Mobile colistin resistance: Prevalence, mechanisms, and current detection methods

Nguyen Quang Truong[1,2*], Le Huyen Ai Thuy[2], Svetlana A Egorova[3], Lidiia A Kaftyreva[3], Hoang Quoc Cuong[1]

[1]Pasteur Institute in Ho Chi Minh City, Ho Chi Minh City, Vietnam
[2]Ho Chi Minh City Open University, Ho Chi Minh City, Vietnam
[3]Laboratory of Enteric Infection, Saint Petersburg Pasteur Institute, Russia
[*]Corresponding author: truongou@rgmail.com

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ABSTRACT

Colistin is considered the last-line antibiotic against multidrug-resistant Gram-negative bacterial infections. The global dissemination of Mobile colistin resistance *mcr*-harboring bacteria is threatening public health. Such isolates have been detected in various reservoirs including animals, food products, the environment, and humans. From these reservoirs, the isolates are approximate: 53% from animals; 39% from humans; 5% from the environment; and 3% from food. Bacterial distributions were: *E.coli* 91%; *Salmonella* 7%; and *Klebsiella* 2%. Among the 10 *mcr* variants, *mcr-1* and *mcr-9* are the most prevalent (up to 95% and 64.53%, respectively, in different studies) while other variants account for 5%. The coexistence of *mcr* and other antibiotic resistance genes in single isolates is a significant concern; *mcr* variants carried by different plasmid types increase antibiotic resistance and transfer of *mcr* genes to other bacteria. The hypothesis that the food chain is intimately involved in *mcr* gene transfer is supported by the presence of *mcr-harboring* isolates in animals and foods relative to humans. Cheaper, quicker, and more effective diagnostic tools for detecting colistin-resistant bacterial phenotypes and genotypes are essential and urgent. Currently, high quality tests include RPNP (99.3% specificity, 96.7% sensitivity) and MRPNP (95.4% specificity, ~100% sensitivity). LBJMR, CHROM agar, COL-APSE, and Super Polymyxin are now the best media to screen the bacteria, with near 100% selectivity. Multiplex PCR is a suitable method to quickly and accurately detect *mcr* genes in *E. coli* and *Salmonella* . Multi-drug resistant Gram-negative bacteria remain a global burden and need to have continuous and effective surveillance.

1. Introduction of colistin

Colistin, a 74-year-old antibiotic, was isolated from the bacterium *Paenibacillus polymyxa* subsp. *colistinus* in 1947. Colistin (polymyxin E) is a polycationic peptide antibiotic of the polymyxin family (Bialvaei & Kafil, 2015). It is considered the first antibiotic effective against Gram-negative bacteria, including *Acinetobacter species, P. aeruginosa, Enterobacter, Klebsiella,*

Salmonella, Shigella, Escherichia coli, Citrobacter, Yersinia pseudotuberculosis, Morganella morganii, and Haemophilus influenzae (Falagas & Kasiakou, 2005). However, colistin is not active against Gram-negative cocci or Gram-positive bacteria, and it is a concentration-dependent antibiotic (Aghapour et al., 2019; Bialvaei & Kafil, 2015).

Colistin was originally used therapeutically in Japan and Europe in the 1950s. It was then approved by the US FDA, with availability since 1959 for the treatment of Gram-negative bacterial infections. In the 1970s, colistin was largely replaced by aminoglycosides because of concerns about nephrotoxicity and neurotoxicity (Nation & Li, 2009). Colistin was gradually abandoned in many places of the world in the early 1980s because of the reported high prevalence of nephrotoxicity. Afterward, intravenous use of colistin has been largely limited during the past 20 years. Since the mid-1990s, with the appearance of multi-drug resistant Gram-negative bacteria (particularly *P. aeruginosa, K. pneumoniae,* and *A. baumannii*), and the absence of new antibiotics, colistin has been reconsidered as an important last-line antibiotic for the treatment of Multi-Drug Resistant (MDR) bacterial infections, especially carbapenem-resistant Gram-negative bacteria (Elbediwi et al., 2019). Although the prevalence of colistin resistance has been relatively low, likely reflecting its uncommon use, resistant isolates have recently been identified in some Gram-negative bacteria (Bialvaei & Kafil, 2015; Falagas & Kasiakou, 2005; Nation & Li, 2009).

1.1. Mechanism of action

The chemical structure of colistin is comprised of a cyclic heptapeptide with a tripeptide side-chain acylated at the N terminus by a fatty acid tail. Remarkably, the antimicrobial activity of colistin, and its essential toxicity, are sharply influenced by the hydrophobicity of the N-terminal fatty acyl segment.

Colistin's activities mainly target the outer cell membrane of Gram-negative bacteria, which is composed of a negatively charged lipopolysaccharide (LPS) membrane responsible for the integrity and stability of the bacterial outer membrane. When colistin interacts with LPS, electrostatic interactions occur between the α, γ-diaminobutyric acid (of colistin), and phosphate groups of the lipid A region of LPS. Disruption of LPS is caused by competitive displacement of divalent cations (Ca^{2+}, Mg^{2+}) from membrane lipid phosphate groups. This leads to increased outer membrane permeability, leakage of intracellular contents, and finally cell death. In addition, this binding also neutralizes Gram-negative bacterial endotoxins because, structurally, endotoxin is the lipid A portion of LPS (Bialvaei & Kafil, 2015).

1.2. Colistin resistance, an alarming situation

MDR bacteria have become a public health threat, a clinical burden, a point of difficulty in food safety, and a factor in socioeconomic development. Colistin is currently considered the last option to treat MDR bacteria-producing carbapenemase. The number of *mcr*-harboring isolates has increased significantly in some parts of the world, leading to alarming conditions. Although some nations have banned the use of colistin in livestock, the proportion of *mcr*-harboring isolates found in livestock is still higher than that in humans. Recently increased usage of colistin may increase the clinical potential for a wide prevalence of *mcr genes* in hospital settings.

Colistin resistance is independent of bacterial metabolic activity, and acquired resistance is relatively rare. It has been suggested that resistance to the antibiotic is related to the modification of LPS via different routes.

These include two main mechanisms described in Gram-negative bacteria. The first is a chromosomal mutation in genes (*phoPQ, pmrAB, mgrB*) involved in the synthesis and

modification of LPS. In most strains, 4-amino-4-deoxy-L-arabinose (LAra-4N), phosphoethanolamine (PETN), or galactosamine are enzymatically inserted into the phosphate group at the 4' position of Lipid A, a combination site with colistin. The addition of one of the three molecules to Lipid A results in a decrease in the net negative charge of phosphate residues, leading to a reduction in colistin affinity for LPS and prevention of cell lysis (Bialvaei & Kafil, 2015; Esposito et al., 2017).

The second route is the acquired colistin-resistance mechanism (plasmid-mediated *mcr* gene). The *mcr* genes encode the enzyme participating directly in LPS modification. Their expression leads to the addition of PETN to lipid A, a reduction in negative charge, and decreased binding of positively charged colistin (Aghapour et al., 2019; Esposito et al., 2017). Remarkably, zinc metalloprotein is like the catalytic domain of the *mcr* phosphoethanolamine transferase. Therefore, PETN is inhibited by metalloenzyme chelators such as Dipicolinic Acid (DA) and ethylenediamine tetra-acetic acid (EDTA) (Esposito et al., 2017; J. Sun et al., 2017). These resistance mechanisms have a remarkable influence on antibiotic treatment regimens. This leads to an absence of effective antibiotics to treat infectious diseases or to support surgical procedures. Plasmid-mediated colistin resistance is a considerable challenge and global concern because of the facile transfer of the *mcr* gene to other bacterial strains (Ngbede et al., 2020).

In many countries, a steadily increasing stream of reports is being published about the presence of *mcr*-harboring Gram-negative bacteria: *Escherichia coli*; *Klebsiella pneumoniae*; *Klebsiella oxytoca*; *Salmonella enterica*; *Cronobacter sakazakii*; *Kluyvera ascorbata*; *Shigella sonnei*; *Citrobacter freundii*; *Citrobacter braakii*; *Raoultella ornithinolytica*; *Proteus mirabilis*; as well as *Aeromonas*, *Moraxella*, and *Enterobacter* species (Elbediwi et al., 2019). *E. coli* accounted for the highest proportion of isolates (91%), with *Salmonella* (7%) and *Klebsiella* (2%) also represented (Nang, Li, & Velkov, 2019). The *mcr* gene has been shown to be present in isolates from various sources: humans; cattle; poultry; fresh and saltwater aquaculture; animal products (pork, beef); environmental sources (hospital sewage, rivers, seas); and even surfaces in public transportation (Anyanwu, Jaja, & Nwobi, 2020; Elbediwi et al., 2019; C. Shen et al., 2018; Zhang, Wei, Huang, Umar, & Feng, 2020). Among the sources, animals are the most common reservoirs, accounting for 53%. Other sources include humans (39%), the environment (5%), and food 3% (Elbediwi et al., 2019).

Alarmingly, the co-occurrence of *mcr* genes and other resistance genes, especially extended-spectrum β- lactamases (ESBL) and carbapenemases, have already been reported. Mobile colistin resistance genes can be carried by diverse plasmids, including prevalent plasmid types such as IncI2, IncHI2, IncX4, IncP, IncF, and IncY (Elbediwi et al., 2019). In addition, the prevalence of *mcr* is shown by its identification in migratory penguins and seagulls. This indicates the possibility of rapid global dissemination as these flying animals are capable of intercontinental migration (Nang et al., 2019). International trade in exotic animals (Unger et al., 2017), and international travel, also play important roles in the global spread of *mcr* genes (Arcilla et al., 2016; Doumith et al., 2016).

Worryingly, colistin resistance genes (*mcr*-1, *mcr*-3, or *mcr*-4) and other resistance genes, including β-lactamase and carbapenemase (*bla*TEM-1, *bla*CTX-M-1, *bla*CTX-M-55, *bla*NDM-5), can coexist in the prevalent IncHI2 plasmid, due to which bacterial strains can obtain both resistant genotypes by horizontal gene transfer (Elbediwi et al., 2019; Kawahara et al., 2019; Nang et al., 2019; Xu et al., 2018). The ability of plasmids to transfer colistin resistance between relevant species and genera has been assessed by conjugation and transformation. In particular, the *mcr*-1-carrying pHNSHP45 plasmid in wine isolates can transfer into human pathogenic

Enterobacteriaceae, such as *E. coli* ST131 and *K. pneumoniae* ST11, as well as into *P. aeruginosa* (Liu et al., 2016). According to the CLSI M100-ED31: 2021 standard, the resistance breakpoint for MIC-colistin is greater than or equal to 4 µg/mL. A recent study, however, showed that several *mcr*-1$^+$ strains recorded MIC-colistin values of less than 3 mg/mL. This indicates that the determination of the colistin resistance breakpoint needs to be considered as well as the intermediated breakpoint (Chew, La, Lin, & Teo, 2017). Such consideration can assist the identification of *mcr*-harboring and colistin-susceptible isolates without confirmation.

In Vietnam, the number of mcr-harboring isolates has considerably increased during the last eight years, from 2013 to 2021. The isolates were observed on both human beings, food animals, and contaminated foods, and their MIC-colistin was 4 mg/L or 8 mg/L (Le et al., 2021; Malhotra-Kumar et al., 2016; N. T. Nguyen et al., 2016; T. V. Nguyen et al., 2017). Particularly, the proportion of the mcr-carrying *E. coli* isolated from pig and chicken farms in Tien Giang Province fluctuated between 22% and 59.4% from 2013 to 2017. Meanwhile, the proportion of those isolated from humans accounted for 20.6%. The other study in 2016 witnessed the proportion of mcr-carrying *E. coli* isolated from chicken farms in Hanoi, at 35%. However, there were not any mcr-harboring *E. coli* isolated from humans to be detected. Besides, there was one *Salmonella* strain isolated pork sample, which was collected from the wet market in Ho Chi Minh City, exhibiting colistin resistance. This is the first colistin-resistant *Salmonella* that has been found in meat in Vietnam (N. T. Nguyen et al., 2018). Retail raw foods in Nha Trang were found mcr-carrying *E. coli* in 2021, including chicken (53.3%), shrimp (22.7%), pork (11.3%), fish (6.4%) (Le et al., 2021).

2. Current global dissemination of *mcr* genes

Studies of *mcr* genes, from 1980 to 2020 show global distribution of such genes. According to NCBI (n.d.), *mcr* genes have appeared in 47 countries over 06 continents: Asia (China, Japan, Laos, Vietnam, Malaysia, Singapore, Cambodia, Bahrain, Taiwan, Hong Kong, Thailand, South Korea, Russia, Pakistan, United Arab Emirates, Saudi Arabia, Oman); Europe (Austria, Estonia, UK, The Netherlands, Norway, Spain, Germany, France, Belgium, Denmark, Italy, Poland, Portugal, Russia, Switzerland, Sweden, Lithuania, Hungary); Africa (Algeria, Egypt, Tunisia, Morocco, South Africa), North America (USA, Canada); South America (Colombia, Argentina, Brazil, Ecuador); and Oceania (New Caledonia, Australia) (Elbediwi et al., 2019; Hassen et al., 2020; Nang et al., 2019; Touati & Mairi, 2021).

Such bacterial strains (*mcr*$^+$) have been isolated from diverse sources. Positive strains were determined from human sources in 44 nations; livestock and poultry in 21 nations; meat and food products in 13 nations; and other sources (pet/exotic/wild animals, the environment) in 11 nations. Only two countries (Estonia, and Tunisia) have reported the occurrence of *mcr*$^+$ strains in livestock, but not in humans. Currently, the first *mcr*$^+$ strain is thought to have appeared in China, and the country also has the highest prevalence of *mcr*-carrying isolates. This may be because colistin is widely used and extensive studies on *mcr* have been carried out in China (Ling et al., 2020; Nang et al., 2019).

In studies from 1980 to 2018, it was observed that the highest average prevalence of *mcr* genes accounted for 22% (2.8 - 47.8%) in the environment, animals 11% (0.3 – 22.4%) food 5.4% (0.6 - 11.6%) and humans 2.5% (0.1 - 5.1%). China shares a similar pattern with the global picture and displayed the proportions: environment 39% (8.3 - 88.5%); animals 14% (0.7 - 30%); humans 4.5% (0.2 - 9.3%); and food products 4.9% (0.7 - 10.7%). Vietnam has a similar pattern to China: 14.7% in animals; 4.5% in humans; and 4.9% in food products (Elbediwi et al., 2019).

3. Appearance of *mcr* variants

After the strain carrying the *mcr*-1 gene was found in chicken meat in China (the 1980s) (Z. Shen, Wang, Shen, Shen, & Wu, 2016), *mcr-1* genes were continuously detected in cattle in many different countries (Elbediwi et al., 2019). The occurrence of an *mcr* gene in *Shigella sonnei* (*mcr-1*) was reported only once from a Vietnamese child with dysentery in 2008 (Pham et al., 2016). The presence of *mcr*-carrying bacteria was detected in wild animals in 2005 (fish, *Aeromonas allosaccharophila*, *mcr*-3.6) and environmental samples (seawater, *E. coli*, *mcr-1*) in 2010 (Eichhorn et al., 2018; Jorgensen et al., 2017). Based on reservoir dynamics, this suggests that *mcr* genes have been circulating for at least 41 years.

MCR family distributions (hosts, bacterial species, plasmids) show significant geographical differences. To date, about 10 *mcr* variants have been detected. Among the MCR family, *mcr-1* and *mcr*-9 are the most widely disseminated, being detected in isolates from 61 nations and 40 nations, respectively. The *mcr*-3 and *mcr*-5 genes were the second most widely spread, being detected in 22 and 15 nations, respectively. Other variants (*mcr*-2, *mcr*-4, *mcr*-8) were disseminated across smaller areas, and *mcr*-7 showed a scattered distribution. To date, *mcr*-6 has only been reported in the UK (Ling et al., 2020). The earliest *mcr*-3 was seen in Germany (2005). Other variants soon followed in various locations: *mcr*-2 (2009); *mcr*-4 (2013); *mcr*-5 (2011); *mcr*-6 (2015); and *mcr*-7 (2014). This supports the hypothesis that mobile colistin resistance existed far earlier than the first report. Studies show that all the variants have been discovered in China, except *mcr*-6 and *mcr*-9. To date, geographic detections include mcr-2 (Belgium, Spain); mcr-3 (Brazil, Denmark, France, Germany, Japan, Spain, Thailand); mcr-4 (Italy, Spain); mcr-5 (Colombia, Japan, Spain, Germany); mcr-6 (United Kingdom), and mcr-9 (US) (Elbediwi et al., 2019; Nang et al., 2019).

According different authors, the percentages of *mcr-1* were up to 95%, *mcr-9* - up to 61.53%, respectively, and other variants (*mcr*-2, *mcr*-3, *mcr*-4, *mcr*-5, *mcr*-6, *mcr*-7, *mcr*-8) only accounted for roughly 5%, and their amino acid sequence identities with *mcr-1* were 81%, 32%, 34%, 36%, 83%, 35%, and 31%, respectively (Elbediwi et al., 2019). The variants have also been detected in different sources, such as aquatic, soil, botanical, wildlife, and animal environments, as well as public places (Anyanwu et al., 2020). Isolates carrying *mcr*-9 and *mcr-1*0 have been recently reported (Borowiak et al., 2020; C. Wang et al., 2020); *mcr*-9 is closely related to *mcr*-3, *mcr*-4, and *mcr*-7 (Carroll et al., 2019; Elbediwi et al., 2019). To date, *mcr*-1 and *mcr*-9 have been considered globally disseminated colistin resistance determinants (Ling et al., 2020). Mobile colistin resistance genes are carried by Gram-negative bacterial isolates: *Escherichia coli; Enterobacter; Klebsiella; Proteus; Salmonella; Citrobacter; Pseudomonas; Acinetobacter; Kluyvera; Aeromonas; Providencia;* and *Raoultella* (Anyanwu et al., 2020).

Studies have shown more heterogeneity in pathogenic *E. coli* (average prevalence of 23%) than in *Salmonella* (average prevalence of 6%) or *Klebsiella* (average prevalence of 8%) (Elbediwi et al., 2019). The *mcr-1* gene has been the most common variant in *E. coli*, followed by *Klebsiella* and *Salmonella*. The prevalence of *mcr-1* accounted for a total of 3% in *E. coli* from animal and human sources (Adiguzel et al., 2021; Dutta et al., 2020; Kawahara et al., 2019; X. Wang et al., 2021; Zhao et al., 2020). *E. coli* carrying *mcr-2* was isolated from a pig in Belgium in 2006 (Xavier et al., 2016). The *mcr-3* gene has been found in *E. coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Aeromonas spp.*; sources have included humans, livestock, aquatic environments, meat, and seafood from 12 countries representing all continents, except Africa and Antarctica (R. Y. Sun et al., 2020). The *mcr-4* gene, and its several recently identified variants, have mainly been discovered in *E. coli* or *Salmonella enterica* isolated from meat

products and humans. Among Italian *Salmonella mcr*-4 variants, there were differences between human and animal isolates (*mcr*-4.2 and *mcr*-4.1, respectively). Regarding other variants: the *mcr*-5 gene is primarily isolated from *Salmonella* species; *mcr*-7 and *mcr*-8 were only reported in *K. pneumoniae* in animals (Elbediwi et al., 2019); and *mcr*-9 is most prevalent in *Salmonella enterica* (Ling et al., 2020).

4. Role of the food chain in transmission of *mcr*$^+$ bacteria

Studies have shown that colistin use in livestock mirrors the emergence of *mcr*-carrying bacteria (Xu et al., 2018; Yuan et al., 2021; Zhao et al., 2020). The persistence and transmission of *mcr*-carrying strains to humans are attributed to bacteria from animal intestines and feces. The highest prevalence of MCR was observed in poultry, mainly in China and Germany. About 33% of overall *mcr*$^+$ strains have been isolated from pigs, mainly distributed in China and Japan (Elbediwi et al., 2019; Nang et al., 2019). Furthermore, bacteria carrying *mcr* genes have also been detected in food products, wastewater, river water, seawater, and in healthy people (Filioussis et al., 2020; Hassen et al., 2020; Ngbede et al., 2020; N. T. Nguyen, Liu, Katayama, Takemura, & Kasuga, 2021). The estimated overall prevalence of *mcr*-carrying isolates in human samples has been lower than those in animal and food samples. This supports the hypothesis that the food chain plays a role in *mcr* transmission. In addition, extensive investigations in China have provided data on the food chain transmission of *mcr*-carrying strains to humans from aquaculture; this may represent another important reservoir (Elbediwi et al., 2019). Detection of *mcr*$^+$ bacteria (*mcr-1*) in infants with no history of livestock exposure and who have not started a solid diet indicates that there are likely other means of transmission besides the food chain and zoonotic transfer (Gu et al., 2016).

5. Colistin combination therapy

Isolates carrying *mcr* exhibit resistance to colistin, but they can feature other antibiotic resistance determinants. As such, *mcr*$^+$ strains should also be handled as potential MDR strains. Remarkably, several reports suggested that, although *E. coli* isolated from humans and pigs were *mcr*$^+$, they had a colistin-susceptible phenotype *in vitro*. The cause may be inhibition of the *mcr* gene, or specific unknown reasons (Elbediwi et al., 2019). Currently, several reports have proposed carbapenem usage for the treatment of infections caused by colistin-resistant bacteria. Combination therapy (colistin with other antibiotics) has shown better cure rates, 14-day survival, and microbiological eradication, relative to monotherapy. Furthermore, combinations of colistin with other antimicrobials (sulbactam, carbapenems, aminoglycosides, tigecycline, rifampicin) have been recommended to avoid the growth of colistin-resistant strains (Aghapour et al., 2019; Batirel et al., 2014).

6. Current tools for detecting colistin-resistant bacterial genotypes and phenotypes

The expansion and dissemination of *mcr* genes have significantly increased and become a major cause of global concern. Therefore, simpler, cheaper, and better diagnostic tools are urgently needed for the rapid and effective detection of colistin-resistant bacteria.

6.1. Phenotypic tests: Screening media and MIC determination tools

Currently, there are many tools to determine the colistin-resistant phenotype, such as screening media: SuperPolymyxin™; CHROMagar COL-APSE; and Lucie-Bardet-Jean-Marc-Rolain (LBJMR). Their advantage is that they enable the isolation of colistin-resistant bacteria from fecal, environmental, food samples, and clinical specimens. MIC determination tools include BMD; UMIC; MMS; MTS; E-test, and automated MIC commercial equipment (MicroScan,

MICRONAUT-S, BD Phoenix, Sensititre, Vitek 2). They are the main routine tools in microbiology laboratories determining MIC values for infectious bacteria isolated from various sources. In addition, cation-modified Mueller-Hinton broth (CAMHB) and Mueller-Hinton agar (CAMHA), with or without colistin and/or metal chelators, are novel tests: Rapid Polymyxin NP test (RPNP); Colistin MAC test; and EDTA-based assays (CDT, CMR, MRPNP, Colispot, Sensitest™ Colistin (STC)). Among the currently available diagnostic tools, the following tools will be very suitable for laboratories lacking resources (Sekyere, 2019).

Diagnostic tests. Rapid polymixin NP (RPNP), with a sensitivity of 99.3% and a specificity of 95.4%, requires about 02 hours to perform (Nordmann, Jayol, & Poirel, 2016a). Modified RPNP (MRPNP), with a sensitivity of 96.7% and a specificity of ~100%, features a turnaround time of 04 hours (Esposito et al., 2017; Nordmann, Jayol, & Poirel, 2016b).

Screening media. LBJMR is an ideal medium to screen colistin-resistant strains (Bardet, Le Page, et al., 2017), followed by SuperPolymyxin, and CHROMagar COL-APSE (Momin et al., 2017; Nordmann et al., 2016a, 2016b). These three media features near 100% sensitivity and specificity. Between SuperPolymyxin and CHROMagar COL-APSE media, CHROMagar COL-APSE has a broader spectrum of activity with agents than SuperPolymyxin medium (Sekyere, 2019). Although phenotypic tests are simple and inexpensive, they require substantial time (18 - 24 hrs). Their specificity and sensitivity of *mcr* detection are also not as high as molecule methods. Moreover, phenotypic tests cannot detect novel drug resistance mechanisms.

6.2. Genotyping tests: Conventional, multiplex PCR, real-time PCR, and WGS

To date, the gold standard for identifying *mcr*-carrying isolates is still PCR and WGS. Conventional PCR or real-time PCR is considered a critical tool for epidemiological surveillance and antimicrobial resistance investigation. This is especially true in settings where laboratory resources (or access to WGS) are limited and where plasmid-mediated colistin resistance may threaten the effectiveness of available antimicrobials. Two recent molecular assays are able to detect *mcr-1*, *mcr*-2, *mcr*-3, *mcr*-4, and *mcr*-5 variants in a single reaction. They were designed to screen for *mcr* genes in *E. coli* and *Salmonella* from animals. They feature ~100% sensitivity and specificity, with a turnaround time of 02 hours. They could also be used to screen agents in well-resourced laboratories (Lescat, Poirel, & Nordmann, 2018; Rebelo et al., 2018).

7. Conclusion

Mobile colistin resistance (*mcr*) genes were first discovered in an *E. coli* strain isolated from chickens in China (November 1980). To date, an alarming number of *mcr*-carrying isolates have been reported in 47 countries on 06 continents. There are now ten variants: mcr-1; mcr-2; mcr-3; mcr-4; mcr-5; mcr-6; mcr-7; mcr-8; mcr-9; and mcr-10. Among variants, *mcr-1* and *mcr*-9 are the most common, accounting for up to 95% and 61.53%, respectively, according to different authors. The remaining variants account for 5%. The most prevalent *mcr*-carrying plasmids include IncI2, IncHI2, IncX4, IncP, IncF, and IncY. These enable the easy horizontal spread of *mcr gene*s to bacterial species such as *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Salmonella enterica, Cronobacter sakazakii, Kluyvera ascorbata, Shigella sonnei, Citrobacter freundii, Citrobacter braakii, Raoultella ornithinolytica, Proteus mirabilis*, as well as *Aeromonas, Moraxella,* and *Enterobacter* species. Among bacterial strains, *E. coli* accounted for the highest percentage (91%), with *Salmonella* (7%) and *Klebsiella* (2%) also well represented. Prevalent reservoirs have been the environment 22% (2.8 - 47.8%); animals 11% (0.3 - 22.4%); food 5.4% (0.6 - 11.6%); and people 2.5% (0.1 - 5.1%).

Although the use of colistin in livestock has been banned in some countries, such as China,

Brazil, and Australia, the isolation rate in livestock and food is still currently much higher than that in humans. This supports the hypothesis that the food chain plays a role in *mcr* transmission. In addition, some studies have also discovered other transmission routes besides 'food chain to human' or 'animal to human'. On the other hand, the increasing usage of colistin in clinical treatment may also raise the risk of *mcr* gene spread in clinical settings. In Vietnam, mcr gene has been detected from different sources such as food animals, contaminated foods, and human beings. The proportion of mcr-harboring isolates is an upward trend (53%, foods, and food animals) and (20.6%, humans). Currently, diagnostic tools used in studies in Vietnam, to determine colistin-resistant isolates are MIC determination tools (E-test, microdilution method) and PCR amplification. However, the turnaround time spends much more.

Better, simpler, and cheaper diagnostic tools that can quickly and effectively detect colistin-resistant bacterial strains are essential and urgently needed for preventing this global, rapidly spreading threat. Currently, diagnostic tests (Rapid polymyxin NP, Modified RPNP) and screening media (LBJMR, SuperPolymyxin, CHROMagar COL-APSE) are *mcr*-detection tools with high sensitivity and specificity. To impede the emergence of colistin resistance, all approaches and efforts should continue to be made. The proportion of bacterial strains featuring drug resistance is constantly increasing, and the lack of new antibiotics (effective against MDR Gram-negative bacteria) currently represents a real global burden. Therefore, a strategy of effective infection prevention and control is essential, and monitoring of *mcr*-harboring strains should be maintained on a constant basis. In addition, the development of novel, effective, inexpensive diagnostics, which can be implemented in a variety of laboratory conditions, continues to be an important factor.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article

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