ORIGINAL PAPER

Polish Journal of Microbiology
2022, Vol. 71, No 1, 83–90
https://doi.org/10.33073/pjm-2022-011

Screening of *mcr-1* among Gram-Negative Bacteria from Different Clinical Samples from ICU Patients in Alexandria, Egypt: One-Year Study

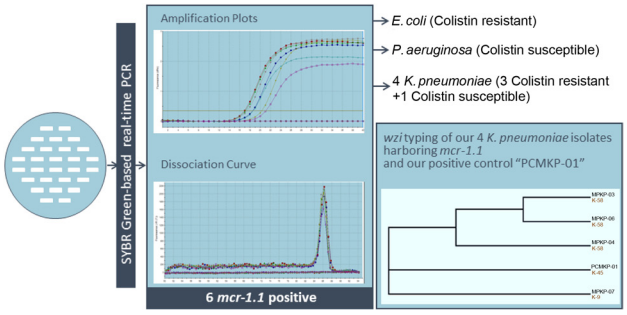
AMIRA ELBARADEI[1, 2]*, MAHROUS S. SAYEDAHMED[3], GAMAL EL-SAWAF[3] and SHERINE M. SHAWKY[3]

[1] Department of Microbiology and Immunology, Faculty of Pharmacy, Pharos University in Alexandria, Alexandria, Egypt
[2] Alexandria University Hospital, Alexandria University, Alexandria, Egypt
[3] Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt

Submitted 10 November 2021, accepted 12 February 2022, published online 22 March 2022

Abstract

Antimicrobial resistance represents a global dilemma. Our present study aimed to investigate the presence of *mcr-1* among different Gram-negative bacteria including *Enterobacteriaceae* (except intrinsically resistant to colistin) and *Pseudomonas aeruginosa*. Gram-negative bacterial isolates were collected from different ICUs in several Alexandria hospitals from June 2019 to June 2020. The identification of these Gram-negative isolates was made using the VITEK-2® system (BioMérieux, France). SYBR Green-based PCR was used to screen for the presence of *mcr-1* using a positive control that we amplified and sequenced earlier in our pilot study. All isolates were screened for the presence of *mcr-1* regardless of their colistin susceptibility. Isolates that harbored *mcr-1* were tested for colistin susceptibility and for the presence of some beta-lactamase genes. *Klebsiella pneumoniae* isolates harboring *mcr-1* were capsule typed using the *wzi* sequence analysis. Four hundred eighty isolates were included in this study. Only six isolates harbored *mcr-1.1*. Of these, four were resistant to colistin, while two (*K. pneumoniae* and *P. aeruginosa*) were susceptible to colistin. Five of the six isolates were resistant to carbapenems. They harbored bla_{OXA-48}, and three of them co-harbored bla_{NDM-1}. K-58 was the most often found among our *K. pneumoniae* harboring *mcr-1.1*. To our knowledge, this is the first time to report colistin susceptible *P. aeruginosa* and *K. pneumoniae* harboring the *mcr-1.1* gene in Egypt. Further studies are needed to investigate the presence of the *mcr* genes among colistin susceptible isolates to shed more light on its significance as a potential threat.



K e y w o r d s: colistin, *K. pneumoniae*, *mcr-1*, SYBR Green, *wzi* typing

Introduction

Resistance to antibacterial agents represents a global threat. Infections caused by multi-drug resistant (MDR) and extensively drug-resistant (XDR) bacteria are growing, representing a major therapeutic challenge. MDR bacteria are defined as those resistant to at least one agent in at least three distinct categories of antibacterial agents. In comparison, XDR bacteria are defined as those resistant to at least one agent in all categories except for two or fewer categories of antibacterial agents (Magiorakos et al. 2012) Currently, colistin remains one of the last resort treatments against these infections (El-Sayed Ahmed et al. 2020; Wang et al. 2020b). Colistin was first used in the 1950s to treat infections caused by Gram-negative bacteria. Then, in the 1970s, it was replaced by other newly discovered antimicrobial agents, which did not have the toxic effects caused by colistin (El-Sayed Ahmed et al. 2020). However, due to the increased resistance to all available antibacterial agents, colistin has resurfaced, in the 1990s, as the last line of defense against infections caused by MDR and XDR Gram-negative bacteria, although its safety profile has not changed and its dosing problem. There

* Corresponding author: A. ElBaradei, Department of Microbiology and Immunology, Faculty of Pharmacy, Pharos University in Alexandria, Alexandria, Egypt; **e-mail: amiraelbaradei@gmail.com**

is a lack of a universal synchronization of colistin dose units, which leads to suboptimal dosing potentially contributing to the resistance problem (Lim et al. 2010; Ahern and Schnoor 2012; Kaye et al. 2016). In addition to MDR and XDR infections, colistin can be combined with other antibacterial agents to manage pan-resistant Gram-negative bacteria (Sayyahfar et al. 2021).

Interestingly, some Gram-negative bacteria are intrinsically resistant to colistin as *Morganella* spp., *Serratia* spp., *Providencia* spp., and *Proteus* spp. Colistin resistance was thought to be only chromosomally mediated; however, in 2015, the plasmid-mediated resistance to colistin was reported for the first time. Since then, different *mcr-1* alleles have been described; furthermore, ten different *mcr* family genes (*mcr-1*-family gene to *mcr-10*-family gene) have been reported worldwide (Hussein et al. 2021). The emergence of horizontally acquired resistance hampers colistin as a last resort against MDR Gram-negative bacteria (Liu et al. 2016; Sun et al. 2018; El-Sayed Ahmed et al. 2020; Xu et al. 2021). Interestingly, colistin susceptible *Escherichia coli* isolates harboring either *mcr-1* or other *mcr* family genes have been reported (Wang et al. 2017; Chen et al. 2019). The aim of our present study was to investigate the presence of *mcr-1* among different Gram-negative bacteria including *Enterobacteriaceae* (except intrinsically resistant to colistin) and *Pseudomonas aeruginosa*.

Experimental

Materials and Methods

Sample collection. We conducted a one-year prospective study, during which Gram-negative bacterial isolates were consecutively collected over a one-year period, starting from June 2019 till June 2020. Non-duplicate isolates were collected from each patient. Isolates were collected from different ICUs in five major tertiary care hospitals in Alexandria, Egypt. Inclusion criteria included being ≥ 18 years old, minimal length of stay in ICU (five days), previous treatment with antibiotics including carbapenems, and failure of treatment denoted by the persistence of signs and symptoms of infection. We excluded *Serratia marcescens*, *Providencia* spp., and *Proteus* spp. because of their intrinsic resistance to colistin. These Gram-negative isolates were identified using the VITEK-2® system (BioMérieux, France).

Antimicrobial susceptibility testing. Susceptibility testing was performed using the disk diffusion method. Antimicrobial agents used were cefepime (FEP), ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), and trimethoprim/sulfamethoxazole (SXT), according to the CLSI guidelines (CLSI 2020). Colistin susceptibility testing was not performed at this point to ensure blind screening for the *mcr-1* gene. Antibiotic disks and culture media were purchased from Oxoid (Cambridge, UK).

Screening for the *mcr-1* gene among Gram-negative bacterial isolates. All collected Gram-negative isolates were screened for the presence of the *mcr-1* gene using SYBR Green-based real-time PCR. The primers used were CLR5-F: CGGTCAGTCCGTTTGTTC and CLR5-R: CTTGGTCGGTCTGTAGGG (Liu et al. 2016). Isolate "PCMKP-01" was used as a positive control. This isolate was found to harbor *mcr-1* (amplified and sequenced earlier in a pilot study that we conducted prior to the start of this study, and the details are shown in Supplementary materials).

Real-time PCR was performed on Stratagene Mx3000P (Agilent Technologies California, USA) using PowerUp SYBR Green Master Mix (Thermo Fischer, California, USA). The thermal profile was: activation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute, followed by melting curve analysis (95°C for 20 seconds, 50°C for 1 minute and 95°C for 20 seconds). For confirmation, any amplicon obtained using the primers mentioned above, was subsequently sequenced using an ABI 3730xl DNA sequencer (Applied Biosystems, California, USA).

Confirmation of identification and subtyping of the *mcr-1* gene. To confirm the specific subtype of the *mcr-1* gene, we amplified the whole *mcr-1* gene using the conventional PCR, and the amplicon size was 1,672 bp. The primers used were: SQmcr-1F: CTCATGATGCAGCATACTTC and SQmcr-1R: CGAATGGAGTGTGCGGTG (Elnahriry et al. 2016). The amplification scheme was: 4 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 90 seconds, and a final elongation step of 72°C for 7 minutes using the Veriti thermal cycler (Applied Biosystems, California, USA), and DreamTaq Green PCR Master Mix (Applied Biosystems, California, USA). Sequencing was performed using the same primers (forward and reverse) with the ABI 3730xl DNA sequencer (Applied Biosystems, California, USA).

Determination of colistin susceptibility. Isolates that were found to harbor *mcr-1* were then tested for colistin susceptibility using the broth microdilution method according to CLSI (CLSI 2020).

Capsule typing of *Klebsiella pneumoniae* isolates. *K. pneumoniae* isolates, which were found to possess *mcr-1* and our isolate "PCMKP-01" were subsequently subjected to capsule typing. The *wzi* gene was amplified and sequenced using primers wzi-F: GTGCC GCGAGCGCTTTCTATCTTGGTATTCC and wzi-R:

GAGAGCCACTGGTTCCAGAA[C or T]TT[C or G] ACCGC (Brisse et al. 2013). Sequencing was performed using the ABI 3730xl DNA sequencer (Applied Biosystems, California, USA). Typing was performed using the K-PAM platform (https://www.iith.ac.in/K-PAM/pred_sertp.php) (Patro et al. 2020).

Genotypic detection of different beta-lactamase genes. All isolates that harbored the *mcr-1* gene were further investigated for the presence of the $bla_{\text{CTX-M}}$ gene (encoding for ESBL), using conventional PCR on the Veriti thermal cycler (Applied Biosystems, California, USA), and DreamTaq Green PCR Master Mix (Applied Biosystems, California, USA). The primers used were F: CGCTTTGCGATGTGCAG, and R: ACCGCGATATCGTTGGT (Gröbner et al. 2009). Then, the isolates that harbored *mcr-1* and were carbapenem-resistant were further investigated for the presence of serine carbapenemase genes (bla_{KPC} and $bla_{\text{OXA-48}}$), and metallo-beta-lactamases genes (bla_{VIM} and $bla_{\text{NDM-1}}$). The primers used were as follows: F: TGTCACTGTATCGCCGTC, and R: CTCAGTGCTCTACAGAAAACC for bla_{KPC} (Wang et al. 2012); F: AAATCACAGGGCGTAGTTGTG, and R: GACCCACCAGCCAATCTTAG for $bla_{\text{OXA-48}}$; F: AGTGGTGAGTATCCGACAG, and R: ATGAAAGTGCGTGGAGAC for bla_{VIM} (Gröbner et al. 2009); and F: GGTTTGGCGATCTGGTTTTC, and R: CGGAATGGCTCATCACGATC) for $bla_{\text{NDM-1}}$ (Nordmann et al. 2011).

Results

A total of 480 Gram-negative isolates were collected from June 2019 to June 2020. Most of the collected isolates were *K. pneumoniae* (62.71%), followed by *E. coli* (22.71 %), *P. aeruginosa* (11.46%), *Enterobacter cloacae* complex, and *Enterobacter asburiae* represented (2.92%) and (0.20 %) of the isolates, respectively. These isolates were collected from different types of clinical samples, including blood cultures (26%), urinary tract infections (17%), aspirates and swabs from surgical site infections (fluid and tissues) (31%), sterile body fluids, including (CSF, pleural fluid and perineal fluid) (2%), and respiratory tract infections (24%). Regarding the susceptibility testing results, resistance to the third and fourth generation cephalosporins was 82.29% and 78.33%, respectively, and they were distributed as follows: *K. pneumoniae* 92.36% and 87.38%, *E. coli* 78.9% and 77.06%, *E. cloacae* complex 64.29% and 71.43%, and *P. aeruginosa* 40% and 34.5%, respectively. Moreover, 49.79% of the isolates were resistant to MEM. They were distributed as follows: *K. pneumoniae* 68.44%, *E. coli* 6.42%, *E. cloacae* complex 21.43%, and *P. aeruginosa* 41.82%. Most of the Gram-negative organisms were resistant to CAZ and FEP, and almost half of the isolates were resistant to carbapenems and gentamicin. Among 480 isolates, 338 (70.4%) were MDR Gram-negative bacteria; of these seven isolates belong to *E. cloacae* complex, 25 were *P. aeruginosa*, 60 were *E. coli*, while 246 of them were *K. pneumoniae* isolates. The susceptibility testing results are shown in (Table I).

Among 480 Gram-negative bacterial isolates, only six (1.25%) harbored the *mcr-1* gene, while 474 (98.75%) did not harbor that gene.

The *mcr-1* gene was sequenced, then the BLASTN (https://blast.ncbi.nlm.nih.gov) tool for was used for confirmation. All isolates were found to harbor *mcr-1.1*. The obtained sequences were deposited in GenBank (accession numbers: MZ820395, MZ820396, MZ820398, MZ820399, MZ820400, MZ820401).

Table I
Resistance profiles of different isolates of Gram-negative bacteria.

Antimicrobial	*Klebsiella pneumoniae* (n = 301)		*Escherichia coli* (n = 109)		*Enterobacter cloaclae* complex (n = 14)		*Enterobacter asburiae** (n = 1)		*Pseudomonas aeruginosa* (n = 55)	
	Resistant		Resistant		Resistant		Resistant		Resistant	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ceftazidime	278	92.36	86	78.90	9	64.29	0	0	22	40.00
Cefepime	263	87.38	84	77.06	10	71.43	0	0	19	34.55
Imipenem	206	68.44	6	5.50	2	14.29	0	0	24	43.64
Meropenem	206	68.44	7	6.42	3	21.43	0	0	23	41.82
Amikacin	180	59.80	4	3.67	0	0	0	0	19	34.55
Gentamicin	177	58.80	36	33.03	6	42.86	0	0	24	43.64
Tobramycin	232	77.08	38	34.86	3	21.43	0	0	24	43.64
Ciprofloxacin	217	72.10	69	63.30	3	21.43	0	0	26	47.27
Trimethoprim/ Sulfamethoxazole	231	76.74	59	54.13	6	42.86	0	0	–	–

* – the isolate was susceptible to all these antibacterial agents

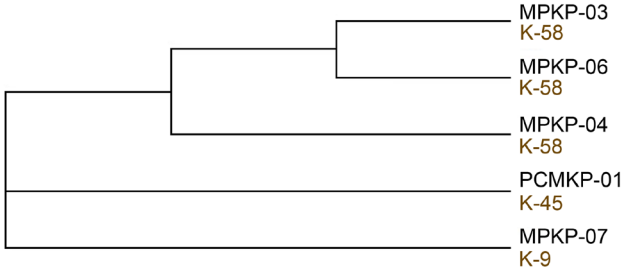


Fig. 1. Phylogenetic tree of four *Klebsiella pneumoniae* isolates and the positive control "PCMKP-01" harboring *mcr-1.1* based on the *wzi* typing.

Then, to confirm the *mcr-1* gene subtype, we amplified and sequenced the whole *mcr-1* gene of all isolates; however, it could not be amplified in one *K. pneumoniae* isolate using this pair of primers. The obtained sequences were deposited in GenBank (accession number: MZ820389, MZ820390, MZ820391, MZ820393, MZ820394).

Remarkably, two isolates out of six isolates harboring *mcr-1.1* were susceptible to colistin. Moreover, one of them could not be amplified using this pair of primers. However, it was successfully amplified and sequenced using the first primers set, and it was found to harbor *mcr-1.1*. Its sequence was submitted to GenBank, as mentioned earlier.

Then, all *K. pneumoniae* isolates harboring *mcr-1.1* and the isolate "PCMKP-01" were capsule typed using the *wzi* gene sequencing; three were K-58, one K-9, and one K-45. A phylogenetic tree was constructed, and it is shown in Fig. 1.

The characteristics of the six isolates that harbor *mcr-1.1* is shown in Table II. Three of the six isolates harbored $bla_{\text{CTX-M}}$. Five of the isolates were resistant to carbapenems. These five isolates harbored the $bla_{\text{OXA-48}}$ gene, $bla_{\text{NDM-1}}$ was present in three of them, while bla_{VIM} and bla_{KPC} were absent in these isolates. The distribution of the different beta-lactamase genes among the six isolates is shown in Table III.

Discussion

Colistin remains our last resort against MDR and XDR Gram-negative bacteria. Moreover, it has been combined with other antibacterial agents to manage

Table II
Characteristics of six bacterial isolates harboring *mcr-1*.

Organism	Isolate name	k-type	Sample Source	*mcr-1* amplified by first set of primers	*mcr-1* amplified by second set of primers	Patient gender	FEP	CAZ	IPM	MEM	AK	GN	TOB	CIP	SXT	MIC value in µg/ml*
P. aeruginosa	MPPS-05	–	BAL	*mcr-1.1*	*mcr-1.1*	F	R	R	R	I	R	R	R	R	–	≤0.5
K. pneumoniae	MPKP-07	K9	Mini BAL	*mcr-1.1*	–	F	R	R	R	R	R	R	R	R	S	≤0.5
K. pneumoniae	MPKP-03	k58	BAL	*mcr-1.1*	*mcr-1.1*	M	R	R	R	R	R	I	R	R	R	4
K. pneumoniae	MPKP-04	k58	Spinal fluid	*mcr-1.1*	*mcr-1.1*	F	R	R	R	R	R	R	R	I	R	4
K. pneumoniae	MPKP-06	k58	Sputum	*mcr-1.1*	*mcr-1.1*	M	R	R	R	R	R	S	R	R	R	4
E. coli	MPEC-02	–	Blood culture	*mcr-1.1*	*mcr-1.1*	M	R	R	S	S	S	R	R	I	R	≥4

* – colistin MIC was interpreted according to EUCAST guidelines (EUCAST 2020)

Table III
Beta-lactamase genes distribution among the *mcr-1* positive isolates.

Organism	Isolate name	*mcr-1* amplified by first set of primers	*mcr-1* amplified by second set of primers	ESBL Gene	Serine carbapenemases genes		Metallo-beta-lactamase genes	
				$bla_{\text{CTX-M}}$	bla_{KPC}	$bla_{\text{OXA-48}}$	bla_{VIM}	$bla_{\text{NDM-1}}$
P. aeruginosa	MPPS-05	*mcr-1.1*	*mcr-1.1*	–	–	+	–	+
K. pneumoniae	MPKP-07	*mcr-1.1*	–	+	–	+	–	–
K. pneumoniae	MPKP-03	*mcr-1.1*	*mcr-1.1*	+	–	+	–	+
K. pneumoniae	MPKP-04	*mcr-1.1*	*mcr-1.1*	–	–	+	–	+
K. pneumoniae	MPKP-06	*mcr-1.1*	*mcr-1.1*	–	–	+	–	–
E. coli	MPEC-02	*mcr-1.1*	*mcr-1.1*	+	NT	NT	NT	NT

NT – organism was not tested as it was sensitive to carbapenems

infections caused by pan-resistant Gram-negative bacteria (Sayyahfar et al. 2021). Different mechanisms of resistance contribute to the reduced susceptibility to colistin. However, the most worrying mechanism is plasmid-borne MCR-mediated resistance due to its ability to horizontally transfer between different species and the speed with which it is evolving. Several *mcr* family genes have been detected since they were first described in 2015 (Feng 2018; Xu et al. 2021).

Some studies reported the presence of *mcr-1* among *E. coli* isolates susceptible to colistin, which would further complicate the situation in health care settings. Patients infected with organisms harboring the *mcr-1* gene represent a potential threat for *mcr-1* transmission because these organisms could escape being detected by conventional phenotypic methods. Furthermore, other resistance genes (beta lactamases and non-beta lactamases genes) could also be transmitted along with the *mcr-1* gene. (Yuan et al. 2021) This study aimed to investigate the presence of *mcr-1* among different Gram-negative bacteria, including *Enterobacteriaceae* (except intrinsically resistant to colistin) and *P. aeruginosa*.

Using SYBR Green-based real-time PCR, we screened for *mcr-1* among the 480 Gram-negative bacterial isolates. Only 6 (1.25%) isolates harbored this gene, which was confirmed by sequencing of the amplicon obtained. Four of these six isolates were *K. pneumoniae*, one isolate was *E. coli*, and the remaining one was *P. aeruginosa*.

The first report of *mcr-1* from a clinical isolate in Egypt was provided in 2016 by Elnahriry et al. (2016). Then, different studies reported the presence of *mcr-1* among *E. coli* clinical isolates in Egypt, including Anan et al. (2021), who found only four (7.5%) *E. coli* harboring *mcr-1* among colistin-resistant isolates, and El-Mokhtar et al. (2021) who reported that all their studied *E. coli* isolates resistant to colistin carried *mcr-1*. Moreover, another study reported the presence of *mcr-1* in one *E. coli* and one *K. pneumoniae* isolate among their 450 enterobacterial isolates (Zafer et al. 2019).

Furthermore, Elmonir et al. (2021) reported that all colistin-resistant *K. pneumoniae* isolates harbored the *mcr-1* gene. Abd El-Baky et al. (2020) reported the presence of *mcr-1* among their *P. aeruginosa* isolates. Additionally, Yanat et al. (2016) reported the presence of *mcr-1* in *E. coli* clinical isolate in Algeria, Alghoribi et al. (2019) also found *mcr-1* gene in uropathogenic *E. coli* in Saudia Arabia, and *mcr-1* was also reported from *E. coli* clinical isolate in Lebanon (Al-Bayssari et al. 2021). However, some studies could not detect *mcr-1* among colistin-resistant bacterial isolates, Ramadan et al. (2020) did not find any of the eight *mcr* family genes (*mcr-1* to *mcr-8*) among 65 Gram-negative bacterial isolates (Soliman et al. 2020b). In a study in Tunisia, they could not find any of the *mcr* genes, from *mcr-1* to *mcr-5* (Jaidane et al. 2018). Sadek et al. (2020b) reported only one *E. coli* isolate harboring *mcr-1* among 128 colistin resistant *E. coli* strains isolated from meat and meat product samples in Egypt.

In this study, six isolates that harbored *mcr-1.1* were tested for colistin susceptibility; four were found to be resistant to colistin, while two isolates (one *K. pneumoniae* and one *P. aeruginosa*) were found to be susceptible to colistin (≤ 0.5 μg/ml). To confirm the *mcr-1* subtype, we attempted to amplify and sequence the whole *mcr-1* gene among the six isolates, using another set of primers. Five isolates harbored *mcr-1.1* and the remaining one (*K. pneumoniae*) could not be amplified using this set of primers. Interestingly, *mcr-1.1* was reported in Egypt before; in one uropathogenic *E. coli* (UPEC) (Zakaria et al. 2021) and five *E. coli* isolated from chicken (Soliman et al. 2021). Moreover, *mcr-1.1* was reported in *E. coli* isolate obtained from wound drainage (Eltai et al. 2020) from Qatar. Girardello et al. (2021) reported the presence of *mcr-1.1* in *E. coli* clinical isolate in Sao Paulo, and *mcr-1.1* was found in two *K. pneumoniae* clinical isolates by Rocha et al. (2020).

In this study, the *K. pneumoniae* isolate, whose *mcr-1* gene could not be amplified using the second set of primers (used to amplify the whole *mcr-1* gene), was susceptible to colistin. Another colistin susceptible isolate was *P. aeruginosa*, which was found to harbor the *mcr-1.1* gene.

Previously, some studies have reported Gram-negative isolates susceptible to colistin and harboring the *mcr-1* gene. Wang et al. (2017) described two colistin-susceptible *E. coli* isolates possessing the *mcr-1* gene. Terveer et al. (2017) also reported a colistin-susceptible *E. coli* harboring *mcr-1*, which was not functioning. Using WGS, they found that the gene was rendered not functional by a transposon (IS10R) insertion (Terveer et al. 2017). Zhou et al. (2018) also described a fluoroquinolone-resistant but colistin-susceptible *E. coli* carrying *mcr-1*, which was also non-functional by inserting a 1.7-Kb IS1294b element. Chen et al. (2019) also reported a colistin-susceptible *E. coli* harboring *mcr-1*, which was non-functional because of the insertion of another gene. Jiang et al. (2020) described a colistin susceptible *E. coli* harboring the *mcr-1* gene, which had mutations in the *mcr-1* promotor sequence.

However, to the best of our knowledge, this is the first study reporting the presence of *mcr-1* in colistin susceptible *K. pneumoniae* and *P. aeruginosa* in Egypt. The whole *mcr-1* gene in the susceptible *K. pneumoniae* could not be amplified using the second set of primers and it was found to be susceptible to colistin; it may be due to an insertion sequence that rendered the

gene non-functional. However, in our case, WGS was not feasible due to its high cost and the fact that this study was not funded. Besides the *mcr-1* gene, other *mcr* family genes have also been described in colistin-susceptible bacteria. Ragupathi et al. (2020) reported the presence of the *mcr-3.30* gene in colistin-susceptible *Aeromonas veronii*. The *mcr-3.30* gene was disrupted due to the insertion of ISAs18 (Ragupathi et al. 2020). The *mcr-9* is another *mcr* family gene that was reported in other studies, including a study conducted by Soliman et al. (2020a), who reported the presence of this gene in colistin susceptible *Enterobacter hormaechei* clinical isolate from Egypt. Other colistin susceptible *E. hormaechei* harboring *mcr-9* were also reported in Egypt in pets with respiratory diseases (Khalifa et al. 2020a) and from food of animal origin (Sadek et al. 2020a). Carroll et al. (2019) reported the presence of *mcr-9* in the MDR *Salmonella enterica* subsp. *enterica* serotype Typhimurium isolate, which was susceptible to colistin. Also, another study conducted by Kananizadeh et al. (2020) reported the presence of *mcr-9* in *E. cloacae* complex in Japan. Khalifa et al. (2020b) found *mcr-9* in colistin-susceptible foodborne *K. pneumoniae*. The *mcr-9* subtype was also reported by Marchetti et al. (2021) who reported *mcr-9.2* in a colistin susceptible *E. cloacae*. The *mcr-10* was described by Wang et al. (2020a) in *Enterobacter roggenkampii* that was susceptible to colistin.

In this study, four *K. pneumoniae* isolates that harbored the *mcr-1.1* gene and "PCMKP-01" were capsule typed using the *wzi* gene sequence analysis. The most common K-type found among these isolates was K-58. It was found in three isolates. K-58 has not been associated with virulence (Turton et al. 2010). These three isolates were MDR strains of *K. pneumoniae* resistant to colistin that harbor the *mcr-1.1* gene. The two remaining K-types were K-9 and K-45. K-45 type *K. pneumoniae* was considered the positive control isolate "PCMKP-01". It was the MDR colistin-resistant isolate, while K-45 type isolate was colistin susceptible *K. pneumoniae* that harbored *mcr-1.1*.

Three of the isolates co-harbored *mcr-1.1* and $bla_{\text{CTX-M}}$. Sadek et al. (2021) reported that nine of the isolates co-harbored the *mcr-1* gene and ESBL genes. In the present study, five isolates harbored *mcr-1.1* and were resistant to carbapenems. These five isolates had $bla_{\text{OXA-48}}$, and three of them had $bla_{\text{NDM-1}}$ (one *P. aeuruginsa* isolate and two *K. pneumoniae* isolates). bla_{VIM} and bla_{KPC} were not found among these five isolates. Singh et al. (2021) reported that all the isolates harboring *mcr-1* in their study co-harbored $bla_{\text{OXA-48}}$. Han et al. (2020) reported the identification of the XDR *E. coli* clinical isolate co-harboring *mcr-1* and $bla_{\text{NDM-1}}$. Al-Bayssari et al. (2021) reported the co-existence of $bla_{\text{NDM-4}}$ and *mcr-1* among *E. coli* clinical isolates.

Conclusion

To our knowledge, it is the first time to report colistin susceptible *P. aeruginosa* and *K. pneumoniae* harboring the *mcr-1.1* gene in Egypt. The *mcr-1.1* gene was fully sequenced in *P. aeruginosa,* while in *K. pneumoniae* it could not be fully sequenced, which indicated some abnormality in this gene. The most frequently found K-type was K-58. Five of the isolates were resistant to carbapenems and co-harbored $bla_{\text{OXA-48}}$ and *mcr-1,* and three of them co-harbored *mcr-1.1*, $bla_{\text{OXA-48}}$, and $bla_{\text{NDM-1}}$. Co-existence of these genes together is a clear therapeutic challenge. Further studies are still needed to investigate the presence of the plasmid-borne *mcr* genes among colistin susceptible isolates to shed more light on its significance as a potential threat.

ORCID
Amira ElBaradei https://orcid.org/0000-0001-6813-7896

Accession numbers

Nucleotide sequences of *mcr-1.1* genes, which were amplified using both primers, were deposited in the GenBank database under accession numbers MZ820389 – MZ820401 (the details together with the appropriate web links are shown in Supplementary materials).

Ethical statement

Ethical approval was obtained from the Ethical Committee of the Medical Research Institute, Alexandria University.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abd El-Baky RM, Masoud SM, Mohamed DS, Waly NGFM, Shafik EA, Mohareb DA, Elkady A, Elbadr MM, Hetta HF. Prevalence and some possible mechanisms of colistin resistance among multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa*. Infect Drug Resist. 2020 Feb;13:323–332. https://doi.org/10.2147/IDR.S238811

Ahern JW, Schnoor JB. Colistin: potential for dosage error. Clin Infect Dis. 2012 Nov 01;55(9):1275, author reply 1275–1276. https://doi.org/10.1093/cid/cis632

Al-Bayssari C, Nawfal Dagher T, El Hamoui S, Fenianos F, Makdissy N, Rolain JM, Nasreddine N. Carbapenem and colistin-resistant bacteria in North Lebanon: coexistence of *mcr-1* and NDM-4 genes in *Escherichia coli*. J Infect Dev Ctries. 2021 Jul 31; 15(07):934–342. https://doi.org/10.3855/jidc.14176

Alghoribi MF, Doumith M, Upton M, Al Johani SM, Alzayer M, Woodford N, Ellington MJ, Balkhy HH. Complete genome sequence of a colistin-resistant uropathogenic *Escherichia coli* sequence type 131 *fimH22* strain harboring *mcr-1* on an IncHI2 plasmid, isolated in Riyadh, Saudi Arabia. Microbiol Resour Announc. 2019 May 2;8(18): e00104-19. https://doi.org/10.1128/MRA.00104-19

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 Aug;14:3063–3070. https://doi.org/10.2147/IDR.S318787

Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, Decré D. *wzi* Gene sequencing, a rapid method for determination of capsular type for *Klebsiella* strains. J Clin Microbiol. 2013 Dec 01;51(12):4073–4078. https://doi.org/10.1128/JCM.01924-13

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 Jun 25; 10(3): e00853-19. https://doi.org/10.1128/mBio.00853-19

Chen CW, Tang HJ, Chen CC, Lu YC, Chen HJ, Su BA, Weng TC, Chuang YC, Lai CC. The microbiological characteristics of carbapenem-resistant *Enterobacteriaceae* carrying the *mcr-1* gene. J Clin Med. 2019 Feb 19;8(2):261. https://doi.org/10.3390/jcm8020261

CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne (USA): Clinical and Laboratory Standards Institute; 2020. p. 16–38.

El-Mokhtar MA, Daef E, Mohamed Hussein AAR, Hashem MK, Hassan HM. Emergence of nosocomial pneumonia caused by colistin-resistant *Escherichia coli* in patients admitted to chest intensive care unit. Antibiotics (Basel). 2021 Feb 24;10(3):226. https://doi.org/10.3390/antibiotics10030226

Elmonir W, Abd El-Aziz NK, Tartor YH, Moustafa SM, Abo Remela EM, Eissa R, Saad HA, Tawab AA. Emergence of colistin and carbapenem resistance in extended-spectrum β-lactamase producing *Klebsiella pneumoniae* isolated from chickens and humans in Egypt. Biology (Basel). 2021 Apr 26;10(5):373. https://doi.org/10.3390/biology10050373

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

El-Sayed Ahmed MAEG, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the era of antibiotic resistance: an extended review (2000–2019). Emerg Microbes Infect. 2020 Jan 01;9(1):868–885. https://doi.org/10.1080/22221751.2020.1754133

Eltai NO, Kelly B, Al-Mana HA, Ibrahim EB, Yassine HM, Al Thani A, Al Maslmani M, Lammens C, Xavier BB, Malhotra-Kumar S. Identification of *mcr-8* in clinical isolates from Qatar and evaluation of their antimicrobial profiles. Front Microbiol. 2020 Aug 24;11:1954. https://doi.org/10.3389/fmicb.2020.01954

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2020. Available from https://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/

Feng Y. Transferability of MCR-1/2 polymyxin resistance: complex dissemination and genetic mechanism. ACS Infect Dis. 2018 Mar 09; 4(3):291–300. https://doi.org/10.1021/acsinfecdis.7b00201

Girardello R, Piroupo CM, Martins J Jr, Maffucci MH, Cury AP, Franco MRG, Malta FM, Rocha NC, Pinho JRR, Rossi F, et al. Genomic characterization of *mcr-1.1*-producing *Escherichia coli* recovered from human infections in São Paulo, Brazil. Front Microbiol. 2021 Jun 9;12:663414. https://doi.org/10.3389/fmicb.2021.663414

Gröbner S, Linke D, Schütz W, Fladerer C, Madlung J, Autenrieth IB, Witte W, Pfeifer Y. Emergence of carbapenem-non-susceptible extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. J Med Microbiol. 2009 Jul 01;58(7):912–922. https://doi.org/10.1099/jmm.0.005850-0

Han S, Kim JS, Hong CK, Park SH, Kim HS, Yu JK, Park J, Kim J, Lee SM, Oh YH. Identification of an extensively drug-resistant *Escherichia coli* clinical strain harboring *mcr-1* and bla_{NDM-1} in Korea. J Antibiot (Tokyo). 2020 Dec;73(12):852–858. https://doi.org/10.1038/s41429-020-0350-1

Hussein NH, AL-Kadmy IMS, Taha BM, Hussein JD. Mobilized colistin resistance (*mcr*) genes from 1 to 10: a comprehensive review. Mol Biol Rep. 2021 Mar;48(3):2897–2907. https://doi.org/10.1007/s11033-021-06307-y

Jaidane N, Bonnin RA, Mansour W, Girlich D, Creton E, Cotellon G, Chaouch C, Boujaafar N, Bouallegue O, Naas T. Genomic insights into colistin-resistant *Klebsiella pneumoniae* from a Tunisian Teaching Hospital. Antimicrob Agents Chemother. 2018 Feb;62(2):e01601-17. https://doi.org/10.1128/AAC.01601-17

Jiang Y, Zhang Y, Lu J, Wang Q, Cui Y, Wang Y, Quan J, Zhao D, Du X, Liu H, et al. Clinical relevance and plasmid dynamics of *mcr-1*-positive *Escherichia coli* in China: a multicentre case-control and molecular epidemiological study. Lancet Microbe. 2020 May; 1(1):e24–e33. https://doi.org/10.1016/S2666-5247(20)30001-X

Kananizadeh P, Oshiro S, Watanabe S, Iwata S, Kuwahara-Arai K, Shimojima M, Ogawa M, Tada T, Kirikae T. Emergence of carbapenem-resistant and colistin-susceptible Enterobacter cloacae complex co-harboring bla_{IMP-1} and *mcr-9* in Japan. BMC Infect Dis. 2020 Dec;20(1):282. https://doi.org/10.1186/s12879-020-05021-7

Kaye KS, Pogue JM, Tran TB, Nation RL, Li J. Agents of last resort: Polymyxin resistance. Infect Dis Clin North Am. 2016 Jun; 30(2):391–414. https://doi.org/10.1016/j.idc.2016.02.005

Khalifa HO, Oreiby AF, Abd El-Hafeez AA, Okanda T, Haque A, Anwar KS, Tanaka M, Miyako K, Tsuji S, Kato Y, et al. First Report of multidrug-resistant carbapenemase-producing bacteria coharboring *mcr-9* associated with respiratory disease complex in pets: potential of animal-human transmission. Antimicrob Agents Chemother. 2020a Dec 16;65(1):e01890-20. https://doi.org/10.1128/AAC.01890-20

Khalifa HO, Soliman AM, Saito T, Kayama S, Yu L, Hisatsune J, Sugai M, Nariya H, Ahmed AM, Shimamoto T, et al. First report of foodborne *Klebsiella pneumoniae* coharboring bla_{VIM-1}, bla_{NDM-1}, and *mcr-9*. Antimicrob Agents Chemother. 2020b Aug 20;64(9):e00882-20. https://doi.org/10.1128/AAC.00882-20

Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A 3rd, Forrest A, Bulitta JB, Tsuji BT. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy. 2010 Dec;30(12):1279–1291. https://doi.org/10.1592/phco.30.12.1279

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

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, 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 Mar;18(3):268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Marchetti VM, Bitar I, Sarti M, Fogato E, Scaltriti E, Bracchi C, Hrabak J, Pongolini S, Migliavacca R. Genomic characterization of VIM and MCR co-producers: the first two clinical cases, in Italy. Diagnostics (Basel). 2021 Jan 06;11(1):79. https://doi.org/10.3390/diagnostics11010079

Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol. 2011 Dec;19(12):588–595. https://doi.org/10.1016/j.tim.2011.09.005

Patro LPP, Sudhakar KU, Rathinavelan T. K-PAM: a unified platform to distinguish *Klebsiella* species K- and O-antigen types, model antigen structures and identify hypervirulent strains. Sci Rep. 2020 Dec;10(1):16732. https://doi.org/10.1038/s41598-020-73360-1

Ragupathi NKD, Sethuvel DPM, Anandan S, Murugan D, Asokan K, Neethi Mohan RG, Vasudevan K, D TK, C GPD, Veeraraghavan B. First hybrid complete genome of *Aeromonas veronii* reveals chromosome-mediated novel structural variant *mcr-3.30* from a human clinical sample. Access Microbiol. 2020 Feb 17;2(4): acmi000103. https://doi.org/10.1099/acmi.0.000103

Ramadan H, Gupta SK, Sharma P, Ahmed M, Hiott LM, Barrett JB, Woodley TA, Frye JG, Jackson CR. Circulation of emerging NDM-5-producing *Escherichia coli* among humans and dogs in Egypt. Zoonoses Public Health. 2020 May;67(3):324–329. https://doi.org/10.1111/zph.12676

Rocha IV, dos Santos Silva N, das Neves Andrade CA, de Lacerda Vidal CF, Leal NC, Xavier DE. Diverse and emerging molecular mechanisms award polymyxins resistance to *Enterobacteriaceae* clinical isolates from a tertiary hospital of Recife, Brazil. Infect Genet Evol. 2020 Nov;85:104584. https://doi.org/10.1016/j.meegid.2020.104584

Sadek M, Nariya H, Shimamoto T, Kayama S, Yu L, Hisatsune J, Sugai M, Nordmann P, Poirel L, Shimamoto T. First genomic characterization of bla_{VIM-1} and *mcr-9*-coharbouring *Enterobacter hormaechei* isolated from food of animal origin. Pathogens. 2020a Aug 22;9(9):687. https://doi.org/10.3390/pathogens9090687

Sadek M, Ortiz de la Rosa JM, Abdelfattah Maky M, Korashe Dandrawy M, Nordmann P, Poirel L. Genomic features of MCR-1 and extended-spectrum β-lactamase-producing Enterobacterales from retail raw chicken in Egypt. Microorganisms. 2021 Jan 19; 9(1):195. https://doi.org/10.3390/microorganisms9010195

Sadek M, Poirel L, Nordmann P, Nariya H, Shimamoto T, Shimamoto T. Draft genome sequence of an *mcr-1*/IncI2-carrying multidrug-resistant *Escherichia coli* B1:ST101 isolated from meat and meat products in Egypt. J Glob Antimicrob Resist. 2020b Mar; 20:41–42. https://doi.org/10.1016/j.jgar.2019.11.015

Sayyahfar S, Choobdar FA, Mashayekhi M, Jazi FM. Successful management of pan-resistant *Acinetobacter baumannii* meningitis without intrathecal or intraventricular antibiotic therapy in a neonate. Infect Chemother. 2021;53(1):146–150. https://doi.org/10.3947/ic.2020.0202

Singh S, Pathak A, Rahman M, Singh A, Nag S, Sahu C, Prasad KN. Genetic characterisation of colistin resistant *Klebsiella pneumoniae* clinical isolates from North India. Front Cell Infect Microbiol. 2021 Jun 21;11:666030. https://doi.org/10.3389/fcimb.2021.666030

Soliman AM, Maruyama F, Zarad HO, Ota A, Nariya H, Shimamoto T, Shimamoto T. Emergence of a multidrug-resistant *Enterobacter hormaechei* clinical isolate from Egypt co-harboring *mcr-9* and bla_{VIM-4}. Microorganisms. 2020a Apr 20;8(4):595. https://doi.org/10.3390/microorganisms8040595

Soliman AM, Ramadan H, Zarad H, Sugawara Y, Yu L, Sugai M, Shimamoto T, Hiott LM, Frye JG, Jackson CR, et al. Coproduction of *tet*(X7) conferring high-level tigecycline resistance, fosfomycin fosa4, and colistin *mcr-1.1* in *Escherichia coli* strains from chickens in Egypt. Antimicrob Agents Chemother. 2021 May 18; 65(6): e02084-20. https://doi.org/10.1128/AAC.02084-20

Soliman AM, Zarad HO, Nariya H, Shimamoto T, Shimamoto T. Genetic analysis of carbapenemase-producing Gram-negative bacteria isolated from a university teaching hospital in Egypt. Infect Genet Evol. 2020b Jan;77:104065. https://doi.org/10.1016/j.meegid.2019.104065

Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance. Trends Microbiol. 2018 Sep;26(9):794–808. https://doi.org/10.1016/j.tim.2018.02.006

Terveer EM, Nijhuis RHT, Crobach MJT, Knetsch CW, Veldkamp KE, Gooskens J, Kuijper EJ, Claas ECJ. 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 Jun 2;12(6): e0178598. https://doi.org/10.1371/journal.pone.0178598

Turton JF, Perry C, Elgohari S, Hampton CV. PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol. 2010 May 01;59(5):541–547. https://doi.org/10.1099/jmm.0.015198-0

Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z. Identification of novel mobile colistin resistance gene *mcr-10*. Emerg Microbes Infect. 2020a Jan 01;9(1):508–516. https://doi.org/10.1080/22221751.2020.1732231

Wang CH, Hsieh YH, Powers ZM, Kao CY. Defeating antibiotic-resistant bacteria: exploring alternative therapies for a post-antibiotic era. Int J Mol Sci. 2020b Feb 05;21(3):1061. https://doi.org/10.3390/ijms21031061

Wang L, Gu H, Lu X. A rapid low-cost real-time PCR for the detection of *Klebsiella pneumonia* carbapenemase genes. Ann Clin Microbiol Antimicrob. 2012 Jan;11(1):9. https://doi.org/10.1186/1476-0711-11-9

Wang Y, Tian GB, Zhang R, Shen Y, Tyrrell JM, Huang X, Zhou H, Lei L, Li HY, Doi Y, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect Dis. 2017 Apr;17(4):390–399. https://doi.org/10.1016/S1473-3099(16)30527-8

Xu Y, Chen H, Zhang H, Ullah S, Hou T, Feng Y. The MCR-3 inside linker appears as a facilitator of colistin resistance. Cell Rep. 2021 May;35(7):109135. https://doi.org/10.1016/j.celrep.2021.109135

Yanat B, Machuca J, Yahia RD, Touati A, Pascual Á, Rodríguez-Martínez JM. First report of the plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate in Algeria. Int J Antimicrob Agents. 2016 Dec;48(6):760–761. https://doi.org/10.1016/j.ijantimicag.2016.09.003

Yuan J, Wang X, Shi D, Ge Q, Song X, Hu W, Wei D, Ge C, Li X, Hu C. Extensive antimicrobial resistance and plasmid-carrying resistance genes in *mcr-1*-positive *E. coli* sampled in swine, in Guangxi, South China. BMC Vet Res. 2021 Dec;17(1):86. https://doi.org/10.1186/s12917-021-02758-4

Zafer MM, El-Mahallawy HA, Abdulhak A, Amin MA, Al-Agamy MH, Radwan HH. Emergence of colistin resistance in multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from cancer patients. Ann Clin Microbiol Antimicrob. 2019 Dec;18(1):40. https://doi.org/10.1186/s12941-019-0339-4

Zakaria AS, Edward EA, Mohamed NM. Genomic insights into a colistin-resistant uropathogenic *Escherichia coli* strain of O23:H4-ST641 lineage harboring *mcr-1.1* on a conjugative IncHI2 plasmid from Egypt. Microorganisms. 2021 Apr 10;9(4):799. https://doi.org/10.3390/microorganisms9040799

Zhou K, Luo Q, Wang Q, Huang C, Lu H, Rossen JWA, Xiao Y, Li L. Silent transmission of an IS*1294b*-deactivated *mcr-1* gene with inducible colistin resistance. Int J Antimicrob Agents. 2018 Jun; 51(6):822–828. https://doi.org/10.1016/j.ijantimicag.2018.01.004

Supplementary materials are available on the journal's website.