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Detection of Plasmid-Mediated Resistance against Colistin in Multi-Resistant Gram-Negative Bacilli Isolated from a Tertiary Hospital

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Abstract: The main aim of this study was to determine the prevalence of plasmid-mediated colistin resistance *mcr*-1 to *mcr*-5 genes among colistin and multi-drug resistant *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter sp* strains isolated from patients in a tertiary hospital in the city of Toluca, Mexico. 241 strains were included in the study. The presence of *mcr* genes among these strains was assessed by PCR and sequencing. In the case of *mcr*-carrying *E. coli*, further PCR tests were performed to determine the presence of *bla*$_{CTX-M}$ and whether the strains belonged to the O25b-ST131 clone. Conjugation experiments were carried to assess plasmid-mediated colistin resistance horizontal transmission. 12 strains (5.0%), of which four were *E. coli*; four, *P. aeruginosa*; three, *K. pneumoniae* and one, *E. cloacae*, were found to be resistant to colistin. Of these strains, two *E. coli* isolates were found to carry *mcr*-1. Both *mcr*-1-carrying *E. coli* strains were found to co-express *bla*$_{CTX-M}$, belong to the O25b-ST131 clone and horizontally transmit their colistin resistance. The results of this study confirm the presence of plasmid-mediated colistin resistance in hospitalized patients in Mexico and demonstrated that the multidrug-resistant O25b-ST131 *E. coli* clone can acquire *mcr* genes and transmit such resistance trait to other bacteria.

Keywords: antibiotic resistance; mcr-1; plasmid mediated colistin resistance; O25b-ST131; CTX-M

1. Introduction

The use of antibiotics in humans and animals represents the cornerstone of modern medicine. Since their discovery, antibiotics have revolutionized the treatment of bacterial infections around the world. However, bacteria have evolved mechanisms to become resistant to these agents, and a steady increase in this phenomenon has made antimicrobial resistance one of the major health problems affecting humankind.

In the case of Gram-negative bacteria, infections caused by multi-drug resistant-*Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter sp*, are on the rise [1]. This reality has led to the use of old antibiotics, such as colistin, as a last-resort antimicrobial against these pathogens. Colistin, a polypeptide antibiotic also known as polymyxin E, was discovered in 1949 and was largely used in the 1950s; however, due to its neuro- and nephro-toxicity, it was abandoned in the 1980s in favor of other antibiotics [2]. Colistin exerts its antibacterial activity against Gram-negative organisms by electrostatically binding the negatively charged phosphate groups of lipid A in the bacterial lipopolysaccharide (LPS), disrupting the integrity of the bacterial membrane [3].

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Currently, antibiotic resistance against colistin is not as worrisome as that against other antimicrobials; however, due to the overuse and misuse of this antibiotic among humans and animals, colistin resistance is on the rise, as described in several reports from different nations [4–6]. Resistance against this agent is attributed to the modification of LPS, through cationic substitutions, which reduces the net negative charge of this molecule, impeding the binding of the antibiotic [7]. Colistin resistance was originally described as being chromosomally encoded; however, in 2015, Liu et al described the first plasmid-mediated resistant gene against this antibiotic, *mcr*-1, in *E. coli* [8]. Shortly after its discovery, *mcr*-1 was identified in over 20 different countries and, to date, nine additional *mcr*-like genes have been described [9]. Furthermore, *mcr* genes have also been identified in *Pseudomonas aeruginosa* and *Acinetobacter sp* [10,11].

With the global spread of *mcr* genes among multi-drug resistant bacterial strains and their further horizontal transmission among bacteria, the effectiveness of colistin is under serious jeopardy. An additional factor that might facilitate the spread of colistin resistance is the likelihood of acquiring this plasmid-mediated resistant trait in multi-resistant *E. coli* clones such as O25b-ST131, the most common multidrug-resistant high-risk clone associated with extra-intestinal *E. coli* infections around the world [12]. This clone commonly carries resistance genes to antibiotics frequently prescribed in general practice, such as cephalosporins, which are mainly mediated by the *bla*$_{\text{CTX-M}}$ gene, as well as quinolones, and can easily be transmitted through the consumption of food [13]. These antibiotics can co-select for colistin-resistant strains and, thus, contribute to the spread of resistance against this agent. ST131 *E. coli* clones carrying colistin resistance genes have already been reported in environmental and clinical samples [14,15].

As the surveillance of antibiotic resistance and research is a key component of the global action plan against antimicrobial resistance [16], the purpose of the current study was to determine the prevalence of the plasmid-mediated colistin resistance genes *mcr* -1 to *mcr* -5 among colistin and multi-drug resistant *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter sp* strains isolated from patients in a tertiary hospital in the city of Toluca, Mexico. Additionally, we sought to assess whether plasmid-mediated colistin-resistant *E. coli* strains isolated from these patients belonged to the ST131 clone and co-expressed *bla*$_{\text{CTX-M}}$ genes.

2. Materials and Methods

This was a prospective study conducted between May and October 2022 in coordination with the Microbiology laboratory from Centro Médico ISSEMYM Toluca, Mexico. Strains included in this research were isolated from cultures obtained by the clinical laboratory of the hospital as part of routine care for hospitalized patients, as instructed by their physician. No additional specimens were obtained for the purposes of this study and no personal information was obtained from patients; therefore, informed consent was not required.

2.1. Bacterial strains, culture, identification and microbial susceptibility testing

Biological samples were plated on blood agar and MacConkey agars and cultured at 35 ± 2 °C for 18 hours. Strains growing on the latter medium were further identified using standard microbiological techniques and the VITEK Compact System (bioMerieux, Marcy l'E´toile, France). Only strains that belonged to the order *Enterobacterales* or the genus *Pseudomonas aeruginosa* or *Acinetobacter sp*p were included in the study.

Minimum inhibitory concentrations (MIC) of different antibiotics against strains included in the study were determined using the VITEK Compact System (bioMerieux, Marcy l'E´toile, France) and compared to Clinical and Laboratory Standard Institute (CLSI) guidelines [17]. Only strains that fulfilled the multidrug-resistance (MDR), extensively drug-resistant (XDR) or pandrug-resistant criteria (PDR) established by Magiorakos et al [18] were included for further testing. MDR, XDR and PDR strains were initially tested for colistin resistance by the colistin broth disk elution test described by Simner et al [19]. The MIC of the strains determined to be resistant by this method (≥ 4 μg/mL) were further measured in duplicate using the micro broth dilution method according to CLSI

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guidelines [17] and only those confirmed to be colistin-resistant (MIC ≥ 4 μg/mL) were included in the study.

2.2. PCR amplification

DNA was extracted using the boil lysis method, and extracted DNA was tested via PCR for plasmid-encoded *mcr* -1 to *mcr* -5 genes using primers described in Table 1. Amplified PCR products were sequenced in both directions and nucleotide sequences were compared against the National Center for Biotechnology Information BLAST database [20]. In the case of colistin-resistant *E. coli* strains carrying *mcr* genes, the presence of the O25b-ST131 clone and *bla*$_{CTX-M}$ genes was determined using previously described PCR primers (Table 1).

Table 1. Primers and PCR conditions used to determine the presence of *mcr* and *bla*$_{CTX-M}$ genes and O25b-ST131 clone.

Amplified gene	Primers sequence (5´- 3´)	Reference
mcr -1-fw	AGTCCGTTTGTTCTTGTGGC	[21]
mcr -1-rv	AGATCCTTGGTCTCGGCTTG	
mcr -2-fw	CAAGTGTGTTGGTCGCAGTT	[21]
mcr 2-rv	TCTAGCCCGACAAGCATACC	
mcr-3-fw	AAATAAAAATTGTTCCGCTTATG	[21]
mcr-3-rv	AATGGAGATCCCCGTTTTT	
mcr-4-fw	TCACTTTCATCACTGCGTTG	[21]
mcr-4-rv	TTGGTCCATGACTACCAATG	
mcr-5-fw	ATGCGGTTGTCTGCATTTATC	[22]
mcr-5-rv	TCATTGTGGTTGTCCTTTTCTG	
pabB-fw	TCCAGCAGGTGCTGGATCGT	[23]
pabB-rv	GCGAAATTTTTCGCCGTACTGT	
bla$_{CTX-M}$-fw	TTTGCGATGTGCAGTACCAGTA	[24]
bla$_{CTX-M}$-rv	CGATATCGTTGGTGGTGCCATA	

2.3. Conjugation experiments

Conjugation experiments were carried out using *E. coli* J53, a sodium azide resistant strain, as the recipient organism, following the broth-mating method. Transconjugants were selected on Mueller Hinton agar plates containing 100 mg/mL sodium azide and 4 μg/mL of colistin. The presence of *mcr* genes in transconjugants was assessed via antimicrobial susceptibility testing using the micro broth dilution method, as suggested by CLSI [17], as well as PCR. The transmission of the *bla*$_{CTX-M}$ gene to transconjugants was confirmed by PCR, and its ESBL phenotype, by the CLSI confirmatory method [17].

For all experiments, a previously identified *E.coli* strain isolated from a swine farm, resistant to colistin carrying *mcr*-1 gene was included as a positive control [25].

3. Results

3.1. Colistin resistance

In total, 241 isolates of MDR, XDR and PDR strains of *Enterobacterales*, *P. aeruginosa* and *Acinetobacter sp* were collected at the clinical laboratory from Centro Médico ISSEMYM Toluca and included in this study. The number of isolates per each bacterial species identified is shown in Figure 1.

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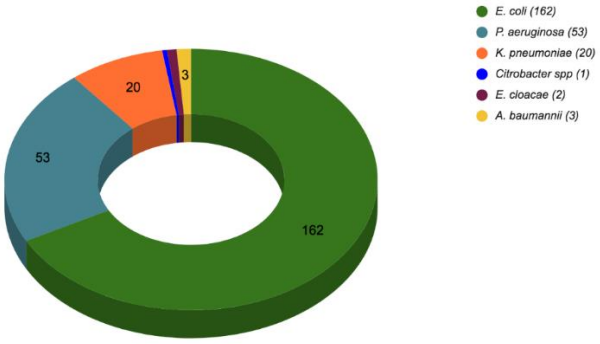

Figure 1. Number of strains isolated per bacterial species.

Among the 241 multi-drug resistant strains included in the study, 12 (5.0%) were found by the micro broth dilution method to be resistant against colistin (MIC ≥ 4 µg/mL) and included *E. coli* (N=4), *P. aeruginosa* (N=4), *K. pneumoniae* (N=3) and *E. cloacae* (N=1). The colistin MIC of these 12 strains and their isolation sites are shown in Table 2.

Table 2. Colistin MIC of strains identified as resistant and their isolation sites.

Strain	Micoorganism	Colistin MIC (µg/mL)	Isolation site
744	*P. aeruginosa*	8	Blood
1308	*K. pneumoniae*	16	Respiratory secretions
2207	*E. coli*	4	Respiratory secretions
2230	*E. coli*	4	Urine
2445	*E. coli*	8	Renal abscess
2892	*P. aeruginosa*	16	Respiratory secretions
3148	*P. aeruginosa*	8	Urine
3172	*K. pneumoniae*	4	Blood
3196	*P. aeruginosa*	4	Urine
3202	*K. pneumoniae*	4	Respiratory secretions
3271	*E. cloacae*	16	Respiratory secretions
5891	*E. coli*	4	Urine

The antibiotic resistance profiles of the 12 strains found to be resistant to colistin against commonly used antibiotics and their respective classification as MDR, XDR or PDR are depicted in Table 3.

Table 3. Antibiotic resistance profile of colistin resistant strains.

Colistin resistant bacteria	Antibiotic	Antibiotic Resistance prevalence	Acquired resistance profile
P. aeruginosa (N=4)	Ceftazidime	4/4 (100%)	MDR: 0
	Cefepime	4/4 (100%)	XDR: 3
	Amikacin	4/4 (100%)	PDR: 1
	Ciprofloxacin	4/4 (100%)	
	Piperacilin/tazobactam	2/4 (50.0%)	
	Imipenem	4/4 (100%)	
	Ceftazidime	4/4 (100%)	
	Meropenem	4/4 (100%)	
	Gentamicin	4/4 (100%)	

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	Tigecyclin	1/4 (25%)	
	Ampicilin/sulbactam	3/4 (75%)	MDR: 2
	Cefuroxime	3/4 (75%)	XDR: 2
	Cefotaxime	3/4 (75%)	PDR: 0
	Ceftazidime	3/4 (75%)	
	Ceftriaxone	3/4 (75%)	
E. coli	Cefepime	2/4 (50%)	
(N=4)	Ertapenem	1/4 (25%)	
	Meropenem	0/4 (0%)	
	Amikacin	0/4 (0%)	
	Gentamicin	2/4 (50%)	
	Ciprofloxacin	4/4 (100%)	
	Trimethoprim/sulfamethoxazol	4/4 (100%)	
	Ampicilin/sulbactam	3/3 (100%)	MDR: 1
	Cefuroxime	2/3 (66.7%)	XDR: 2
	Cefotaxime	2/3 (66.7%)	PDR: 0
	Ceftazidime	2/3 (66.7%)	
	Ceftriaxone	2/3 (66.7%)	
K. pneumoniae	Cefepime	2/3 (66.7%)	
(N=3)	Ertapenem	1/3 (33.3%)	
	Meropenem	0/3 (0%)	
	Amikacin	0/3 (0%)	
	Gentamicin	2/3 (66.7%)	
	Ciprofloxacin	3/3 (100%)	
	Trimethoprim/sulfamethoxazol	3/3 (100%)	
	Cefuroxime	1/1 (100%)	MDR: 1
	Cefotaxime	0/1 (0%)	XDR: 0
	Ceftazidime	0/1 (0%)	PDR: 0
	Ceftriaxone	0/1 (0%)	
	Cefepime	0/1 (0%)	
E. cloacae	Ertapenem	0/1 (0%)	
(N=1)	Meropenem	0/1 (0%)	
	Amikacin	0/1 (0%)	
	Gentamicin	1/1 (100%)	
	Ciprofloxacin	1/1 (100%)	
	Trimethoprim/sulfamethoxazol	0/1 (100%)	

3.2. mcr prevalence

Of all 12 strains resistant to colistin, the multiplex PCR protocol amplified one fragment of approximately 320 bp in two *E. coli* isolates (2207 and 5891) and in the control strain, suggesting the presence of the *mcr* -1 gene. Sequencing in both directions of these amplicons confirmed that both strains carry this gene. PCR targeting was negative on all strains for the *mcr* -2 to – 5 genes. Both *mcr* -1-carrying *E.coli* strains were shown to be ESBL producers carrying the *bla*$_{\text{CTX-M}}$ gene and belonging to the O25b-ST131 clone.

3.3. Conjugation experiments

Conjugation experiments were conducted on strains 2207 and 5891, with both isolates transmitting the *mcr* -1 plasmid-mediated colistin resistance trait to the recipient J53 *E. coli* strain. The two parental strains also transmitted their *bla*$_{\text{CTX-M}}$ gene. Both transconjugants were shown to be PCR positive for the *mcr* -1 gene and *bla*$_{\text{CTX-M}}$ genes of the donor bacterial cell and confirmed to be ESBL producers by the CLSI confirmatory test [17] as well de novo resistant to colistin, as assessed by the

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micro broth dilution method (≥4 ug/mL). The strain 2207 transconjugant presented an identical MIC to that of the parental strain, while the 5891 transconjugant presented a one-two fold higher MIC.

4. Discussion

Due to the increasing rates of resistance among Gram-negative bacilli against commonly used antibiotics, mainly in bacterial strains isolated form hospitalized patients, colistin has emerged as one of the last-resort antimicrobials in the treatment of these infections. Unfortunately, resistance against this agent is on the rise, particularly in Asia, which is mainly attributed to its overuse in veterinary medicine [26].

One of the main objectives of the current study was to determine the prevalence of colistin resistance among multi-resistant *Enterobacterales*, *P. aeruginosa* and *Acinetobacter sp* strains isolated from patients in a tertiary hospital in Mexico. In total, 241 strains were included in the study, of which 12 (5.0%) were found to be resistant to colistin according to the CLSI guidelines. Of the 12 colistin-resistant strains, 4 were *P. aeruginosa*, with a colistin-resistance prevalence of 7.5%, (4/53); 4 were *E. coli*, with a prevalence of 2.5% (4/162); 3 were *K. pneumoniae*, with a prevalence of 15.0% (3/20) and one, *E. cloacae*, with a prevalence of 50.0% (1/2). Although *K. pneumoniae* presented one of the highest prevalence levels of colistin resistance, no PDR strains were found, unlike *P. aeruginosa*, for which one strain was found to be resistant to all tested antibiotics. In this study, no *Acinetobacter sp* isolates were found to be colistin-resistant.

The antibiotic resistance profiles of the colistin-resistant strains found in this study are shown in Table 3. As all 241 strains included in the current study were multi-resistant isolates, antibiotic resistance to different types among them was high. In the case of *P. aeruginosa*, colistin-resistant strains presented an extremely high prevalence of resistance against carbapenems (100%), ceftazidime (100%), ciprofloxacin (100%) and amikacin (100%) and low resistance to tigecycline (25%). For *Enterobacterales*, higher rates of resistance were found against ciprofloxacin (100.0%), trimethoprim/sulfamethoxazole (87.5%), cephalosporines (62.5%), and ceftazidime (62.0%); no resistance was found against amikacin. Finally, resistance against at least one of the carbapenems was found in one *E. coli* (1/4) and one *K. pneumoniae* (1/3) strain.

The prevalence of colistin resistance in this study is in agreement with reports in different geographical areas of the world among muti-resistant strains isolated from hospitalized patients [10,27,28], but higher than the 1.26% worldwide prevalence reported by Dadashi et al [29]. In Mexico, reports on the prevalence of colistin-resistant strains are scarce; however, in a recent report by the Red Temática de Investigación y Vigilancia de la Farmacorresistencia (INVIFAR network), that included clinical strains isolated from different parts of the country, the colistin resistance prevalence among *K. pneumoniae* strains was 17.8% [30], a rate similar to that found in the current study among isolates of this bacterial species (3/20 = 15.0%). Unlike the INVIFAR report, where no resistance to colistin was found among *E. coli* strains, we found that 2.5% of *E. coli* strains included in the current study presented this resistance trait. These results confirm that colistin resistance among clinical isolates in our country is already a reality and should be carefully monitored.

As the world has witnessed an increase in resistance against colistin, this phenomenon has been mainly attributed to the emergence and dissemination of plasmid-mediated (*mcr* -1 to 10) genes among bacteria. The first *mcr* gene (*mcr* -1) was originally described in 2015 in China [8], and in Mexico, *mcr* -1 was first identified in 2019 in an *E. coli* strain isolated from a fecal sample from a child [31]. The presence of this plasmid-mediated gene in our country was recently confirmed in *K. pneumoniae* strains isolated from different clinical samples [30]. In the present study, only two *E. coli* strains, both isolated from female patients with urinary tract infections that required hospitalization, were found to carry the *mcr* -1 gene. No other *mcr* gene was found in the strains analyzed and no other bacterial species in addition to *E. coli* were found to carry plasmid-mediated colistin resistance genes. In this study, plasmid-mediated colistin resistance genes were not found in *P. aeruginosa*. However, as colistin is the last-resource antibiotic against strains of this organism resistant to carbapenems, reports of *mcr* genes among multi-resistant strains of this species have started to emerged [32,33] and horizontal transmission of *mcr* -1 genes has been shown to occur from *P.*

aeruginosa to other bacterial species [34], the surveillance of *mcr* -carrying *P. aeruginosa* strains remains highly encouraged.

The relatively low prevalence of *mcr* genes among colistin-resistant Gram-negative bacteria found in this study (16.7%) is higher to the values reported for *E.coli* in Egypt (7.5%) [35] and similar to those described in Ecuador (20.0%) [36], but lower than the prevalence shown in a study in Nepal [37] and Peru [38], where the prevalence of strains that serve as carriers of *mcr* genes was reported to be high among colistin-resistant *E. coli* and *K. pneumoniae*. The results of the current research confirm the fact that plasmid-mediated colistin resistance has spread at different rates among different geographical areas of the world. It is also important to note that in this study, *mcr* genes were searched for in colistin-resistant strains and only multi-resistant isolates were included, but different studies have shown that colistin susceptible *Enterobacterales* can carry *mcr* genes [39,40]. Thus, the prevalence of *mcr* -carrying strains at the Microbiology laboratory from Centro Médico ISSEMYM Toluca, Mexico, could be higher than the levels indicated by the results in this study. The low prevalence of *mcr* genes found in this investigation among colistin-resistant strains suggest that in our population, resistance to this agent is mainly driven by chromosomal mutations or plasmid-mediated genes not included in the study. Further research is needed to understand additional mechanisms of colistin resistance to those identified in the current study.

Several reports have shown that Gram-negative bacilli can co-harbor *mcr* genes and other plasmid-mediated antibiotic resistance traits such as those encoding for carbapenemase and ESBL production. Since colistin is mainly indicated as a last-resource antibiotic, the co-expression of colistin and carbapenemase resistance is worrisome among the medical community and, understandably, has been more thoroughly studied and more commonly demonstrated among *Enterobacterales* [30,41] than its co-expression with extended-spectrum-β-lactamases. However, bacterial strains co-harboring *mcr* and ESBL genes should also be carefully monitored. In addition to encode for intrinsic resistance against cephalosporines, penicillins and monobactams, plasmids carrying ESBL-encoding genes, can also harbor resistance genes against other commonly used antibiotics such as ciprofloxacin, trimethoprim/sulfametoxazol and aminoglycosides [42]. When administered, any of these antibiotics could co-select for colistin-resistant strains and contribute to the spread of resistance against this antimicrobial. In the present study, the two *mcr*-1-carrying *E.coli* strains were additionally found to carry *bla*$_{\text{CTX-M}}$ genes, a phenomenon that has also been demonstrated in human isolates in Qatar [43], Peru [44] and the Indian Ocean Commission [45], showing that the co-expression of plasmid-mediated resistance against colistin and ESBL production has spread to different regions of the world.

In the current study, both *mcr*-1- and CTXM-carrying *E. coli* strains were able to horizontally transmit their colistin resistance and ESBL production to the recipient strain, suggesting that conjugation may play a role in the spread of colistin resistance. In this study, only a minority of colistin-resistant isolates were found to carry *mcr* genes and in the two *mcr*-1-carrying strains no carbapenem resistance was detected, leaving other therapeutic options available for the treatment of infections caused by these strains. However, as different studies have shown that the horizontal transmission of *mcr*-1 genes occurs in food [46] and animals [47], this mechanism might be responsible around the globe for the steady increase in colistin resistance among Gram-negative bacilli.

The highly antibiotic resistant clone ST131, predominantly serogroup O25b, is considered the dominant extraintestinal pathogenic *E. coli* around the world, including being a frequent cause of urinary tract infections [12,13]. In the current study, the two *E. coli* strains carrying *mcr*-1 and *bla*$_{\text{CTX-M}}$ genes were isolated from patients with urinary tract infections and were resistant to most classes of antibiotics, demonstrating sensitivity only to the carbapenems and, in the case of one strain (2207), to cefepime. In addition, these two strains were found to belong to the ST131-O25b clone, an *E. coli* clone that commonly exhibits resistance to quinolones, trimethoprim-sulfamethoxazole and aminoglycosides, and is recognized as the primary lineage responsible for the spread of *bla*$_{\text{CTX-M}}$ genes [48]. The results of this study support previous findings on strains isolated from humans [15,49], showing that the already highly resistant clone ST131 can acquire plasmid-mediated colistin

resistance genes. As this clone can be transmitted from person to person and through the consumption of contaminated food [13], and given that its prevalence in the feces of healthy humans is on the rise [50], the spread of this multi-resistant clone could be facilitated by a lack of hygiene, which suggests that less privileged areas of the world might see an increase in the prevalence of this clone. If *mcr*-1-carrying O25b-ST131 *E. coli* clones expand to different geographical areas, commonly used antibiotics, such as ciprofloxacin, cephalosporines and trimethoprim-sulfametoxazol, could co-select for colistin-resistant strains and contribute to the spread of resistance against this last-resort antibiotic.

Limitations of the current study include its small sample size, unicentric design and short timeframe. Lastly, not all known *mcr* variants were analyzed; thus, the actual plasmid-mediated colistin resistance prevalence at our institution might be higher than the levels suggested by these results.

In conclusion, this study, albeit small, demonstrated that the emergence and spread of *mcr*-carrying strains among humans is a reality in Mexico. In addition, the presence of this resistant trait among the highly resistant and easily transmissible O25b-ST131 *E. coli* clone further complicates the antibiotic resistance scenario in our country. Further surveillance studies are needed among other hospitals and ambulatory patients in Mexico to determine the magnitude of the problem of colistin resistance, especially that mediated by mcr genes, in order to establish policies aimed at optimizing antibiotic stewardship programs to reduce the dissemination of resistance against this last-resource antibiotic.

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References

1. Nagvekar V, Sawant S, Amey S. Prevalence of multidrug-resistant Gram-negative bacteria cases at admission in a multispeciality hospital. J Glob Antimicrob Resist. 2020, 22:457-461. DOI: 10.1016/j.jgar.2020.02.030.
2. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014, 5:643. DOI: 10.3389/fmicb.2014.00643
3. Andrade FF, Silva D, Rodrigues A, Pina-Vaz C. Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges. Microorganisms. 2020, 8(11):1716. DOI: 10.3390/microorganisms8111716.
4. Monaco M, Giani T, Raffone M, Arena F, García-Fernández A, Pollini S. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill. 2014, 19 (42):20939. DOI: 10.2807/1560-7917.es2014.19.42.20939.
5. Pena I, Picazo JJ, Rodríguez-Avial C, Rodríguez-Avial I. Carbapenemase-producing Enterobacteriaceae in a tertiary hospital in Madrid, Spain: high percentage of colistin resistance among VIM-1-producing *Klebsiella pneumoniae* ST11 isolates Int J Antimicrob Agents. 2014, 43(5):460-464. DOI: 10.1016/j.ijantimicag.2014.01.021
6. Meletis G, Oustas E, Botziori C, Kakasi E, Koteli A. Containment of carbapenem resistance rates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in a Greek hospital with a concomitant increase in colistin, gentamicin and tigecycline resistance. New Microbiol. 2015, 38(3):417-421.
7. Binsker U, Käsbohrer A, Hammerl JA. Global colistin use: a review of the emergence of resistant *Enterobacterales* and the impact on their genetic basis. FEMS Microbiol Rev. 2022, 46(1):fuab049. DOI: 10.1093/femsre/fuab049.

8. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. 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. 2016, 16(2):161-168. DOI: 10.1016/S1473-3099(15)00424-7.
9. Baron S, Hadjadj L, Rolain JM, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents. 2016, 48(6):583-591. DOI: 10.1016/j.ijantimicag.2016.06.023
10. Ejaz H, Younas S, Qamar MU, Junaid K, Abdalla AE, Abosali KOA, et al. Molecular Epidemiology of Extensively Drug-Resistant mcr Encoded Colistin-Resistant Bacterial Strains Co-Expressing Multifarious β-Lactamases. Antibiotics (Basel). 2021, 10(4):467. DOI: 10.3390/antibiotics10040467.
11. Hameed F, Khan MA, Muhammad H, Sarwar T, Bilal H, Rehman TU. Plasmid-mediated mcr-1 gene in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: first report from Pakistan. Rev Soc Bras Med Trop. 2019, 52:e20190237. DOI: 10.1590/0037-8682-0237-2019.
12. Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. Int J Antimicrob Agents. 2010, 35(4):316-321. DOI: 10.1016/j.ijantimicag.2009.11.003
13. Nicolas MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev. 2014, 27(3):543-574. DOI: 10.1128/CMR.00125-13.
14. Lopes R, Furlan JPR, Dos Santos LDR, Gallo IFL, Stehling EG. Colistin-Resistant mcr-1-Positive *Escherichia coli* ST131-H22 Carrying blaCTX-M-15 and qnrB19 in Agricultural Soil. Front Microbiol. 2021, 12:659900. DOI: 10.3389/fmicb.2021.659900.
15. Ortiz de la Tabla V, Ortega A, Buñuel F, Pérez-Vázquez M, Marcos B, Oteo J. Detection of the high-risk clone ST131 of *Escherichia coli* carrying the colistin resistance gene mcr-1 and causing acute peritonitis. Int J Antimicrob Agents. 2017, 49(1):115-116. DOI 10.1016/j.ijantimicag.2016.10.003.
16. World Health Organization. Global action plan on antimicrobial resistance. 2016;45. Accessed January 10, 2023.
17. Clinical and Laboratory Standards Institute. M100 Performance Standards for Antimicrobial Susceptibility Testing, 33rd Edition; 2023.
18. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske GC, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012, 18(3):268-281. DOI: 10.1111/j.1469-0691.2011.03570.x.
19. Simner PJ, Bergman Y, Trejo M, Roberts AA, Marayan R, Tekle T, et al. Two-Site Evaluation of the Colistin Broth Disk Elution Test To Determine Colistin In Vitro Activity against Gram-Negative Bacilli. J Clin Microbiol. 2019, 57(2):e01163-18. DOI: 10.1128/JCM.01163-18.
20. BLAST Sequence Analysis Tool. National Center for Biotechnology Information. 2013. Accessed February 17, 2023.
21. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill. 2018, 23(6):17-00672. DOI: 10.2807/1560-7917.ES.2018.23.6.17-00672.
22. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. enterica serovar Paratyphi B. J Antimicrob Chemother. 2017, 72(12):3317-3324. DOI: 10.1093/jac/dkx327.
23. Clermont O, Dhanji H, Upton M, et al. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. J Antimicrob Chemother. 2009, 64(2):274-277. DOI: 10.1093/jac/dkp194.
24. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob Agents Chemother. 2003, 47(12):3724-3732. DOI: 10.1128/AAC.47.12.3724-3732.2003
25. Garza U, Tamayo E, Arellano DM, et al. Draft Genome Sequence of a Multidrug- and Colistin-Resistant mcr-1-Producing *Escherichia coli* Isolate from a Swine Farm in Mexico. Genome Announc. 2018, 6(10):e00102-18. DOI: 10.1128/genomeA.00102-18.
26. Gogry FA, Siddiqui MT, Sultan I, Haq QMR. Current Update on Intrinsic and Acquired Colistin Resistance Mechanisms in Bacteria. Front Med (Lausanne). 2021, 8:677720. DOI: 10.3389/fmed.2021.677720.
27. 27. Zafer MM, El-Mahallawy HA, Abdulhak A, Amin MA, Al-Agamy MH, Radwan HH. Emergence of colistin resistance in multi-drug resistant *Klebisella pneumoniae* and *Escherichia coli* strains isolated from cancer patients. Ann Clin Microbiol Antimicrobiano. 2019, 18(1):40. DOI: 10.1186/s12941-019-0339-4.
28. Saavedra SY, Diaz L, Wiesner M, Correa A, Arévalo SA, Reyes J, et al. Genomic and Molecular Characterization of Clinical Isolates of *Enterobacteriaceae* Harboring mcr-1 in Colombia, 2002 to 2016. Antimicrob Agents Chemother. 2017, 61(12):e00841-17. DOI: doi: 10.1128/AAC.00841-17.

29. Dadashi M, Sameni F, Bostanshirin N, Yaslianifard S, Khosravi-Dehaghi N, et al. Global prevalence and molecular epidemiology of mcr-mediated colistin resistance in *Escherichia coli* clinical isolates: a systematic review. J Glob Antimicrob Resist. 2022, 29:444-461. DOI: 10.1016/j.jgar.2021.10.022.
30. Garza-Ramos U, Silva-Sánchez J, López-Jácome LE, Hernández-Durán M, Colín-Castro CA, Sánchez-Pérez A, et al. Carbapenemase-Encoding Genes and Colistin Resistance in Gram-Negative Bacteria During the COVID-19 Pandemic in Mexico: Results from the Invifar Network. Microb Drug Resist. 2022, 10.1089/mdr.2022.0226. DOI: 10.1089/mdr.2022.0226.
31. Merida-Vieyra J, De Colsa-Ranero A, Arzate-Barbosa P, Arias-de la Garza E, Méndez-Tenorio A, Murcia-Garzón J, et al. First clinical isolate of *Escherichia coli* harboring mcr-1 gene in Mexico. PLoS One. 2019, 14(4):e0214648. DOI: 10.1371/journal.pone.0214648.
32. Abd El-Baky RM, Masoud SM, Mohamed DS, Waly NG, Shafik EA, Mohareb DA, et al. Prevalence and Some Possible Mechanisms of Colistin Resistance Among Multidrug-Resistant and Extensively Drug-Resistant *Pseudomonas aeruginosa*. Infect Drug Resist. 2020, 13:323-332. DOI: 10.2147/IDR.S238811.
33. Snesrud E, Maybank R, Kwak YI, Jones AR, Hinkle MK, McGann P. Chromosomally Encoded mcr-5 in Colistin-Nonsusceptible *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2018, 62(8):e00679-18. DOI: 10.1128/AAC.00679-18.
34. Chen H, Mai H, Lopes B, Wen F, Patil S. Novel *Pseudomonas aeruginosa* Strains Co-Harbouring blaNDM-1 Metallo β-Lactamase and mcr-1 Isolated from Immunocompromised Paediatric Patients. Infect Drug Resist. 2022, 15:2929-2936. DOI: 10.2147/IDR.S368566.
35. Anan MMG, El-Seidi EA, Mostafa MS, Rashed LA, El-Wakil DM. Detection of Plasmid-Mediated Mobile Colistin Resistance Gene (mcr-1) in *Enterobacterales* Isolates from a University Hospital. Infect Drug Resist. 2021, 14:3063-3070. DOI: 10.2147/IDR.S318787.
36. Ortega-Paredes D, Barba P, Zurita J. Colistin-resistant *Escherichia coli* clinical isolate harbouring the mcr-1 gene in Ecuador. Epidemiol Infect. 2016, 144(14):2967-2970. DOI: 10.1017/S0950268816001369.
37. Karki D, Dhungel B, Bhandari S, Kunwar A, Joshi PR, Shesthra B, et al. Antibiotic resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. Gut Pathog. 2021, 13(1):45. DOI: 10.1186/s13099-021-00441-5.
38. Ugarte RG, Olivo JM, Corso A, Pasteran F, Albornoz E, Sahuanay ZP. Resistencia a colistín mediado por el gen mcr-1 identificado en cepas de *Escherichia coli* y *Klebsiella pneumoniae*. Primeros reportes en el Perú. An Fac Med. 2018, 79(3):213-217. DOI:10.15381/ANALES.V79I3.15313
39. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of Novel Mobilized Colistin Resistance Gene mcr-9 in a Multidrug-Resistant, Colistin-Susceptible *Salmonella enterica* Serotype *Typhimurium* Isolate. mBio. 2019, 10(3):e00853-19. DOI: 10.1128/mBio.00853-19.
40. Terveer EM, Nijhuis RHT, Crobach MJT, Knetsch C, Veldkamp KE, Gooskens J, et al. Prevalence of colistin resistance gene (mcr-1) containing *Enterobacteriaceae* in feces of patients attending a tertiary care hospital and detection of a mcr-1 containing, colistin susceptible *E. coli*. PLoS One. 2017, 12(6):e0178598. DOI: 10.1371/journal.pone.0178598.
41. Mendes AC, Novais Â, Campos J, Rodrigues C, Santos C, Antunes P, et al. mcr-1 in Carbapenemase-Producing *Klebsiella pneumoniae* with Hospitalized Patients, Portugal, 2016-2017. Emerg Infect Dis. 2018, 24(4):762-766. DOI: 10.3201/eid2404.171787.
42. Östholm Å, Tärnberg M, Monstein HJ, Hällgren A, Hanberger H, Nilsson LE. High frequency of co-resistance in CTX-M-producing *Escherichia coli* to non-beta-lactam antibiotics, with the exceptions of amikacin, nitrofurantoin, colistin, tigecycline, and fosfomycin, in a county of Sweden. Scand J Infect Dis. 2013, 45(4):271-278. DOI:10.3109/00365548.2012.734636
43. Tsui CKM, Sundararaju S, Mana HA, Hasan MR, Tang P, Perez A. Plasmid-mediated colistin resistance encoded by mcr-1 gene in *Escherichia coli* co-carrying blaCTX-M-15 and blaNDM-1 genes in pediatric patients in Qatar. J Glob Antimicrob Resist. 2020, 22:662-663. DOI: 10.1016/j.jgar.2020.06.029.
44. Yauri-Condor K, Zavaleta-Apestegui M, Sevilla-Andrade CR, Piscoya-Sara J, Villoslado-Espinosa C, Taboada WV, et al. Extended-spectrum beta-lactamase-producing *Enterobacterales* carrying the mcr-1 gene in Lima, Peru. Rev Perú Med Exp. Salud Publica. 2020, 37(4), 711-715. DOI 10.17843/rpmesp.2020.374.5832
45. Leroy AG, Naze F, Dortet L, Naas T, Jaubert J. Plasmid-mediated colistin resistance gene mcr-1 in a clinical *Escherichia coli* isolate in the Indian Ocean Commission. Med Mal Infect. 2018, 48(6):426-428. DOI: 10.1016/j.medmal.2018.04.388
46. Luo X, Matthews KR. The conjugative transfer of plasmid-mediated mobile colistin resistance gene, mcr-1, to *Escherichia coli* O157:H7 and *Escherichia coli* O104:H4 in nutrient broth and in mung bean sprouts. Food Microbiol. 2023, 111:104188. DOI: 10.1016/j.fm.2022.104188.
47. Li XP, Sun RY, Song JQ, Fang LX, Zhang RM, Lian XL, et al. Within-host heterogeneity and flexibility of mcr-1 transmission in chicken gut. Int J Antimicrob Agents. 2020, 55(1):105806. DOI: 10.1016/j.ijantimicag.2019.09.010.

48. Novais A, Pires J, Ferreira H, Costa L, Montenegro C, Vuotto C, et al. Characterization of globally spread *Escherichia coli* ST131 isolates (1991 to 2010). Antimicrob Agents Chemother. 2012, 56(7):3973-3976. DOI: 10.1128/AAC.00475-12.
49. Li G, Li X, Wu Y, Xu J, He F. Genomic Insights into the Colistin Resistant mcr-Carrying *Escherichia coli* Strains in a Tertiary Hospital in China. Antibiotics (Basel). 2022, 11(11):1522. DOI: 10.3390/antibiotics11111522.
50. Kudinha T, Kong F. Possible step-up in prevalence for *Escherichia coli* ST131 from fecal to clinical isolates: inferred virulence potential comparative studies within phylogenetic group B2. J Biomed Sci. 2022, 29(1):78. DOI: 10.1186/s12929-022-00862-7.