



# Plasmid-Mediated Colistin Resistance Due to *mcr-1*- Update

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## Background

The continued evolution of highly resistant Gram-negative bacteria is an ongoing public health threat worldwide. The combination of increasing resistance to such antimicrobial classes as the carbapenems, with limited development of new antimicrobials, has resulted in the reintroduction of antimicrobials previously considered last resort. Although historically polymyxins have had limited clinical efficacy and are associated with substantial risk of nephrotoxicity, these agents are still used despite the availability of newer non-polymyxin treatment options. In 2019, an international consensus guideline for the optimal use of polymyxins was published to aid healthcare providers in the selection and dosing of polymyxin agents (1).

The emergence of plasmid-mediated mobile colistin resistance (*mcr*) genes in Enterobacterales occurred in 2015 (2). This raised worldwide concerns due to the possibility of rapid horizontal spread making colistin treatment ineffective. Soon after, the Centers for Disease Control and Prevention (CDC) released an alert to increase awareness among clinical laboratories of the discovery of the *mcr* genes and emphasized the importance of monitoring the prevalence of these genes in clinical isolates of Enterobacterales (3).

The *mcr-1* *mcr* gene was the first plasmid-mediated colistin resistance gene reported and, since then, ten different variants of the *mcr* gene (named *mcr-1* through *mcr-10*) have been identified around the world (4, 9). Because the *mcr-1* gene continues to be the most predominant *mcr* type and the most frequently associated with clinical infections in humans, this guideline will focus on the laboratory testing and epidemiology of the *mcr-1* gene.

## Mode of Action of Colistin and Mechanisms of Resistance

Polymyxins are polypeptide antibiotics; the class contains five compounds, polymyxin A-E, two of which have been used clinically in humans (polymyxin B and E). Polymyxin B and colistin (polymyxin E) differ from each other only by one amino acid and have comparable biological activity. Colistin binds the outer membrane lipopolysaccharide (LPS) of Gram-negative bacteria, disrupts cell membrane integrity, and leads to leakage of the cytoplasmic content and ultimately cell death (5).

Colistin has been extensively used in animal production and veterinary medicine and has led to the emergence of chromosomal- and plasmid-mediated colistin resistance (6). Acquired resistance to the polymyxins through chromosomal mutation has long been reported in organisms such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These chromosomal mutations typically mediate colistin resistance by modifying LPS to change the overall charge of LPS and reduce affinity of polymyxins to the outer membrane (6).

Plasmid-mediated colistin resistance is related to the presence of *mcr* genes. The *mcr-1* gene modulates lipid A residues and leads to a lower binding affinity of colistin to its target site.

## Emergence and evolution of the *mcr-1* gene

In November 2015, Liu et al (2) published the first case of plasmid-mediated colistin resistance conferred by the *mcr-1* gene in Enterobacterales from animals and humans. Since the initial report, *mcr-1* has been reported on all continents from humans, animals, food products, and environmental sources (7). The wide dissemination of *mcr* from food animals to meat, manure, the environment, and wastewater samples has increased the risk of transmission to humans via food products. The *mcr-1* gene has been found primarily in *E. coli* but it has also been reported in other members of the Enterobacterales including *K. pneumoniae*, *Salmonella enterica*, and *Enterobacter cloacae*. The description of the first detailed genomic analysis of an *E. coli* ST95 isolate with both high virulence potential and resistance to multiple antibiotics emphasizes the importance of continued monitoring of new and emergent antibiotic resistance determinants (8). Additionally, *mcr-1* has demonstrated natural interspecies spread within the Enterobacterales and can coexist with other drug resistance mechanisms, such as carbapenemases and extended spectrum beta-lactamases (ESBL) (5). Since *mcr-1* was first identified, variants of the *mcr* gene, referred to as *mcr-1* to *mcr-10* have been reported in several countries (9). Although these variants have been detected mainly in bacteria isolated from animal sources, they have the potential to spread to human bacterial isolates. Therefore, it is important to continue monitoring for the presence of *mcr* genes in colistin-resistant Gram-negative pathogens.

## Prevalence and epidemiology of *mcr-1* in the United States

Since the initial discovery of *mcr-1*, several reports of *mcr-1*-positive clinical isolates recovered from U.S. patients have been published. The first case, described in May 2016 of an *E. coli* urinary tract isolate from a patient in Pennsylvania, demonstrated a colistin minimal inhibitory concentration (MIC) of 4 Qg/ml as measured by broth microdilution (10). As of January 2020, Clinical and Laboratory Standards Institute (CLSI) colistin breakpoints for Enterobacterales are intermediate,  $\leq 2$  Qg/ml and resistant,  $\geq 4$  Qg/ml. The isolate had an ESBL phenotype but tested susceptible to carbapenems, amikacin, nitrofurantoin, and piperacillin-tazobactam. Basic local alignment search tool (BLAST) analysis indicated that the *mcr-1* gene was carried on an IncF plasmid harboring 7 antibiotic resistance genes, including *bla*<sub>CTX-M-14</sub>.

The second isolate was also reported in 2016 but was detected from an isolate collected in the U.S. in May 2015 as part of a retrospective analysis of 59 *E. coli* and 331 *K. pneumoniae* isolates with elevated MICs to colistin ( $\geq 4$  Qg/ml) through the SENTRY Antimicrobial Surveillance program (11). The 2015 *E. coli* isolate was also recovered from the urinary tract and was susceptible to amikacin, ertapenem, imipenem, meropenem, nitrofurantoin, piperacillin-tazobactam, cefepime, gentamicin, tobramycin, fosfomycin, tigecycline, ceftolozane-tazobactam and ceftazidime-avibactam. The colistin MIC of this isolate was 8 Qg/ml.

The third case was identified from a urine sample of a U.S. patient whose *E. coli* isolate harbored both *mcr-1* and *bla*<sub>NDM-5</sub> (12). This isolate tested resistant to the carbapenems and fluoroquinolones and tested susceptible to amikacin, aztreonam, gentamicin, nitrofurantoin, tigecycline, and trimethoprim-sulfamethoxazole.

The fourth case was a pediatric patient with diarrhea; the *mcr-1* gene was identified from non-Shiga toxin-producing *E. coli* O157 isolated from stool. The case appears to have been travel-associated; there was no identified transmission, and only transient colonization of the patient (13). The SENTRY program retrospectively identified two additional clinical *E. coli* isolates from U.S. patients from the 2016 surveillance data (14).

A more recent clinical case was recovered from a urine sample in a patient diagnosed with urinary tract infection. The *E. coli* isolate was resistant to beta-lactams, macrolides, fosfomycin, aminoglycosides, trimetho-



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prim-sulfamethoxazole, tetracycline, and colistin (colistin MIC was  $\geq 4$  Qg/ml). Although the *mcr-1* gene is predominantly located on an IncX4 plasmid in the U.S., this strain was co-producing ESBL genes and *mcr-1* that were carried on an IncHI2-type plasmid (15).

The SENTRY project showed that of the 21,006 *E. coli* and *K. pneumoniae* isolates collected worldwide in 2014-2015, only nineteen were positive for *mcr-1* with a total prevalence of only 0.1% (11). Additionally, in 2016, among 11,493 *E. coli* and *K. pneumoniae* isolates tested, 199 were found to be resistant to colistin displaying colistin MIC values of  $\geq 4$  Qg/ml. Of these, only 10 *E. coli* and two *K. pneumoniae* isolates were *mcr-1* positive (14). These global data demonstrate that the overall prevalence of *mcr-1* continues to be very low, and the clinical isolates harboring it are often multi-drug resistant. Importantly, none of the cases reported thus far from the U.S. have been pan-resistant.

The first *mcr-1* was reported in the IncI2 plasmid named pHNSHP45. Since then, *mcr* genes have been observed in a wide range of plasmid types, including IncI2, IncHI2, IncHI2A, IncX4, IncP, and IncF. IncI2, IncHI2, and IncX4 are the most common plasmid types associated with human and animal isolates. The conjugative properties of these plasmids may play an important role in the transferability and dissemination of *mcr* genes to human and animal isolates of diverse clones (16).

### Laboratory Testing

CLSI has included a warning stating that clinical and PK/PD data demonstrate that colistin and polymyxin B have limited efficacy, even if an MIC in the intermediate range is obtained (17). Colistin susceptibility testing may be considered when treating patients with infections due to multi-drug resistant organisms when limited antimicrobial options are available.

Challenges in laboratory testing and interpretive reporting of colistin are related to specific physicochemical properties of this antimicrobial agent and the relative lack of clinical data to correlate with isolate MICs. The large size and amphipathic (hydrophobic and hydrophilic) nature of the polymyxins hamper the performance of disk diffusion and agar gradient diffusion. The polymyxins are also cationic, which increases adsorption of these agents to plastic surfaces. Broth microdilution is the only approved method for polymyxin B. For colistin, CLSI states that broth microdilution, broth disk elution and the colistin agar test MIC method (in Table 3D of the M100 document) are the only acceptable test methods (17). The colistin broth disk elution and the colistin agar test MIC method is approved for Enterobacterales and *P. aeruginosa* only. For *Acinetobacter* spp., only broth microdilution can be used for testing. Neither disk diffusion, conventional agar dilution, or agar gradient diffusion accurately detect colistin resistance. Therefore, CLSI does not recommend the use of these methods for susceptibility testing of colistin currently. Some automated antimicrobial susceptibility testing systems have been evaluated. None have been found to meet CLSI standards, and none are FDA-approved.

Investigations of the most accurate method for testing polymyxins are ongoing. If laboratories receive requests for colistin testing, it is recommended to use one of the methods validated by CLSI using current interpretive breakpoints or to send the isolate to a reference laboratory.

Due to technical challenges associated with susceptibility testing and the variability of colistin *in vivo* concentrations in patients with altered renal function, CLSI reevaluated colistin interpretive criteria, and established in January 2020 an intermediate colistin breakpoint of  $\leq 2$  Qg/ml and a resistant breakpoint of



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≥4 Qg/ml for Enterobacterales, *Acinetobacter* spp., and *P. aeruginosa*. The CDC advises that laboratories which test Enterobacterales for colistin resistance should confirm the presence of the *mcr-1* gene in isolates with elevated colistin MICs, particularly if other risk factors exist, such as a recent history of travel outside the United States to a country where *mcr-1* has been found to be more common (18). If needed, laboratories may send the isolate to their state public health laboratory or CDC for testing. Currently, there are limited FDA approved/cleared tests available for molecular detection of *mcr-1*. It is not necessary to test isolates that are intrinsically resistant to colistin (e.g., *Proteus*, *Providencia*, *Morganella*, and *Serratia*) for the presence of the *mcr-1* gene.

### Infection Control

Given the potential consequences of widespread colistin resistance, it is imperative that every effort be made to control the spread of this resistance mechanism. As such, in their alert to healthcare facilities in 2016, the CDC offered several infection control recommendations which included following standard and contact precautions for any patient colonized or infected with strains found to harbor *mcr-1*, and immediately notifying local and state public health authorities when these isolates are encountered. The CDC also states that all *mcr-1*-harboring isolates can safely be handled in a biosafety level-2 (BSL-2) laboratory (18).

At this time, very little is known about the risk factors for infection or colonization with *mcr-1*-positive isolates. However, point-prevalence studies have identified isolates with *mcr-1* in cases of true infection as well as asymptomatic colonization. Of note, a Hong Kong-based point-prevalence study identified *mcr-1* positive isolates from the stool of two asymptotically colonized individuals (19). Other studies have also demonstrated human fecal carriage of *mcr-1* (20, 21). Colonization with *mcr-1*-positive organisms is not surprising, but it is nonetheless concerning because it suggests that *mcr-1* may follow the same rapid pattern of spread that has already been observed for other plasmid-borne mechanisms of resistance, such as the *Klebsiella pneumoniae* carbapenemase (KPC) and the New Delhi metallo-beta-lactamase (NDM).

### Summary

The polymyxin antibiotic class serves as an alternative option for treating infections caused by multi-drug resistant Gram-negative organisms when preferred agents are not available or are not active *in vitro*. The emergence and spread of plasmid-borne colistin resistance threatens to eliminate this treatment option and minimizing *mcr-1* dissemination is key. Unfortunately, laboratory methods for susceptibility testing of colistin are limited and because polymyxins are not preferred agents for front-line treatment, colistin antimicrobial susceptibility testing is not routinely performed. Laboratories that choose to test for colistin susceptibility should validate a broth microdilution method, or another CLSI-approved method, and should report antimicrobial susceptibility testing results according to the most up-to-date CLSI guidelines. Laboratories should continue to reference the CLSI M100 standards for guidance on colistin susceptibility testing. If laboratories detect isolates with elevated colistin MICs (4 Qg/mL or greater) in the Enterobacterales, it is strongly recommended that those strains be forwarded to public health laboratories for testing for the *mcr-1* gene.

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