Narrative Review
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mcr Genes Conferring Colistin Resistance in *Enterobacterales*; a Five Year Overview

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Abstract

The present review aims to study and detect the global emergence of *mcr* genes in *E. coli, K. pneumoniae* and *Salmonella* spp., isolates from human specimens over the last six years. Nowadays the rise of multidrug-resistant superbugs has made essential the return of drugs that were previously abandoned. A clear example is colistin, which acts against multidrug - resistant gram - negative pathogens, including *Enterobacterales*. Colistin resistance is an unfortunate fact, with the emergence of *mcr* genes conferring resistance to colistin in *Enterobacterales* posing the most recent threat. Literature about *mcr* genes and their spread in *E. coli, K. pneumoniae* and *Salmonella spp.* is cited, focusing on the emergence of *mcr* genes in human specimens since 2015. The data were taken from the *PubMed* and *Scopus* databases. It seems that the *mcr*-1 gene continues to be the protagonist among the three species. *E. coli* is the dominant species harbouring *mcr* genes. Moreover, plasmid - mediated colistin resistance is also conferred upon other species that carry different genes resistant to antibiotics. There are only scarse reports on human *Salmonella spp* isolates harbouring *mcr* genes. Finally, the emergence of the *mcr-9* gene in all of them is quite remarkable. **Conclusion.** Plasmid - mediated colistin resistance in *Enterobacterales* is a global issue and has been worsening over the years. The continuous mutations of *mcr* gene subtypes underline the need for better surveillance, constant investigation and wise use of colistin, especially in countries with high levels of antibiotic resistance.

Key Words: Colistin ▪ Resistance ▪ *mcr* Genes ▪ *Enterobacterales.*

Introduction

Antibiotic resistance is one of the greatest open battle fronts of modern medical science and humanity. In the 1990s, the need to add more antibiotics to the therapeutic quiver resulted in the restoration of outmoded drugs, such as polymyxins. Polymyxins are a group of 15 peptide molecules of similar function and morphology, which are divided into 5 categories (polymyxins A - E), and they generally act against gram - negative microorganisms (1, 2).

Among them, two are used in clinical practice: polymyxin B and colistin (polymyxin E). Both have been administered systematically and in various ways since their discovery. However, in the late 1970s, they were considered responsible for causing nephrotoxicity and neurotoxicity. Therefore, their widespread use was limited to the treatment of *Pseudomonas spp*. lung infections in patients with cystic fibrosis, as well as to ear and eye infections (2). Unfortunately, the development of multidrug-resistant (MDR) superbugs of the *P. aeruginosa, A. baumannii* and *K. pneumoniae* species, gave rise to the need for the return of polymyxins, and particularly colistin, in therapeutic regimens. In recent decades, intravenous administration of colistin has increased, while the incidence of its side effects has significantly decreased. Regarding its local administration, its use continues successfully (3). Nevertheless, it should not be forgotten that nephrotoxicity still remains one of colistin's side effects. Therefore, pharmacokinetics are essential in order to determine the optimal therapeutic and non-nephrotoxic concentrations of co-

listin. Planning the appropriate directed antibiotic therapy could also prevent the emergence and proliferation of new resistant species (4). Specifically, heteroresistance, a phenomenon responsible for the increase of colistin resistance, makes colistin an inadequate antibiotic. Therefore, colistin is recommended to be administrated in combination with other antibiotics, and not as monotherapy (2, 4).

Morphologically, colistin is characterized by a cyclic heptapeptide and a tripeptide side chain. The *N*-terminus of the side chain is acetylated by a fatty acid tail, which is responsible for colistin's side effects. On the basis of structural differences, colistin is classified into two chemical molecules: colistin A (polymyxin E1) and colistin B (polymyxin E2) (2).

Colistin is a narrow - spectrum antibiotic that is ineffective against gram - positive bacteria. It acts against most *Enterobacterales,* including multidrug-resistant *E. coli, Enterobacter spp., Klebsiella spp., Citrobacter spp., Salmonella spp.,* and *Shigella spp*. It also acts against some non - fermenters, such as *A. baumannii, P. aeruginosa* and *Stenotrophomonas maltophilia,* but is inactive against *Burkholderia cepacia* and *Pseudomonas mallei* (3, 5).

Despite the need for colistin due to the proliferation of resistant bacteria, its prolonged use has brought undesirable results. Emergence of colistin resistant isolates in clinical medicine, the agriculture sector and animal husbandry has become frequent in many geographical areas. The most worrisome effects of this phenomenon have become apparent in the increasing infections due to colistin - resistant pathogens (6).

Colistin Resistance

Over the past 10 years, the rate of colistin resistance has increased. For this reason, extensive research and studies are being conducted on the mechanisms of colistin resistance. Initially, resistance was attributed exclusively to intrinsic mechanisms. More specifically, genetic modifications in the chromosomal genome of pathogens were considered to be responsible for their resistance (3).

Paradigms of intrinsic resistance mechanisms have been observed in a variety of colistin target pathogens. *P. aeruginosa* uses a kinase system (PmrA / PmrB and PhoP / PhoQ) which controls the transcription of *pmrHIJKLM* operon, resulting in the production of an enzyme (N4-aminoarabinosis) that modifies the lipid load of the cell wall. *K. pneumoniae* regulates the action of regulatory *PhoPQ* and *PmrAB*, by inactivating a gene, *mgrB* (6). Finally, the *Proteeae* tribe, which includes *Proteus spp*., *Providencia spp.* and *Morganella morganii*, seems to share the same intrinsic mechanisms for colistin resistance (7). Specifically, *P. mirabilis* and *S. marcesens* present resistance based on the coding of its *arnBCADTEF* operon genes and the *eptB* gene, aiming to substitute cations in the lipopolysaccharide (LPS) layer (5).

A notable phenomenon of colistin resistance is heteroresistance. In the case of heteroresistance, different degrees of sensitivity to the antibiotic are observed in subpopulations of the same bacterium, and the resistance is not detectable by conventional susceptibility methods (8). It is attributed to mutations that occur in chromosomal genes, such as the *lpxA, lpxC* and *lpxD* of *A. baumannii*. Heteroresistance is thought in some cases to be responsible for the development of colistin resistance, especially during colistin treatment. Apart from *A. baumannii,* colistin heteroresistance has also been observed in other species, including *K. pneumoniae* (2).

Over recent decades, chromosomal resistance mechanisms have been the only explanation for colistin resistance. However, 2015 was a milestone, as researchers found clear scientific evidence regarding the plasmid - mediated colistin resistance mechanisms. A pioneer study was conducted in China by Liu et al. where the researchers highlighted the existence of a plasmid that carries a colistin resistance gene in *Enterobacterales,* and named it *mcr*-1, although it should not be overlooked that the first reports of *mcr* genes and their enzymes emerged in the 1980s in E. coli isolates. These isolates were from poultry in China (2, 9).

The newly discovered mobile *mcr*-1 gene is responsible for reducing the negative charge of lip-

id A. This action is carried out by the transfer of glucosamine from lipid A through the mediation of the enzyme phosphoethanolamine transferase, which is encoded by *mcr*-1. The reduction in load results in the inability of colistin to adhere to lipid A (6). In subsequent years, reports of the *mcr-1* gene in the family of *Enterobacterales* increased globally. At the same time, various publications demonstrated the evolution of the series of *mcr* genes, including a plethora of variants and subtypes. Particularly, in recent years around 22 variants of *mrc*-1 (*mcr*-1.2 to *mcr*-1.22) have been discovered, which differ only by a few amino acids and they are up to 99% identical. It seems that these similarly structured variants provide the same ratio of colistin resistance to pathogens. Furthermore, in later years, 10 novel *mrc* alleles emerged, from the original *mcr-1* to the novel *mcr*-10 gene, which enhanced *Enterobacteriaceae* resistance to colistin even more (2).

Detecting colistin resistance determinants in gram - bacteria has become a significant laboratory challenge. The disc diffusion method and agar dilution method have proven to be inaccurate in determining colistin susceptibility. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the broth dilution method is more reliable. Nowadays, the broth microdilution method is recommended and performed by the majority of routine laboratories to test colistin susceptibility. Moreover, the innovative rapid polymyxin NP test is similarly effective as the broth dilution method. Nowadays, efforts have been made to expand its use in non-fermenting bacilli as well. Regarding the bacteria's minimum inhibitory concentrations (MICs), they seem to be affected by the plasma colistin concentrations; a concentration of 2μg/mL is the optimal dose, resulting in MICs ≤1μg/mL (2, 4).

Nowadays, the scientific community is focusing on finding new strategies to reverse colistin resistance. Several approaches have been made. The reduction of *mcr* gene expression at the gene level is one of the most popular. Additional approaches are the discovery and use of new antibiotics (eravacycline, plazomicin, and artilysin) and the determination of the optimal colistin concentrations, combined with extra agents (amikacin, aztreonam, rifampin etc.). However, it is important to underline that the reduction in the spread of colistin resistance depends on wise colistin consumption, and perseverance in hospital hygiene measures (4).

mcr Genes and *Enterobacterales*

This study aims to review the current data and bring together the most recent research relating to the detection of *mcr* genes in *Enterobacterales* isolates in human specimens. Specifically, an effort was made to deposit studies over the last six years, which present the progress of *mcr* genes and their subtypes detected in *E. coli, K. pneumoniae* and *Salmonella spp.* Recent research studies detecting *mcr* genes in *Enterobacterales* exclusively from human specimens are listed below. The data were taken from *PubMed* and *Scopus* databases, using the key words: "*mcr*", "genes", "humans", "*Escherichia coli*", "*Klebsiella pneumoniae*" and "*Salmonella*" for the search query. The search limitations included only medical results and full text articles. The timeline was set between 2015 and 2021. Studies which did not include human specimens were excluded.

E. Coli

E. coli was the first pathogen in which a plasmid-mediated *mcr* gene was isolated in 2015 (9). The same year, Carattoli *et al.* monophasic variant of serovar Typhimurium (4,5,12:i:- confirmed the spread of the *mcr*-1 gene in both animals and humans. They also reported the presence of the *mcr*-2 gene, exclusively in *E. coli* strains from animals in Belgium, and highlighted the spread of the *mcr*-3 gene from Asia and the United States. They also predicted the danger of the emergence of *mcr*-4 (10).

During 2016 - 2017, the spread of the *mcr*-1 gene in *E. coli* strains increased dramatically. Several studies reported clinical isolates of colistin - resistant *E. coli* harboring the *mcr*-1 gene and its wide spread in South America (11, 12). In the same

year, Italy and Algeria reported resistant *E. coli* strains carrying the *mcr*-1 gene on a clinical level (13, 14). Similar clinical reports of *mcr*-1 genes in *E. coli* were published in both England and Wales (15), in the USA (16), in Japan (17), in Egypt (18), in Taiwan (19), and Malaysia (20), and expanded globally to numerous countries. Additionally, in December 2017, Liu et al. (21) identified a colistin-resistant *E. coli* clinical isolate carrying two plasmid-borne colistin-resistant genes, *mcr*-1 and the newly identified *mcr*-3. It is noteworthy that many studies reported *E. coli* isolates harbouring *mcr* genes together with multiple resistance mechanisms (22, 23).

Over the past three years, worldwide reports of colistin - resistant *E. coli* clinical isolates have soared. The *mcr*-1 gene has remained the protagonist, with reports from both clinical and community studies (24, 25). However, in 2019 Kieffer *et al.* reported the role of the *mcr*-5 gene in the colistin resistance of *E. coli*. Finally, in the same year, the novel *mcr*-9 gene was identified. According to Kieffer *et al.* the way that the *mcr*-9 gene in *E. coli* confers resistance was rather bizarre. Although the *mcr*-9 gene in wild-type *E. coli* strains seemed only to reduce susceptibility, it led to resistance once induced by small concentrations of colistin (26).

K. Pneumoniae

K. pneumoniae is one of the most clinically important bacteria in terms of resistance to antibiotics and nosocomial infections. However, on a smaller scale, plasmid - mediated colistin resistance manifests a common course with that of *E. coli*. In 2016, many studies identified resistant strains of *K. pneumoniae* carrying the *mcr*-1 gene in various countries, e.g., in European, North American, and southeast Asian areas (27). A similar report was also made by Guetet *et al.* during their study of a stool specimen of an infant with diarrhea in China (28). In Italy, in the same year, the *mcr-1.2* variant of a KPC-3-producing ST512 *K. pneumoniae* isolate from a leukemic child was detected for the first time (29). The *mcr*-1 gene was also reported in France, China, Laos and Lebanon (30-33). It is widely known that strains of *K. pneumoniae* commonly carry various types of resistance genes. A characteristic paradigm was recorded by Dalmolin et al. (34) in 2017 in Southern Brazil, who isolated a clinical strain that harbored both the *mcr*-1 and $bla_{\text{KPC-2}}$ genes. In 2020, the *mrc*-8.1 gene was detected in clinical *K. pneumoniae* isolates in various countries, such as Lebanon, Qatar and Morocco (35-37). Finally, the same year, Wang *et al.* isolated 28 *K. pneumoniae* strains which harbored the novel *mcr*-9 gene from patients in Belgium, Denmark, Montenegro, Poland, Romania, Serbia, Slovenia and Spain (38).

Salmonella Spp.

The human records in which *Salmonella* strains were found to bear plasmid - mediated colistin resistance mechanisms, are obviously fewer than those of *E. coli* and *K. pneumonia*e. In Portugal 2016, a 4 - year study detected the presence of the *mcr*-1 gene in *Salmonella* serotypes recovered from human clinical specimens (39). The same year in China, both *mcr*-1 and $bla_{\text{CTX-M-55}}$ were detected on a single plasmid in *S. enterica* for the first time (40). Furthermore, Carnevali et al. (41) demonstrated the occurrence of *mcr*-1 in colistin-resistant *S. enterica* isolates gathered from humans and animals, between 2012 and 2015. Similar reports were made from Doumith et al. in England and Wales (15). In 2020, reports indicated the emergence of colistin-resistant *S. enterica* carrying the *mcr*-9 gene (42). Finally, in 2019 a novel *mcr* homologue *mcr-9*, identified in a *Salmonella enterica* serotype *Typhimurium (*S. *Typhimurium*) genome, was reported by Carroll et al., isolated from a human patient in Washington State in 2010 (43).

Discussion

The use of the "formerly abandoned" colistin as a last - line antibiotic to treat gram-negative bacterial infections emphasizes the need to develop new antibiotics and also the necessity for prudent use of the existing ones. Despite the short period of colistin re-use (5-6 years), studies have shown that

Table 1. Molecular Epidemiology of Colistin Resistance in Human Isolates

Pathogen	*Mrc* genes	Year (Area) of detection	References
E. coli	*mrc*-1	2015 (China), 2016 (globally)	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20
	mrc-3	2017 (China)	21
	mrc-5	2019 (Czech Republic)	26
	mrc-9	2019 (Czech Republic)	26
K. pneumoniae	*mrc*-1	2016 (Europe, North America, Asia, China, Brazil)	27, 28, 30, 31, 32, 33, 34
	mrc-1.2	2016 (Italy)	29
	mrc-8.1	2020 (Lebanon, Qatar and Morocco)	35, 36, 37
	mrc-9	2020 (Belgium, Denmark, Montenegro, Poland, Romania, Serbia, Slovenia, Spain)	38
Salmonella spp.	*mrc*-1	2016 (Portugal, China, England and Wales)	15, 39 , 40
	mrc-9	2019-2020 (USA)	42 , 43

resistant bacterial strains against it have increased alarmingly.

Regarding *Enterobacterales,* especially *E. coli* and *K. pneumoniae*, the emergence of novel *mcr* genes has become a global threat (Table 1). Over recent years, *E. coli* and *K. pneumoniae* human isolates which carry the *mcr*-1 gene, have been observed increasingly around the globe. In fact, it seems that plasmid - mediated colistin is acquired by isolates harbouring other resistance traits, such as ESBLs or carbapenemases, resulting in the multidrug or extensively drug resistant phenotype. Even though the most commonly identified *mcr* gene appears to be *mcr*-1, isolates have been recorded to carry other types as well. The emergence of the *mcr*-9 gene is remarkable and its prevalence is increasing significantly.

Although human clinical samples for *Salmonella* spp. have not been extensively recorded and studied, *mcr* genes seem to seem to spread through the food producing chain (41). In fact, the discovery of the *mcr*-9 gene in food routed for human consumption underscores the need for further investigation. Plasmid - mediated colistin resistance in *Enterobacterales* is an issue that has worsened over the years. The latest news about the identification of the novel *mcr*-10 gene in strain 090065 of *Enterobacter roggenkampii* in 2020 underlines this global issue and the need for more strict surveillance of colistin use (24).

Conclusion

To conclude, in this review an effort was made to study and detect the global emergence of *mcr* genes in *E. coli, K. pneumoniae, Salmonella* spp. isolates from human specimens. *E. coli* strains are the most resistant among *Enterobacterales*, since they carry several *mcr* genes and their subtypes. Moreover, *E. coli, K. pneumoniae* and *Salmonella* spp strains which carry the *mcr*-1 gene have been reported simultaneously on different continents around the globe. It is remarkable that a plethora of countries have underlined the increasing threat of colistin resistant *Enterobacterales.* This could sound the alarm for several other areas and countries as well. Particularly, Greece is one of the countries with the highest antibiotic resistance rates in Europe, so it is of paramount importance for Greece to investigate further colistin resistance in *Enterobacterales,* among others (44-46). Surveillance and screening for colistin resistant *Enterobacterales* is highly recommended in livestock, animal farms, imported meat and poultry, along with monitoring antibiotic use. Future work could aim at well-organized universal surveillance programs of colistin resistance, in order to prevent the dangerous spread of the life-threatening superbugs.

What Is Already Known on This Topic:

There is currently a continuous and dangerous augmentation of colistin resistance. Studies have focused on the mechanisms used by pathogens in order to become colistin resistant. The general emergence of mcr genes is a fact.

What This Study Adds:

Collective data were gathered from the global biography, and a review performed about the emergence of different types of mcr genes, specifically focused on E. coli, K. pneumoniae and Salmonella spp, over the last five years. Additionally, this study underlines the severity of the global spread of colistin and multi-resistant Enterobacterales, which is often combined with a lack of surveillance programs.

Conflict of Interest: The authors declare that they have no conflict of interest.

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