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Antimicrobial Susceptibility Studies

Low level of polymyxin resistance among nonclonal *mcr*-1–positive *Escherichia coli* from human sources in Brazil

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ABSTRACT

We report 26 human isolates of *mcr*-1–positive *Escherichia coli,* most of them (65.4%) with a polymyxin B MIC of 2 mg/L. Seventeen out of the 24 *mcr*-1–positive *E. coli* proved to be nonclonal by rep-PCR which strengthens the hypothesis of environmental or animal origin of these strains and reinforces the one health context of antimicrobial resistance.

Polymyxin resistance is being increasingly reported in clinical isolates of *Enterobacteriaceae*. This resistance is usually related to chromosomal mutations, but recently, it was also associated to a gene located in a plasmid, the *mcr*-1 gene (Liu et al., 2016). MCR-1 was originally detected in *Escherichia coli* isolates from food animals, food, and patients in China. The *mcr*-1 gene was further identified in isolates causing infections in humans and has been reported among other species of *Enterobacteriaceae* (Castanheira et al., 2016). In Brazil, the *mcr*-1 gene was first reported among chicken and pork isolates of *E. coli* on April 2016 (Fernandes et al., 2016b), and the first clinical isolate was reported on October 2016 (Fernandes et al., 2016a). Moreover, very recently, the *mcr*-1 gene was reported in a high-risk clone of KPC-2–producing *Klebsiella pneumoniae* from a rectal swab of a patient hospitalized at an emergence room in southern Brazil (Dalmolin et al., 2017).

In this context, we standardized a real-time polymerase chain reaction (PCR) assay to detect the *mcr-1* gene among clinical isolates received at a public health laboratory (LACEN-PR) in the Paraná State, Brazil. The *mcr*-1–positive isolates were also submitted to molecular typing by rep-PCR.

Between August 2016 and October 2017, a total of 2150 isolates of Gram-negative rods previously defined as resistant to carbapenems and/or polymyxins by phenotypic methods were referred to LACEN/PR by different laboratories in the state of Paraná/Brazil for resistance genes detection. The majority of the isolates (84%) belonged to the species *K. pneumoniae* (1438–66.9%) and *E. coli* (368–17.1%). The identification of all isolates was confirmed by MALDI-TOF–Vitek MS® (bioMérieux, France). Antibiotic susceptibility of carbapenems (meropenem and imipenem) and polymyxin B was determined by the Vitek 2® system (bioMérieux) and by broth microdilution, respectively, according to EUCAST (2018). Extended-spectrum beta-lactamase (ESBL) presence was determined by a phenotypic evaluation using VITEK 2®. The DNA of all isolates was extracted and subjected to PCR with specific primers for *mcr*-1, according to Chabou et al. (2016), and for bla_{KPC} and bla_{NDM}, according to CDC (https://www.cdc.gov/hai/settings/lab/kpc-ndm1-lab-protocol.html). The *mcr*-1–positive isolates were further evaluated using rep-PCR (DiversiLab®, bioMeriéux). The relatedness was determined by cluster analysis, and the isolates were considered clonally related when they presented ≥95% of similarity (Fluit et al., 2010).

The *mcr-1* gene was detected in 26/368 (7.1%) *E. coli* isolates from 19 patients from 5 different cities at Paraná State (Curitiba, Londrina, Cascavel, Irati, and Maringa). None of the *mcr*-1–positive isolates were positive for bla_{KPC} or bla_{NDM} genes. The polymyxin-B MIC of *mcr*-1–positive isolates indicated that the majority (17/26; 65.4%) was

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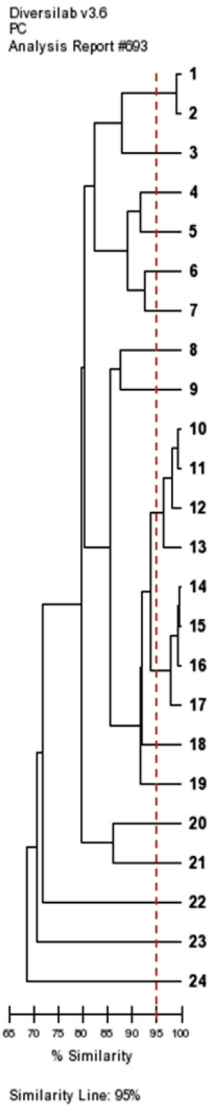
susceptible (MIC ≤2 mg/L). The remaining 9 isolates presented a borderline MIC of 4 mg/L. Most *mcr*-1–positive strains (18/26) exhibited a positive result for ESBL, and almost all of them (25/26) were susceptible to carbapenems. Only 1 isolate of *E. coli* showed resistance to ertapenem and susceptibility to meropenem and imipenem, being detected as an ESBL-producing carbapenemase-negative strain.

The molecular typing of 24/26 *E. coli* harboring the *mcr-1* gene indicated that no clonal relation was observed among 17 isolates. Only isolates from the same patient were clonally related, with the exception of the clonal group #9, which comprised isolates from 2 different patients from 2 different cities (Fig. 1). The ertapenem-resistant *E. coli* isolate was not evaluated for clonality.

Polymyxins are one of the last resorts for treatment of carbapenem-resistant *Enterobacteriaceae* infections, and the emergence of polymyxin resistance seriously limits treatment options. This is a major concern because KPC and NDM carbapenemases are widely present at many hospitals globally and whether these isolates acquire the *mcr*-1 plasmid they would become untreatable. There is a growing concern about the rising in colistin usage due to the increase of carbapenem-resistant bacteria, that are susceptible only to this drug. This pressure is leading to the selection of polymyxin-resistant isolates. There is no consensus yet about the ideal treatment for infections due to polymyxins resistant isolates since only limited antibiotic options are available. In addition, new drugs for CRE have been tested such as cefatzidime-avibactam, but their use in polymyxin-resistant CRE is still uncertain. Besides that, the use of polymyxins in cases of infections due to isolates presenting the *mcr*-1 gene in vitro is not recommended due to therapeutic failure (Paul et al., 2018; van Duin et al., 2018).

According to our findings, *E. coli* strains harboring *mcr*-1 have been present in Paraná State (Brazil) since October 2016, the same time of the first report of the presence of this gene in Brazil, highlighting the potential of *mcr-1* to continuously spread (Fernandes et al., 2016a, 2016b). However, the fact that we found a high clonal diversity of the *mcr*-1–positive isolates of *E.coli* by rep-PCR strengthens the hypothesis that these isolates present environmental or food/animal origin. Although we have found the *mcr*-1 gene in 26/368 (7.1%) of the *E. coli* isolates, this may not represent a true prevalence since the isolates sent to LACEN-PR have a potential bias: they were supposedly resistant to carbapenems and/or polymyxins.

We did not detect KPC nor NDM in our *mcr*-1–harboring *E. coli* strains, but the co-ocurrence of carbapenemase (*bla*KPC and *bla*NDM) and the *mcr-1* gene has already been described in *E. coli* and *K. pneumoniae* in southern Brazil (Dalmolin et al., 2017). This emphasizes the need for continuous surveillance programs to identify the



	P1	Isolate	Patient	Hospital	City	Sample	Polymyxin-B MIC[2]	ESBL[3]	Carbapenems[4]
1	1	14459RM	P1	1	Londrina	Sterile fluid	2 mg/L	+	S
2	1	14460RM	P1	1	Londrina	Prosthesis	4mg/L	+	S
3	2	14071RM	P2	2	Curitiba	Prosthesis	4 mg/L	+	S
4	3	17256RM	P3	3	Curitiba	Rectal swab	2 mg/L	+	S
5	4	13891RM	P4	4	Cascavel	Rectal swab	2 mg/L	-	S
6	5	17185RM	P5	5	Curitiba	Rectal swab	4 mg/L	+	S
7	6	17252RM	P6	6	Curitiba	Rectal swab	2 mg/L	+	S
8	7	17084RM	P7	7	Londrina	Urine	4 mg/l	+	S
9	8	14571RM	P8	4	Cascavel	Rectal swab	4 mg/L	-	S
10	9	15088RM	P9	10	Curitiba	Rectal swab	2 mg/L	+	S
11	9	15486RM	P9	10	Curitiba	Urine	2 mg/L	+	S
12	9	15487RM	P9	10	Curitiba	Rectal swab	4 mg/L	+	S
13	9	14005RM	P10	7	Londrina	Urine	4 mg/L	+	S
14	10	16879RM	P11	8	Londrina	Urine	2 mg/L	-	S
15	10	16974RM	P11	8	Londrina	Urine	2 mg/L	-	S
16	10	17217RM	P11	8	Londrina	Urine	2 mg/L	-	S
17	10	16720RM	P11	8	Londrina	Urine	2 mg/L	-	S
18	11	14075RM	P12	2	Curitiba	Rectal swab	2 mg/L	+	S
19	12	15796RM	P13	3	Curitiba	Rectal swab	2 mg/L	+	S
20	13	15784RM	P14	9	Curitiba	Rectal swab	2 mg/L	-	S
21	14	14937RM	P15	3	Curitiba	Rectal swab	2 mg/L	+	S
22	15	16452RM	P16	10	Curitiba	Rectal swab	2 mg/L	+	S
23	16	14462RM	P17	11	Irati	Purulent discharge	4 mg/L	-	S
24	17	16939RM	P18	12	Maringa	Rectal swab	4 mg/L	+	S

Fig. 1. Dendrogram representing the clonal relatedness and antimicrobial resistance characteristic of *E. coli* harboring the *mcr-1* gene. [1] Clonal group. [2] MIC: Minimum Inhibitory Concentration (mg/L). [3] (+) positive; (−) negative. [4] (S) susceptible; (R) resistant.

risk of bacteria with these genes to human health. Noteworthy, most of the *mcr*-1–positive isolates from our study presented borderline susceptibility (MIC = 2 mg/L) or low-level resistance to polymyxin B (MIC = 4 mg/L), reinforcing the importance of a sensitivity and precise method for determining the polymyxin MICs of potential MCR-1 isolates such as broth microdilution, especially in surveillance studies.

Finally, molecular detection of *mcr*-1 should be considered as part of any antimicrobial resistance surveillance program because, according to our results, most of the *mcr*-1–positive isolates presented susceptibility or low-level resistance to polymyxins, which may hinder their detection. The presence of the *mcr-1* gene in nonclonal *E. coli* from patients from the community, which have never been hospitalized, highlights its supposed environmental/animal origin.

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