Supplementary materials for the article:

ElBaradei A. et al. Screening of *mcr-1* among Gram-Negative Bacteria from Different Clinical Samples from ICU Patients in Alexandria, Egypt: One-Year Study
Pol J Microbiol. 2022, Vol. 71, No 1, 83–90

Details of the pilot study

Determination of *mcr-1* positive control. To screen for *mcr-1* gene, using real-time PCR, a positive control is required. So, we investigated the presence of *mcr-1* among 13 colistin-resistant *Klebsiella pneumoniae* isolates. It was performed using conventional PCR and the primers CLR5-F: CGGTCAGTCCGTT TGTTC and CLR5-R: CTTGGTCGGTCTGTAGGG (Liu et al. 2016). The susceptibility testing was performed using the broth microdilution method for colistin, and according to the disk diffusion method for the remaining antibacterial agents; all tests were done according to CLSI 2020 guidelines (CLSI 2020). The details of the antibiotic susceptibilities are shown in supplementary Table SI.

The amplification scheme was as follows: 3 minutes activation at 95°C, followed by 30 cycles of: denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, and a final elongation step of 72°C for 7 minutes. The Veriti thermal cycler (Applied Biosystems, California, USA) and DreamTaq Green PCR Master Mix (Thermofischer, California, USA) were used. The whole gene was further sequenced using both forward and reverse primers on the ABI 3730xl DNA sequencer (Applied Biosystems, California, USA). The sequence obtained was deposited in GenBank (accession number: MZ820397).

For confirmation, the whole gene was amplified and sequenced using primers SQmcr-1F: CTCATGATGCAGCATACTTC and SQmcr-1R: CGAATGGAGTGTGCGGTG, (Elnahriry et al. 2016), and blastn tool was used. It was confirmed that it is *mcr-1.1*. The obtained sequence was deposited in GenBank (accession number: MZ820392).

Table SI

	Organism	Colistin	FEP	CAZ	IPM	MEM	AK	CN	TOB	CIP	SXT	*mcr-1*
1	*Klebsiella pneumoniae*	R	R	R	I	S	R	R	R	R	R	Negative
2	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	R	Negative
3	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	R	Negative
4	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	S	Negative
5	*Klebsiella pneumoniae*	R	R	R	I	R	R	R	R	R	S	Negative
6	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	S	Negative
7	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	S	Negative
8	*Klebsiella pneumoniae*	R	R	R	I	R	R	R	R	R	S	Negative
9	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	R	Negative
10	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	R	Negative
11	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	S	Negative
12	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	S	Negative
13	*Klebsiella pneumoniae*	R	S	R	S	S	S	R	S	R	S	Positive

FEP – cefepime, CAZ – ceftazidime, IPM – imipenem, MEM – meropenem, AK – amikacin, TOB – tobramycin, CIP – ciprofloxacin, SXT – trimethoprim/sulfamethoxazole

Nucleotide sequence accession numbers. In this study, nucleotide sequences were deposited in the GenBank database under accession numbers MZ820389 to MZ820401 (all links are shown in the table SII).

Table SII
The accession numbers and their corresponding weblinks.

Accession number	Links
MZ820389	https://www.ncbi.nlm.nih.gov/nuccore/MZ820389.1/
MZ820390	https://www.ncbi.nlm.nih.gov/nuccore/MZ820390.1/
MZ820391	https://www.ncbi.nlm.nih.gov/nuccore/MZ820391.1/
MZ820392	https://www.ncbi.nlm.nih.gov/nuccore/MZ820392.1/
MZ820393	https://www.ncbi.nlm.nih.gov/nuccore/MZ820393.1/
MZ820394	https://www.ncbi.nlm.nih.gov/nuccore/MZ820394.1/
MZ820395	https://www.ncbi.nlm.nih.gov/nuccore/MZ820395.1/
MZ820396	https://www.ncbi.nlm.nih.gov/nuccore/MZ820396.1/
MZ820397	https://www.ncbi.nlm.nih.gov/nuccore/MZ820397.1/
MZ820398	https://www.ncbi.nlm.nih.gov/nuccore/MZ820398.1/
MZ820399	https://www.ncbi.nlm.nih.gov/nuccore/MZ820399.1/
MZ820400	https://www.ncbi.nlm.nih.gov/nuccore/MZ820400.1/
MZ820401	https://www.ncbi.nlm.nih.gov/nuccore/MZ820401.1/

Literature

CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne (USA): Clinical and Laboratory Standards Institute; 2020.

Elnahriry SS, Khalifa HO, Soliman AM, Ahmed AM, Hussein AM, Shimamoto T, Shimamoto T. Emergence of plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate from Egypt. Antimicrob Agents Chemother. 2016 May;60(5):3249–3250. https://doi.org/10.1128/AAC.00269-16

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, 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 Feb;16(2):161–168. https://doi.org/10.1016/S1473-3099(15)00424-7