1 Colistin Resistance Gene, *Antimicrob Agents Chemother*, vol. 60, no. 12, pp. 7498–7501, Dec.
88. R. L. Skov and D. L. Monnet, (2016). Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds, *Eurosurveillance*, vol. 21, no. 9, Mar.
89. Z. Shen, Y. Wang, Y. Shen, J. Shen, and C. Wu, (2016). Early emergence of mcr-1 in Escherichia coli from food-producing animals, *Lancet Infect Dis*, vol. 16, no. 3, p. 293, Mar.
90. L. Poirel, N. Kieffer, N. Liassine, D. Thanh, and P. (2016). Nordmann, Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of Escherichia coli, *Lancet Infect Dis*, vol. 16, no. 3, p. 281, Mar.
91. D. Muloi, M. J. Ward, A. B. Pedersen, E. M. Fèvre, M. E. J. Woolhouse, and B. A. D. van Bunnik, (2018). ¿Are Food Animals Responsable for Transfer of Antimicrobial-Resistant Escherichia coli or Their Resistance Determinants to Human Populations? A Systematic Review, *Foodborne Pathog Dis*, vol. 15, no. 8, pp. 467–474, Aug.
92. X. Huang et al., (2017). High Prevalence of Colistin Resistance and mcr-1 Gene in Escherichia coli Isolated from Food Animals in China, *Front Microbiol*, vol. 8, Apr.
93. M. Kawanishi et al., (2017). Prevalence of Colistin Resistance Gene mcr-1 and Absence of mcr-2 in Escherichia coli Isolated from Healthy Food-Producing Animals in Japan, *Antimicrob Agents Chemother*, vol. 61, no. 1, Jan.
94. Y.-Y. Liu et al., (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*, vol. 16, no. 2, pp. 161–168, Feb.
95. M. S. Arcilla et al., (2016). Dissemination of the mcr-1 colistin resistance gene, *Lancet Infect Dis*, vol. 16, no. 2, pp. 147–149, Feb.
96. H. E. Webb et al., (2016). Dissemination of the mcr-1 colistin resistance gene, *Lancet Infect Dis*, vol. 16, no. 2, pp. 144–145, Feb.
97. K. Zurfuh, L. Poirel, P. Nordmann, M. Nüesch-Inderbinen, H. Hächler, and R. Stephan, (2016). Occurrence of the Plasmid-Borne mcr-1 Colistin Resistance Gene in Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in River Water and Imported Vegetable Samples in Switzerland, *Antimicrob Agents Chemother*, vol. 60, no. 4, pp. 2594–2595, Apr.
98. K. Zurfluh, M. Nüesch-Inderbinen, M. Morach, A. Zihler Berner, H. Hächler, and R. Stephan, (2015). Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae Isolated from Vegetables Imported from the Dominican Republic, India, Thailand, and Vietnam, *Appl Environ Microbiol*, vol. 81, no. 9, pp. 3115–3120, May.
99. V. Manageiro, D. Jones-Dias, E. Ferreira, and M. Caniça, (2020). Plasmid-Mediated Colistin Resistance (mcr-1) in Escherichia coli from Non-Imported Fresh Vegetables for Human Consumption in Portugal, *Microorganisms*, vol. 8, no. 3, p. 429, Mar.
100. F. Yang et al., (2019). Plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli and Klebsiella pneumoniae isolated from market retail fruits in Guangzhou, China , *Infect Drug Resist*, vol. Volume 12, pp. 385–389, Feb.
101. L. R. Castellanos et al., (2017). High Heterogeneity of Escherichia coli Sequence Types Harbouring ESBL/AmpC

Genes on IncI1 Plasmids in the Colombian Poultry Chain, *PLoS One*, vol. 12, no. 1, p. e0170777, Jan.

102. J. D. D. Pitout, P. Nordmann, and L. Poirel, (2015). Carbapenemase-Producing Klebsiella pneumoniae, a Key Pathogen Set for Global Nosocomial Dominance, *Antimicrob Agents Chemother*, vol. 59, no. 10, pp. 5873–5884, oct.
103. B.-T. Liu, X. Li, Q. Zhang, H. Shan, M. Zou, and F.-J. Song, (2019). Colistin-Resistant mcr-Positive Enterobacteriaceae in Fresh Vegetables, an Increasing Infectious Threat in China, *Int J Antimicrob Agents*, vol. 54, no. 1, pp. 89–94, Jul.
104. B.-T. Liu and F.-J. Song, (2019). Emergence of two Escherichia coli strains co-harboring mcr-1 and blaNDM in fresh vegetables from China , *Infect Drug Resist*, vol. Volume 12, pp. 2627–2635, Aug.
105. M. E. Falagas, A. C. Kastoris, A. M. Kapaskelis, and D. E. Karageorgopoulos, (2010). Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review, *Lancet Infect Dis*, vol. 10, no.1, pp. 43–50, Jan.
106. Z. Shen et al., Emerging Carriage of NDM-5 and MCR-1 in Escherichia coli From Healthy People in Multiple Regions in China: A Cross Sectional Observational Study, *E-ClinicalMedicine*, vol. 6, pp. 11–20, Dec. *2018*.
107. S.-S. Oh, J. Song, J. Kim, and J. Shin, (2020). Increasing prevalence of multidrug-resistant mcr-1-positive Escherichia coli isolates from fresh vegetables and healthy food animals in South Korea, *International Journal of Infectious Diseases*, vol. 92, pp. 53–55, Mar.
108. W. Chelaghma, L. Loucif, E. Bendjama, Z. Cherak, M. Bendahou, and J.-M. Rolain, (2022). Occurrence of Extended Spectrum Cephalosporin-, Carbapenem- and Colistin-Resistant Gram-Negative Bacteria in Fresh Vegetables, an Increasing Human Health Concern in Algeria, *Antibiotics*, vol. 11, no. 8, p. 988, Jul.
109. C.-A. Li, C.-H. Guo, T.-Y. Yang, F.-Y. Li, F.-J. Song, and B.-T. Liu, (2023). Whole-Genome Analysis of blaNDM-Bearing Proteus mirabilis Isolates and mcr-1-Positive Escherichia coli Isolates Carrying blaNDM from the Same Fresh Vegetables in China, *Foods*, vol. 12, no. 3, p. 492, Jan.
110. E. Aklilu and K. Raman, (2020). MCR-1 Gene Encoded Colistin-Resistant Escherichia coli in Raw Chicken Meat and Bean Sprouts in Malaysia, *Int J Microbiol*, vol. 2020, pp. 1–5, Jul.
111. S. S. Devan et al., (2023). emergence of mcr -1, -3, -6, -8 and -9) in escherichia coli isolated from live chickens, raw chicken meat and vegetables from kelantan, malaysia, *Journal of microbiology, biotechnology and food sciences*, p. e9829, Mar.
112. A. Ghafur et al., (2019). Detection of chromosomal and plasmid-mediated mechanisms of colistin resistance in Escherichia coli and Klebsiella pneumoniae from Indian food samples, *J Glob Antimicrob Resist*, vol. 16, pp. 48–52, Mar.
113. Y. Hu et al., (2023). Antimicrobial Resistance in Non-typhoidal Salmonella from Retail Foods Collected in 2020 in China, *Zoonoses*, vol. 3, no. 1.
114. C. Díaz-Gavidia et al., (2021). Isolation of Ciprofloxacin and Ceftazidime-Resistant Enterobacterales from Vegetables and River Water Is Strongly Associated with the Season and the Sample Type, *Front Microbiol*, vol. 12, Sep.
115. S. H. Moon et al., (2022). Isolation of AmpC- and extended spectrum β-lactamase-producing Enterobacterales from fresh vegetables in the United States, *Food Control*, vol. 132, p. 108559, Feb.

DOI:10.31579/2690-4861/659