

## BRIEF REPORT

EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIALES CARRYING THE *mcr-1* GENE IN LIMA, PERU

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## ABSTRACT

We analyzed the presence of the *mcr-1* gene in 165 extended-spectrum beta-lactamase-producing enterobacteriales (ESBL-PE) obtained during 2017, from blood (40), urine (57), lower respiratory secretions (12) and rectal swabs (56) of patients hospitalized in the Instituto Nacional de Enfermedades Neoplásicas (Peru). Antimicrobial identification and susceptibility were determined by the Phoenix M50 automated system; colistin resistance by Colistin Agar-Spot (CAS); *mcr-1* detection by colistin pre-diffusion and inhibition with EDTA test (CPD-E) and by polymerase chain reaction (PCR). We found that from the 165 ESBL-PE, 25 were positive for *mcr-1* by the CPD-E method and confirmed by PCR. Colistin resistance was found in 20/165 by using the CAS method. Additionally, they showed resistance to fluoroquinolones and gentamicin, while remaining sensitive to amikacin; two isolates presented metallo-carbapenemases. Obtaining data on resistance to last-line antimicrobials (colistin) is crucial to establish measures for its control.

**Keywords:** Enterobacteriaceae; beta-Lactamases; Colistin; Drug Resistance, Microbial. (Source: MeSH NLM)

## INTRODUCTION

The worldwide spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) gram-negative bacteria, including carbapenemase-producing enterobacteriales (CPE), has led to the reinsertion of colistin as a last resort therapy; this antibiotic interacts directly with the outer lipopolysaccharide membrane<sup>(1)</sup>. The main resistance mechanisms involve the modification of lipid A, mediated by mutations in genes of the PhoPQ-PmrAB regulatory system, even during treatment of clinically relevant microorganisms such as *Klebsiella pneumoniae*<sup>(2,3)</sup>. In 2015, a mechanism of plasmid-mediated colistin resistance (PMCR), related to the *mcr-1* gene (Mobile Colistin Resistance) was described among enterobacteriales isolated from animals and humans in China<sup>(4)</sup>. This gene encodes a phosphoethanolamine transferase that modifies the colistin target, by adding phosphoethanolamine, which reduces the affinity for colistin<sup>(5)</sup>.

Several studies have shown a worldwide distribution of *mcr-1*, especially in *Escherichia coli*, and occasionally in other bacterial species<sup>(6)</sup>. As with other resistance genes, different allelic variants of *mcr-1* have been detected (*mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-8*, *mcr-9*, and *mcr-10*)<sup>(5,6)</sup>. The presence of *mcr-1* genes has been reported from several countries in South America, in isolates obtained from humans, animals and food<sup>(7-9)</sup>. In 2016, the World

**Cite as:** Yauri-Condor K, Zavaleta Apestegui M, Sevilla-Andrade CR, Piscocoya Sara J, Villoslado Espinoza C, Vicente Taboada W, *et al.* Extended-spectrum beta-lactamase-producing enterobacteriales carrying the *mcr-1* gene in Lima, Peru. Rev Peru Med Exp Salud Publica. 2020;37(4):711-5. doi: <https://doi.org/10.17843/rpmesp.2020.374.5832>.

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**Received:** 19/05/2020  
**Approved:** 09/09/2020  
**Online:** 05/11/2020

Health Organization (WHO) recommended implementing and strengthening the surveillance and epidemiological research of the PMCR<sup>(10)</sup>. In Peru, the presence of *mcr-1* in *E. coli* clinical isolates has been reported so far<sup>(11,12)</sup>.

Therefore, this study is part of the project “Epidemiological surveillance of resistant bacteria in healthcare-associated infections” and is aimed at identifying the extended-spectrum beta-lactamase-producing enterobacterales (ESBL-PE) that carry the *mcr-1* gene in Instituto Nacional de Enfermedades Neoplásicas (INEN) of Perú.

## THE STUDY

This is a descriptive study, in which we collected 165 single consecutive isolates of ESBL-PE between January and December 2017. The samples included *Escherichia coli* (112), *Klebsiella pneumoniae* (41), *Enterobacter cloacae* (5), *Proteus mirabilis* (4), *Klebsiella oxytoca* (2) and *Klebsiella ozanoneae* (1); recovered from blood samples (40), urine (57), lower respiratory secretions (12) and rectal swabs (56) from patients hospitalized at INEN.

Antimicrobial identification and measurement of susceptibility were carried out with the Phoenix M50 automated system. ESBLs were confirmed with the BD-Expert system (BD Diagnostics, Sparks, MD). We interpreted the results following the recommendations of the Clinical and Laboratory Standards Institute (CLSI)<sup>(13)</sup>. Colistin resistance was measured with Colistin Agar-Spot (CAS) screening (Mueller-Hinton Agar [Merck, Germany]), a method developed by the Antimicrobial Service, INEI ANLIS “Dr. Carlos G. Malbrán”<sup>(14)</sup> which uses colistin sulfate (Sigma-Aldrich, Germany). For detecting *mcr* gene, we used the phenotypic method of colistin pre-diffusion and inhibition with ethylenediaminetetraacetic acid (EDTA) (CPD-E) (Mueller-Hinton Agar [Merck, Germany]). This method was described by Yauri *et al.*<sup>(15)</sup> and uses 10 µg colistin discs (Oxoid, England); 372/900 µg and EDTA/SMA discs (Britannia, Argentina).

Total bacterial DNA was used as a mold for the molecular detection carried out in the laboratory of Molecular Epidemiology and Genetics of the Institute of Tropical Medicine “Daniel A. Carrión” in the Center for Technological, Biomedical and Environmental Research - CITBM of Universidad Nacional Mayor de San Marcos. For the identification of the resistance genes (*mcr-1*, *bla*<sub>CTX-M</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub>) we used the polymerase chain reaction (PCR) as described above<sup>(4,16,17)</sup> (Figure 1).

## KEY MESSAGES

**Motivation for the study:** Resistance to last-resort drugs against multidrug-resistant (MDR) bacteria is a cause for concern. It is necessary to investigate the resistance to colistin in extended-spectrum beta-lactamase-producing enterobacterales (ESBL-PE) to know the situation of this type of microorganisms in our environment.

**Main findings:** From ESBL-PE isolates, 15.2% were positive for *mcr-1* gene, presented resistance to fluoroquinolones and gentamicin, and remained sensitive to amikacin; two of these isolates were producers of metallo-carbapenemases.

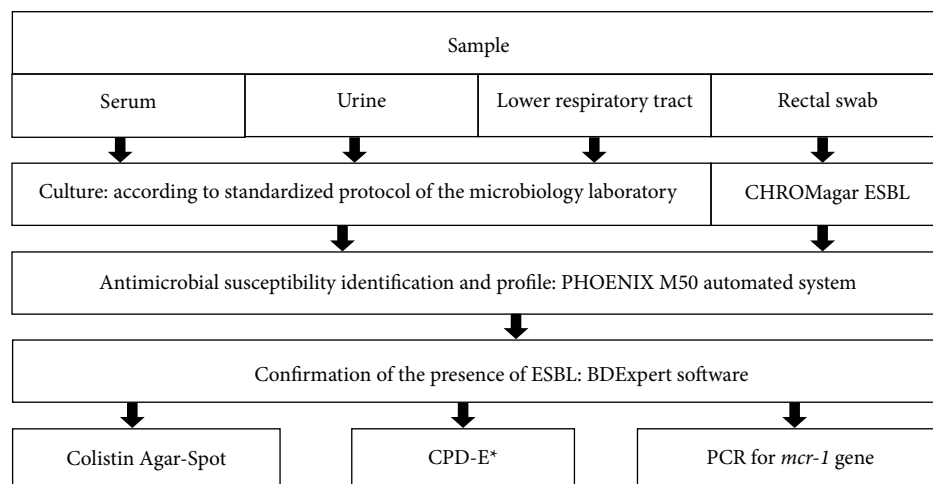
**Implications:** The results of this study show the importance of the data collection regarding plasmid-mediated colistin resistance in MDR bacteria, which supports the need for the implementation of molecular epidemiology studies for an effective control and to avoid dissemination in the hospital environment

This report has been conducted as part of the study by the Science and Technology Research Circles of the Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT), approved by the INEN Research Department. This study follows the guidelines of good practice and ethics in biomedical research. The variables of interest were expressed as absolute and relative frequencies. We used Microsoft Excel for the descriptive analysis.

## FINDINGS

Of the 165 ESBL-PE isolates, 25 (15.2%) were positive for the *mcr-1* gene; 20 (12.1%) were resistant to colistin by the CAS screening method. Phenotypic detection of *mcr* with the CPD-E method had 100% correlation with the genotypic method (25 positive). The presence of the *mcr-1* gene, per species, was as follows: *E. coli* (18), *K. pneumoniae* (4), *E. cloacae* (2) and *K. oxytoca* (1). Depending on the type of sample, *mcr-1* was detected more frequently in isolates from rectal swabs (11/25) and in blood (9/25) (Table 1).

In addition, the susceptibility profile of ESBL-PE carrying the *mcr-1* gene showed resistance to fluoroquinolones and gentamicin and remained sensitive to amikacin. Notably, two isolates of *K. pneumoniae* showed resistance to carbapenems due to the presence of metallo-carbapenemases (New Delhi Metal Beta-Lactamase [NDM]) (Figure 2).



\*CPD-E: pre-diffusion of colistin and inhibition with EDTA.

**Figure 1.** Detection of extended-spectrum beta-lactamase-producing enterobacteriales carrying the *mcr-1* gene.

## DISCUSSION

PMCR due to the *mcr-1* gene has been widely reported throughout the world and in some cases has been related to other resistance markers, such as beta-lactamases (ESBL or carbapenemases) (2,7,11,18-20). In our study, 15.2% of the isolates recovered from samples of different infections and colonizations (carriers) in hospitalized patients presented ESBL-PE carrying the *mcr-1* gene. In 2017, Ugarte *et al.* (12) made the first Peruvian report about *mcr-1* on seven *E. coli* isolates recovered from urine cultures of community patients. In 2019, Deshpande *et al.* (11) reported three *E. coli* isolates producing *mcr-1* recovered in Peru, 2016 from blood and skin infection samples, one of the isolates was a producer of ESBL ( $bla_{CTX-M-55}$ ).

The presence of beta-lactamase-producing enterobacteriales carrying the *mcr-1* gene is increasing in the region (18-20),

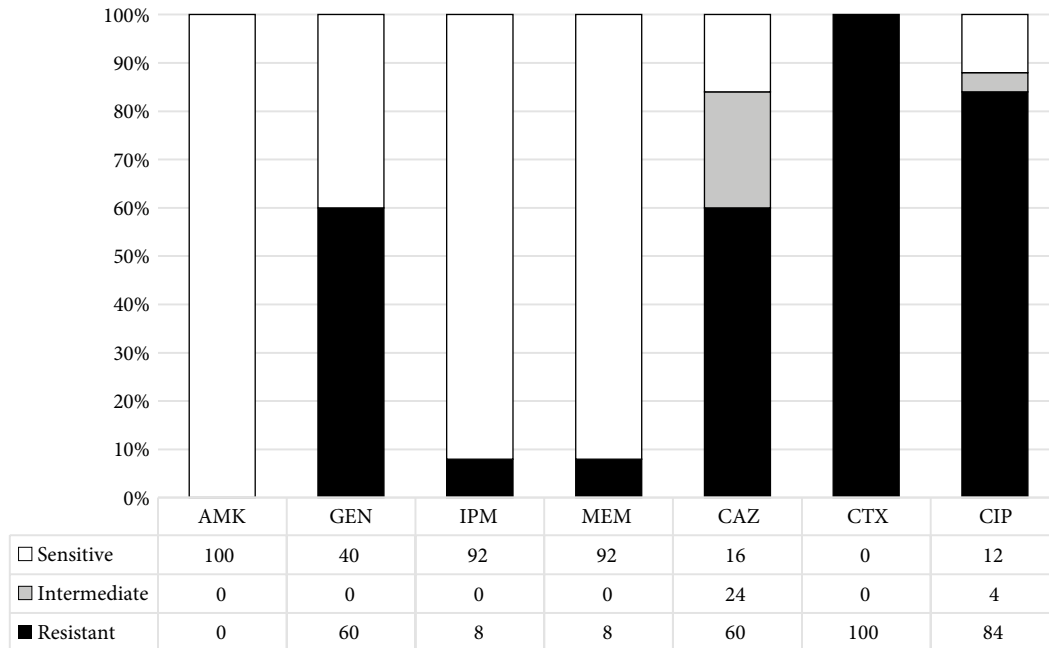
not only co-producers of ESBL, but also of carbapenemases, as observed in two of our MDR *K. pneumoniae* isolates, with resistance ranging from beta-lactams (CTX-M, NDM), fluoroquinolones, gentamicin and even colistin, which is considered an antibiotic of last resort against MDR bacteria, leaving only amikacin as a therapeutic alternative.

The CAS screening method shows a 99.5% accuracy compared to the broth microdilution method, considered as the reference method for the determination of colistin resistance. In our study, colistin resistance was detected in 17/25 *mcr-1*-producing isolates, probably because these isolates had a minimum inhibitory concentration (MIC) <3 µg/mL, which is the colistin concentration used by the CAS screening method (14).

A *K. pneumoniae* isolate classified as colistin resistant did not carry the *mcr-1* gene; its resistance to colistin could be related to mutations in genes of the PhoPQ-PmrAB regu-

**Table 1.** Distribution of isolates of extended-spectrum beta-lactamase-producing enterobacteriales carrying the *mcr-1* gene by sample type and source.

Source	Sample type				Total
	Serum	Urine	Low respiratory secretions	Rectal swab	
Abdomen	1	1	1	-	3
Gynecology	-	1	-	-	1
Oncology	3	1	-	10	14
Pediatric Oncology	4	1	-	-	5
Thorax and breast	-	-	-	1	1
Urology	1	-	-	-	1



AMK: amikacin; GEN: gentamicin; IPM: imipenem; MEM: meropenem; CAZ: ceftazidime; CTX: cefotaxime; CIP: ciprofloxacin.

\* 22 isolates were carriers of bla<sub>CTX-M</sub> genes, and 2 isolates were also carriers of bla<sub>NDM</sub> genes.

**Figure 2.** Antimicrobial susceptibility profile of extended-spectrum beta-lactamase-producing enterobacterales carrying the *mcr-1* gene (n = 25)\*.

latory system<sup>(2,3)</sup>; or to the presence of allelic variants of the *mcr* gene not investigated in this study<sup>(5,6)</sup>. In 2020, the CLSI changed the cut-off points for the interpretation of colistin in enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* spp., which can now only be classified in two categories: intermediate ( $\leq 2 \mu\text{g/mL}$ ) and resistant ( $\geq 4 \mu\text{g/mL}$ ). The CLSI also specifies that the only acceptable methodologies are broth microdilution for colistin (BMD), colistin agar test (CAT) and colistin broth disc elution (CBDE)<sup>(13)</sup>.

The CPD-E method<sup>(15)</sup> is based on the property of the zinc-dependent metalloprotein of the phosphoethanolamine transferase encoded by the *mcr-1* gene, which allows it to be inhibited by chelators, such as EDTA. Therefore CPD-E method had an optimal correlation with *mcr-1* gene carrier isolates, proving to be a good phenotypical alternative for detecting MCR producers.

The presence of the *mcr-1* gene in clinical MDR isolates that caused infections is alarming, the gastrointestinal presence of bacteria with this gene has already been demonstrated in hospital or community environment<sup>(10)</sup>. In our study, the frequency of colonization with ESBL PE carriers of *mcr-1* gene was 44%; this colonization is very dangerous, since plasmids may be the cause of its dissemination to other to other virulent strains or epidemic clones<sup>(10)</sup>.

This study has some limitations. The results obtained correspond to a collection of MDR isolates (producers of ESBL) from a specialized health institution and cannot be extrapolated to other institutions. It is necessary to evaluate the presence of *mcr-1* gene with a higher number of non-MDR isolates from different hospitals to know the real impact of this resistance marker in our country. Besides, we did not analyze allelic variants different from the *mcr-1* gene and other causes of resistance to colistin, such as mutations in the genes of the regulatory system of lipid A from the bacterial membrane.

In conclusion, data collection on bacterial resistance to last-resort antimicrobials is crucial to establish policies according to the local context and to compare them at regional and global level. Although our results show the panorama of a single health institution, the appearance of MDR isolates with colistin resistance supports the need to carry out molecular epidemiology studies to prevent the establishment of healthcare-associated infections by this type of microorganisms.

**Authorship contributions:** KYC and EGE collected data and samples, and conceived the article. MZA, CRSA and JPS contributed to the idea for the research, as well as to the writing, and technical and

administrative assistance. CBE and WVT participated in the data and study material collection and writing of the article. All authors conducted the critical review of the article, approved the final version and assumed responsibility for the contents of the manuscript.

**Acknowledgements:** To the staff of the Laboratory of Microbiology of Instituto Nacional de Enfermedades Neoplásicas for their collaboration in the process of collecting the isolates; to the research team of Biotechnology and Health of Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales for their contribution

in the molecular biology; and to the staff of the Laboratory of Molecular Epidemiology and Genetics of Universidad Nacional Mayor de San Marcos for their continuous support.

**Funding:** Study funded by FONDECYT for the Science and Technology Research Circles (RD No. 142-2015-FONDECYT-DE) and by the Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales - CITBM.

**Conflicts of Interest:** The authors have no conflict of interest to declare.

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