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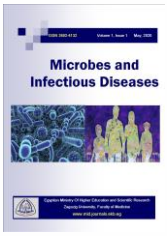

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Original article

Detection of plasmid-borne *mcr-1* gene conferring colistin resistance in MDR and XDR Gram-negative bacterial isolates from an Egyptian hospital

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ABSTRACT

Background: Infections with multi-drug resistant (MDR) Gram-negative bacteria represent a serious public health risk, especially with the emergence of colistin resistance. Colistin resistance is mainly mediated by chromosomal mutations; however, there are reports of transferable plasmid-mediated colistin resistance genes, namely *mcr*, which have been universally documented. Thus, our study aimed to examine the incidence of mobile colistin resistance genes (*mcr-1* and *mcr-2*) among MDR and extensively drug-resistant (XDR) Gram-negative bacteria. **Methods:** Two hundred and forty-two Gram-negative clinical bacterial isolates were obtained in our study. Using standard microbiological methods, the bacteria were isolated and identified. Colistin resistance was phenotypically detected utilizing the broth microdilution technique. The colistin-resistant isolates were examined for their antimicrobial susceptibility profile using the Kirby-Bauer disk diffusion method. We used PCR to identify the *mcr-1* and *mcr-2* genes. **Results:** Our data revealed that up to 18 (7.8%) isolates were colistin-resistant, including 11 *Klebsiella* spp. isolates, six *Pseudomonas* spp. isolates and only one *Escherichia coli* isolate. The PCR results revealed that *mcr-1* was found in two isolates (11.1%), including one isolate of *Klebsiella* spp. (colistin MIC=32 µg/ml) and one isolate of *Escherichia coli* (colistin MIC=4 µg/ml). None of the colistin-resistant isolates carried *mcr*-2. **Conclusions:** Based on our data, a relatively low incidence of colistin resistance was observed among clinical isolates. However, the detection of *mcr-1* in two isolates of different species is concerning because of the possibility of spreading to susceptible strains. Public Health authorities should implement colistin resistance monitoring programs and infection control strategies in healthcare settings.

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Introduction

One of the biggest problems facing humanity today is antimicrobial resistance [1]. The majority of the pathogens on the World Health Organization's (WHO, 2017) list of antibiotic-resistant bacteria were Gram-negative bacteria [2]. Gram-negative bacteria have a higher level of antibiotic resistance than Gram-positive bacteria

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due to their distinct structure, and they are a significant cause of disease and death worldwide [3]. Gram-negative bacteria have the potential to seriously harm people, especially those with weakened immune systems [4]. Gram-negative bacteria cause nosocomial infections, which is a great healthcare challenge due to resistance to antibiotics [4]. Multidrug-resistant (MDR) Gram-negative bacteria are considered the major cause of most ventilator-associated pneumonia cases, bloodstream infections related to catheter use, and other cases of intensive care units (ICUs) acquired sepsis such as urinary tract infections (UTIs) [2].

Now, physicians are forced to use colistin as a final choice in the treatment of infections resulting from MDR Gram-negative bacteria due to the decline in the discovery of novel antibiotics and the increase in extensively drug-resistant (XDR) Gram-negative bacteria [5–7]. Colistin or Polymyxin E is a cationic polypeptide that attaches to anionic lipopolysaccharide molecules of the outer membrane of Gram-negative cell walls by competing with calcium and magnesium cations, which leads to an increase in the permeability of the outer membrane, resulting in the death of cells [8–10]. Due to neurotoxicity and nephrotoxicity, colistin's usefulness was limited in the 1970s [11]. However, polymyxins have been reintroduced into human medicine as one of the last resort options for treating MDR Gram-negative organisms [10].

Colistin resistance emerged because of the increased use of colistin in managing infections resulting from Gram-negative organisms that resist numerous medications [12]. Furthermore, the issue of colistin resistance has been made worse by the widespread usage of colistin in animal production facilities [12]. Chromosomal mutations are the most prevalent cause of acquired colistin resistance [9]. In 2015, Liu et al. [13] reported a new transferable plasmid-mediated colistin resistance gene, *mcr-1*, harbored by *E.coli* in China. This plasmid encodes phosphoethanolamine transferase, which modifies lipid A and thereby reduces susceptibility to colistin [12]. Other colistin resistance genes encoded by plasmids were found, such as *mcr-2*, which shares 76.7% of its nucleotide similarity with *mcr-1* [14, 15]. In 2018, plasmid-mediated colistin resistance genes *mcr-3* to *mcr-8* were found and shared some nucleotide similarities with *mcr-1* [16]. Now, 10 variants of *mcr* genes are known [17]. As a result of horizontal gene transfer of the plasmid carrying the *mcr* genes to other bacterial strains, plasmid-mediated colistin resistance is a serious threat and worldwide concern [13, 18].

In Egypt, the high incidence of infectious diseases and misuse of antibiotics in both veterinary and medical settings may lead to the emergence of incurable diseases because of the spread of colistin resistance in bacterial pathogens [19]. In 2016, *mcr-1* was first identified in a clinical human isolate from Egypt [20]. Numerous investigations subsequently verified the presence of *mcr-1* in Gram-negative clinical isolates from Egypt. Different species can acquire colistin resistance through the *mcr* genes. To reduce the spread of isolates carrying these genes, two key strategies including implementing appropriate infection control measures and running surveillance programs for *mcr* gene detection, are absolutely required [21]. Therefore, our research aimed to determine the incidence of colistin resistance both phenotypically and genotypically through the *mcr-1* and *mcr-2* genes among Gram-negative isolates recovered from clinical specimens in El-Mahalla El-Kobra General Hospital in Egypt.

Methods

Isolation and identification of the tested isolates

From November 2022 to April 2023, a total of 242 Gram-negative clinical isolates of bacteria were recovered from various clinical specimens taken from patients admitted to different departments in El-Mahalla El-Kobra General Hospital. Under strict aseptic conditions, specimens including blood, pus, sputum, urine, endotracheal tube (ETT), pleural fluid lavage, surgical wounds, and sore beds were gathered. On MacConkey agar plates (Oxoid Ltd., Basingstoke, Hampshire, England), the isolates were cultured for 24 hours at 37°C. The isolates were identified using the following biochemical tests as previously described [22]; triple sugar iron agar (TSI); lysine iron agar (LIA); motility, indole, ornithine (MIO); urease; citrate; and oxidase assays. Cetrimide agar was used to confirm *Pseudomonas* identity [23]. In nutrient broth containing 25% v/v glycerol, the isolates were kept at -80 °C for long-term preservation.

Phenotypic detection of colistin resistance

The Kirby-Bauer disc diffusion method is incapable of detecting colistin resistance due to the insufficient diffusion of colistin molecules [24]. The minimum inhibitory concentration (MIC) of colistin was determined in accordance with the Clinical and Laboratory Standards Institute (CLSI) criteria, utilizing a standardized broth microdilution

procedure [25]. In accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations, isolates with MIC > 2 μg/mL were recorded as resistant, and isolates with MIC ≤ 2 μg/mL were recorded as sensitive [26]. *Escherichia coli* A 1-22-2 served as the positive control, and *E. coli* ATCC 25922 was the negative control [27]. For broth microdilution experiments, 96-well polystyrene microplates were used. Dilutions of colistin (Sigma Aldrich, USA) ranging from 0.25 μg/mL to 32 μg/mL were prepared in cation-adjusted Mueller-Hinton broth (HiMedia Laboratories Pvt., Mumbai, India) by means of serial two-fold dilutions and the tested isolates were incorporated into each well to obtain 0.5 McFarland equal to 1–2 × 10^8 cfu /mL as the ultimate bacterial concentration. To determine the MIC values, the bacterial cultures were visually inspected for microbial growth after incubation for 18–20 hours at 37°C [28, 29].

Antimicrobial susceptibility testing

Using the Kirby-Bauer disc diffusion method, the antimicrobial susceptibility of 18 isolates that showed resistance to colistin was tested in compliance with the standards of CLSI [25]. The tigecycline test was conducted in accordance with (EUCAST 2022) recommendations. The isolates were tested against the following antibiotic discs: imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (5μg), amoxicillin/clavulanic acid (20/10 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), ampicillin/sulbactam (10/10 μg), piperacillin/tazobactam (100/10 μg), aztreonam (30 μg), cefotaxime (30 μg), tetracycline (30 μg), ceftriaxone (30 μg), cefazoline (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), ceftazidime (30μg) (HiMedia Laboratories Pvt., Mumbai, India), while ampicillin (10 μg), ceftaroline (30 μg), tigecycline (15 μg), and fosfomycin (200 μg) (Oxoid Ltd; Basingstoke; Hampshire, England). Mueller-Hinton agar plates were incubated (Oxoid Ltd., Basingstoke, Hampshire, England) at 35ºC ± 2˚C for 16–18 hours. The inhibitory zone's diameter, which developed around the disc, was recorded in millimeters and compared to the (CLSI 2020) susceptibility tables, and results were documented as resistant (R), intermediate (I), or susceptible (S). According to Magiorakos et al. [30], isolates that were not susceptible to at least one antimicrobial agent from three or more antibiotic groups were classified as MDR, while isolates that exhibited non-susceptibility to at least one antimicrobial agent across all but two or fewer antimicrobial groups were assigned as XDR. In our investigation, the colistin-resistant isolates were tested for susceptibility to the antibiotics in all the antimicrobial groups designated by Magiorakos et al. [30].

Genotypic identification of plasmid-mediated colistin resistance (*mcr-1* and *mcr-2* genes) via PCR

Extraction of bacterial DNA

Total genomic DNA was extracted using the boiling lysis technique [31]. A DNA template for PCR was created for each isolate by heating three to six pure colonies suspended in 200 μl of nuclease-free water to 95˚C for ten minutes and then quickly placing the suspensions on ice for five minutes. After that, the mixture was centrifuged at 13,000 rpm for 30 seconds to remove any cell debris. After that, the supernatants containing DNA were kept in tiny aliquots at -20 °C until needed.

The identification and amplification of *mcr-1* and *mcr-2* genes

The primers (Eurofins Genomics, Huntsville, AL, USA) used for *mcr-1 and mcr-2* amplification and the size of each amplicon were listed in Table 1. To summarize, the used process was as follows: 15 μL of a 2× PCR premixture was mixed with 2 μL of produced bacterial DNA, 10 pmol of each primer (1 μL), and deionized water was added until the total volume was 30 μL. The Bio-Rad T100 thermocycler was utilized to amplify *mcr-1 and mcr-2*.

For *mcr-1* amplification, the reactions were first denatured for 15 minutes at 94 °C followed by 25 cycles of amplification consisting of denaturation at 94 °C for 30 s, 90 s of annealing at 55 °C, and 60 seconds for the extension at 72 °C, and a final elongation for 10 min at 72 °C [32]. The procedures for *mcr-2* amplification were as follows: there were 33 cycles of denaturation at 95°C for 3 minutes, annealing at 65°C for 30 seconds, DNA extension at 72°C for one minute, followed by one cycle for final extension at 72°C for 10 minutes [15]. Under ultraviolet light, the anticipated amplicons for *mcr-1* (309 bp) and *mcr-2* (1626 bp) were visible following 1.5% agarose gel electrophoresis and ethidium bromide staining.

Results

Identification of the tested isolates

Two hundred and forty-two Gram-negative bacterial isolates were collected from various clinical specimens: urine (n=88), blood (n=67), sputum (n=42), ETT (n=30), pus (n=11), wound swab (n=2), sore beds (n=1), and pleural fluid (n=1). Identification of the collected isolates using conventional biochemical and microbiological tests revealed that the most predominant Gram-negative bacteria were *E. coli*, followed by *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Proteus* spp., *Citrobacter* spp., and *Serratia* spp., while *Salmonella* spp., *Aeromonas* spp., and *Morganella Morganii* were the least frequent (Fig. 1).

Prevalence of the bacterial species in the different clinical specimens

The prevalence of the recovered bacterial isolates in the different clinical samples was recorded and presented in (Table 2). The highest incidence of *E. coli and Pseudomonas* spp. was recorded from urine specimens, while the highest incidence of *Klebsiella* spp. and *Enterobacter* spp. was detected from blood specimens.

Phenotypic detection of colistin resistance

Twelve out of 242 isolates were intrinsically resistant to colistin, including *Proteus, Serratia, and Morganella*, and hence, were not included in the current investigation [33, 34]. The broth microdilution method was used to estimate the MIC of colistin for the remaining isolates (n=230) in accordance with CLSI recommendations and EUCAST instructions for the colistin breakpoints [25, 26]. A total of 18 out of 230 (7.8%) isolates were found to be colistin resistant. Among 18 colistin-resistant isolates, 11 were *Klebsiella* spp., six were *Pseudomonas* spp., and only one was *E. coli*. Of 18 colistin-resistant isolates, four were found to have MICs greater than 32 μg/mL, 5 isolates had MIC of 32 μg/mL, 6 isolates had MIC of 16 μg/mL, 2 isolates had MIC of 8 μg/mL, and one isolate had MIC of 4 μg/mL. Colistin resistance distribution among the tested isolates is displayed in (Table 3).

Notably, 10 colistin-resistant isolates were from urine specimens (10/18, 55.6%), which represents (10/88, 11.4%) of the isolates collected from all urine specimens; 4 isolates were from sputum (4/18, 22.2%), which represents (4/42, 9.5%) of the isolates collected from sputum specimens;2 isolates were from blood (2/18, 11.1%) which represents (2/67, 3%) of the isolates collected from blood specimens; one isolate was from ETT (1/18, 5.6%)which represents (1/30, 3.3%) of the isolates collected from ETT specimens; and the only isolate recovered from the pleural fluid (1/18, 5.6%).

Analysis of antimicrobial resistance of colistin-resistant isolates

Among 18 colistin-resistant isolates,12 isolates belonged to the family *Enterobacteriaceae,* including *Klebsiella* spp., and *E.coli.* The antimicrobial resistance pattern of 16 individual antibiotics and 4 commonly used combined antibiotics was determined for these enteric colistin-resistant isolates according to CLSI (2020) and the EUCAST instructions (2022). It is to be noted that isolates showing either resistance or intermediate resistance to certain antibiotics were recorded as non-susceptible. All the enteric colistin-resistant isolates were not susceptible to amikacin, amoxicillin/clavulanic acid, ampicillin, ampicillin/sulbactam, ceftaroline, cefazoline, cefotaxime, ceftriaxone, ciprofloxacin, and trimethoprim/sulfamethoxazole, while only 7 isolates were non-susceptible to chloramphenicol. The enteric colistin-resistant isolates were classified as MDR or XDR as previously described [30]. Interestingly, out of 12 enteric colistin-resistant isolates 11 (91.7%) isolates were considered XDR, and only one (8.3%) isolate was MDR.

Table 4 displays the antibiotic susceptibility profile of the enteric isolates that were resistant to colistin. It is noteworthy that four enteric isolates show non-susceptibility to all antibiotics tested.

The remaining six colistin-resistant isolates belonged to the *Pseudomonadaceae* family. The antimicrobial resistance pattern of 7 individual antibiotics was determined for the colistin-resistant *Pseudomonas* spp. isolates based on CLSI (2020) guidelines. The isolates were categorized as XDR or MDR as previously described [30]. All the isolates were non-susceptible to ceftazidime or fosfomycin, 5 isolates were non-susceptible to aztreonam,4 isolates were non-susceptible to imipenem, 3 isolates were non-susceptible to gentamicin and ciprofloxacin and 2 isolates were non-susceptible to piperacillin/tazobactam. Our findings showed that of the six colistin-resistant isolates belonging to the family *Pseudomonadaceae*, 3 (50%) isolates were

considered MDR, and 3 (50%) isolates were considered XDR.

Table 5 displays the antibiotic susceptibility profile of colistin-resistant *Pseudomonas* spp. isolates. Remarkably, two *Pseudomonas* spp. isolates showed non-susceptibility to all tested antibiotics.

PCR detection of *mcr-1* and *mcr-2* in colistin-resistant isolates

The *mcr-1* and *mcr-2* genes were screened by PCR in 18 isolates that exhibited resistance to colistin. According to our findings, two (11.1%) isolates, had the *mcr-1 gene*, including one *E. coli* isolate and one *Klebsiella* spp. isolate (Fig. 2). These isolates showed resistance with values of 4 µg/ml and 32 µg/ml for *E. coli* and *Klebsiella* spp. isolates, respectively. However, none of the tested 18 colistin-resistant isolates harbored the *mcr-2* gene.

Table 1. The primers used for PCR detection of *mcr-1* and *mcr-2* genes.

Gene	Primer nucleotide sequence	Size of the amplicons (bps)	Reference
mcr-1	F: 5′-CGGTCAGTCCGTTTGTTC 3′ R: 5′-CTTGGTCGGTCTGTAGGG 3′	(309 bp)	[13]
mcr-2	F: 5′-TGGTACAGCCCCTTTATT-3′ R: 5′-GCTTGAGATTGGGTTATGA-3′	(1626 bp)	[15]

Table 2. Incidence of bacterial species in different clinical specimens

Bacterial species	Incidence (n (%)) in different clinical specimens*								
	Urine	Blood	Sputum	ETT	Pus	Wound swab	Pleural fluid	Sore beds	Total number of isolates
E. coli (n=69)	40 (58%) *	14 (20.3%)	10 (14.5%)	3 (4.3%)	1 (1.4%)	1 (1.4%)	0 (0%)	0 (0%)	69 (28.5%)
Klebsiella spp. (n=64)	11 (17.2%)	21 (32.8%) *	11 (17.2%)	14 (21.9%)	6 (9.4%)	0 (0%)	1 (1.6%)	0 (0%)	64 (26.4%)
Enterobacter spp. (n=40)	8 (20%)	17 (42.5%) *	8 (20%)	5 (12.5%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)	40 (16.5%)
Pseudomonas spp. (n=40)	23 (57.5%) *	6 (15%)	5 (12.5%)	4 (10%)	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)	40 (16.5%)
Acinetobacter spp. (n=11)	0 (0%)	2 (18.2%)	6 (54.5%) *	3 (27.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (4.5%)
Proteus spp. (n=9)	3 (33.3%) *	3 (33.3%) *	1 (11.1%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)	9 (3.7%)
Citrobacter spp. (n=4)	1 (25%)	2 (50%) *	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (1.7%)
Serratia spp. (n=2)	1 (50%) *	1 (50%) *	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.8%)
Salmonella spp. (n=1)	0 (0%)	1 (100%) *	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)
Aeromonas spp. (n=1)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%) *	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)
Morganella Morganii(n=1)	1 (100%) *	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0(0%)	1 (0.4%)
Total number	88/242(36.4 %)	67/242(27.7 %)	42/242(17.4 %)	30/242(12.4 %)	11/242(4. 5%)	2/242(0.8 %)	1/242(0. 4%)	1/242(0. 4%)	242(100%)

* The highest incidence of each species among the different clinical specimens

Table 3. Incidence of colistin resistance among the tested isolates

Bacterial species	Number of isolates	Incidence of resistant isolates *	Resistant isolates code	The clinical source of resistant isolates
E.coli	69	1 (1.4%)	E240	Urine
Klebsiella spp.	64	11 (17.2%)	K69, K106, K166 K13, K37, K124, K234 K92, K238 K220 K217	Urine Sputum blood ETT pleural fluid
Pseudomonas spp.	40	6 (15%)	P35, P44, P45, P93, P139, P161	Urine

* The percentage calculated relative to the corresponding number of isolates in each bacterial species

Table 4. Antibiotic susceptibility Profile of the enteric colistin-resistant isolates.

Antibiotics / Isolate code	Gentamycin	Amikacin	Ceftaroline	Piperacillin/tazobactam	Imipenem	Meropenem	Cefazoline	Cefotaxime	Ceftriaxone	Cefoxitin	Ciprofloxacin	Trimethoprim/sulfamethoxazole	Tigecycline	Aztreonam	Ampicillin	Ampicillin-sulbactam	Amoxicillin- clavulanic acid	Cloramphinchol	Fosfomycin	Tetracycline	Resistance profile
K13	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	XDR
K 37	R	R	I	I	S	R	R	R	R	R	R	R	R	S	R	R	R	I	R	I	XDR
K69	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	XDR
K92	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	I	XDR
K106	R	R	R	I	I	R	R	R	R	R	R	R	R	I	R	R	R	S	R	S	XDR
K124	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	XDR
K166	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	XDR
K217	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	I	XDR
K220	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	XDR
K234	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	XDR
K238	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	XDR
E240	S	I	I	S	R	S	R	R	R	S	R	R	S	R	R	R	R	R	S	R	MDR

R: resistant, I: intermediate, S: sensitive

Table 5. Antibiotic susceptibility Profile of colistin-resistant *Pseudomonas* spp. Isolates.

Antibiotics / Isolate code	Gentamycin	Imipenem	Ceftazidime	Ciprofloxacin	Piperacillin/tazobactam	Aztreonam	Fosfomycin	Resistance profile
P35	S	S	R	S	S	R	R	MDR
P44	R	S	R	R	S	R	R	XDR
P45	R	R	R	R	R	R	R	XDR
P93	R	R	R	R	R	R	R	XDR
P139	S	R	R	S	S	S	R	MDR
P161	S	R	R	S	S	R	R	MDR

R: resistant, I: intermediate, S: sensitive

Figure 1. Proportions of the isolated bacterial species.

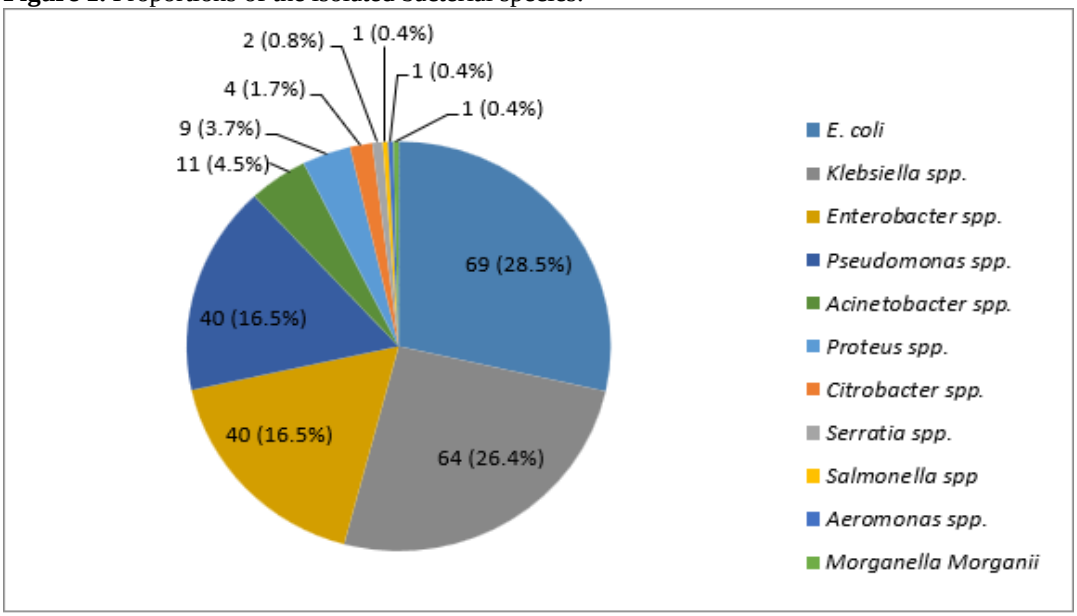
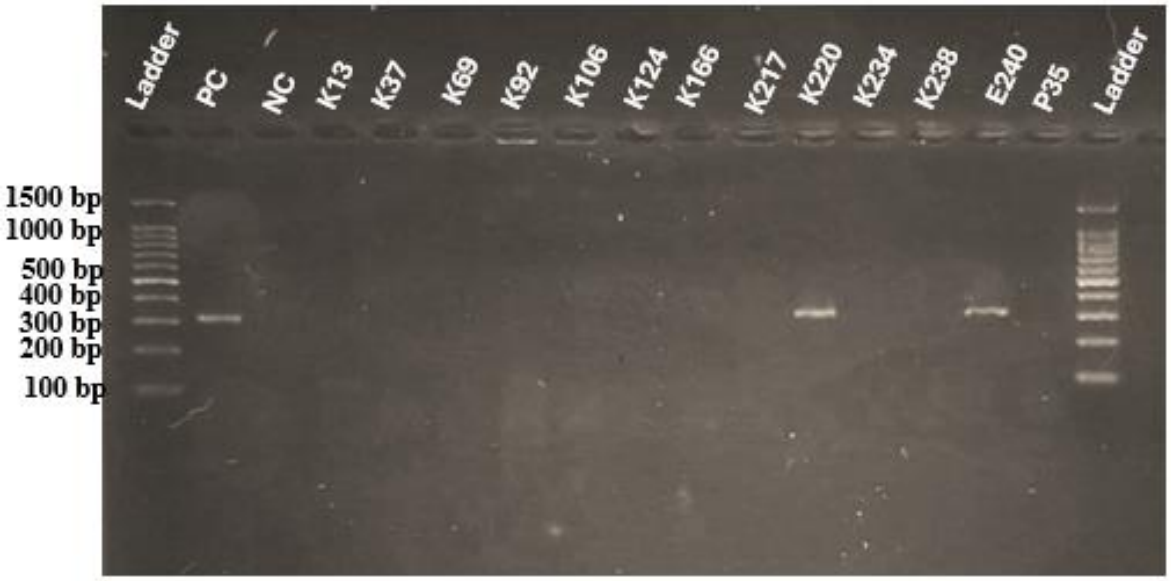


Figure 2. Detection of *mcr-1* gene (309 bp) among the colistin-resistant isolates. Only two isolates (one *Klebsiella* spp. K220 and one *E. coli* E240) were shown to be *mcr-1*-positive. PC is the positive control and NC is the negative control.



Discussion

It is essential to understand the colistin's resistance local epidemiology and resistance mechanism in MDR Gram-negative bacteria to establish therapy regimens for critically ill patients [35]. In this study, *E. coli* (28.5%), *Klebsiella* spp. (26.4%), *Pseudomonas* spp.(16.5%) and *Enterobacter* spp. (16.5%), were the most common Gram-negative isolates. Consistently, Abo-State et al. [36] reported that *E. coli* (41.9%) was the most prevalent pathogen among 210 Gram-negative isolates recovered from different clinical samples from various hospitals, followed by *Klebsiella* spp. (27.1%) and *Pseudomonas* spp. (17.6%). Similarly, Amandeep et al. [37] found that *E. coli* (41.6%) was the most widespread isolate in 276 Gram-negative isolates obtained from different clinical samples in a tertiary care Indian hospital, followed by *K. pneumoniae* (24%) and *Pseudomonas* spp. (17.7%). However, *K. pneumoniae* was the most prevalent Gram-negative isolate, accounting for 43.4% of 244 Gram-negative isolates from different clinical samples recovered from different wards of Tanta University Hospitals in Egypt, while *E. coli* came second (29.1%), then *P. aeruginosa*(13.5%) [33]. Also, Fahim et al. [38] found that *Klebsiella* (39.1%) was the most often isolated Gram-negative

pathogen, followed by *E. coli* (23.4%) among the Gram-negative pathogens recovered from various microbiological samples of ICUs patients at Hospitals of Ain Shams University (ASUHs). These variations may be due to varying types of specimens, variations in the general health of the patient, variations between nations, or the level of adherence to infection control methods [33, 39].

The majority of the isolated Gram-negative bacteria in our study were recovered from urine specimens (36.4%) with the predominance of *E. coli* (45.5%), followed by *Pseudomonas* spp. (26.1%) while *Klebsiella* spp. represents (12.5%). According to several studies conducted in Egypt, the most frequent cause of UTIs was *E. coli.* For instance, the most prevalent Gram-negative bacteria in UTIs were *E. coli* (35.8%), *Klebsiella* spp. (34.1%), and *Pseudomonas* spp. (16.6%) among patients admitted at different ASUHs [38]. Abou-Dobara et al. [40] found that the most common isolate recovered from 77 urine clinical samples in Mansoura was *E. coli* (50%) followed by *K. pneumoniae* (29%) and *P. aeruginosa* (21%). However, Khalifa et al. [41] reported that the most frequent pathogen was *Klebsiella* spp. (53.6%) followed by *E. coli* (35.7%) in the urine cultures recovered from patients in different hospitals in Cairo and Kafrelsheikh.

In our study, 18 of 230 Gram-negative isolates (7.8%) were resistant to colistin. Other studies from Egypt reported similar percentages. According to Shabban et al. [21], colistin resistance was reported in 6.7% of MDR Gram-negative bacterial isolates obtained from patients hospitalized in different wards and ICUs at Ain Shams University Hospital. Furthermore, 10.4% of Gram-negative isolates recovered from clinical specimens of patients referred to different departments of Cairo University Hospitals were found to be colistin-resistant [42]. However, El-khatib et al. [43] found that colistin resistance was detected in 4.4% of Gram-negative isolates collected from different clinical specimens from immunocompromised patients in some hospitals in Cairo. Moreover, Emara et al. [33] found that colistin resistance was detected in 16.4% of Gram-negative isolates from different clinical specimens from patients admitted to different departments in Tanta University Hospitals. Also, in a study carried out in India, Panigrahi et al. [44] found that 19.6% of MDR Gram-negative isolates from various clinical samples of ICUs patients, had colistin resistance. The degree to which Gram-negative isolates are susceptible to colistin may vary depending on the geographic location, the antibiotic regimen used, or the number of specimens used in each study [29]. Colistin resistance rates in our study were 1.4% for *E. coli* isolates, 17.2% for *Klebsiella* spp., and 15% for *Pseudomonas* spp. In a study conducted at Cairo University Hospitals, 12.5% of *E. coli* isolates, 9.5% of *Klebsiella* spp., and 13.8% of *Pseudomonas* spp., were found to be colistin-resistant [42]. El-Mahallawy et al. [35] reported that 21% of *K. pneumoniae* isolates and 20.2% of *E. coli* isolates were colistin-resistant in a study of MDR enterobacterial isolates at the National Cancer Institute (NCI), Cairo University, Egypt. This high level of colistin resistance might be due to the widespread use of colistin in hospitals for high-risk [35]. Globally, according to an Indian study, Panigrahi et al. [44] demonstrated that the frequencies of colistin resistance were 5% among *E. coli* isolates, 9.2% among *K.pneumoniae*, and 1.4% among *Pseudomonas* spp. According to a study including 28 tertiary hospitals in China, Quan et al. [45] found that colistin resistance was 1.5% among *E. coli* isolates whereas the colistin resistance among *K. pneumoniae* isolates was 0.7%. The increased incidence of colistin resistance among MDR isolates in some investigations might be due to the frequent use in inappropriate dosages for illnesses that are treatable with less potent antibiotics. Colistin is widely employed in agriculture, pisciculture, farm and dairy animals. As a result, tiny amounts of colistin leak into the environment and cause saprophytic organisms to produce colistin resistance, which subsequently enters the human body in various ways [44, 45]. Out of 18 colistin-resistant isolates, 10 (55.6%) isolates were from urine and six (33.3%) isolates were from the respiratory tract, which is explained by the high prevalence of the highly resistant organisms *Pseudomonas* spp. and *Klebsiella* spp. in urine specimens and *Klebsiella* spp. in the respiratory tract specimens. This is in line with a retrospective research carried out on 24 patients in a South Indian hospital with tertiary care, who had Gram-negative isolates resistant to colistin, where the most common source of the isolates was found to be urine (33%), followed by blood (25%), respiratory (20.8%), and pus (16.67%) [46]. Rabie et al. [47] found that urine catheters were the most frequent source of colistin-resistant isolates (37.5%), then blood (25%), sputum (20.8%), and wounds (16.7%). In contrast, El-Khatib et al. [43] recorded that the most common

source of isolates that were resistant to colistin was wound swab specimens, followed by blood, sputum, and urine specimens. These variations may be attributed to differences in the patients' diseases, which affect the types of specimens taken and the antibiotics administered [43]. In our investigation, the most frequent colistin-resistant isolated organisms were *Klebsiella* spp. (11/18, 61.1%), followed by *Pseudomonas* spp. (6/18, 33.3%), and *E. coli* (1/18, 5.6%). Similar results were found in another study conducted at Cairo University Hospitals, Egypt, where the most prevalent colistin-resistant isolated organisms were *Klebsiella* spp. and *Pseudomonas* spp. (33.3%) simultaneously, while *E.coli* represented (25.0%) [42]. In an Indian retrospective research, Arjun et al. [46] reported that *K. pneumoniae* represented 87.5% among 24 Gram-negative bacteria resistant to colistin, while *Enterobacter*, *Acinetobacter*, and *E. coli* represented the 3 remaining isolates. Conversely, Prim et al. [48] demonstrated that *Enterobacter* spp. was the most frequent (4.2%) colistin-resistant organism, while *K. pneumoniae* was the least common (0.4%) colistin-resistant isolate. All colistin-resistant *Enterobacteriaceae* isolates in the current investigation were non-susceptible to amikacin, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, ceftaroline, cefazoline, cefotaxime, ceftriaxone, ciprofloxacin, and trimethoprim/sulfamethoxazole. Of the colistin-resistant *Enterobacteriaceae*, 41.7% were sensitive to chloramphenicol, 25% to gentamicin, and 16.7% to tetracycline, while the isolates sensitive to carbapenems, cefoxitin, aztreonam, and fosfomycin represented only 8.3% for each. Sorour et al. [42] reported that all of the isolates of *Enterobacteriaceae* that were resistant to colistin, were not susceptible to amoxicillin-clavulanic acid, ampicillin-sulbactam, ceftriaxone, cefotaxime, and Pipercillin-tazobactam, but 14.3% were sensitive to imipenem, ciprofloxacin, trimethoprim/sulfamethoxazole, 57.1% of the isolates were sensitive to gentamicin and doxycycline, and 42.9% were sensitive to meropenem. In a study on 24 isolates resistant to colistin, including 23 isolates from *Enterobacteriaceae* and one isolate of *Acinetobacter*, Arjun et al. [46] found that 4.2% of the isolates were sensitive to ciprofloxacin, 20.8% were sensitive to doxycycline, 62.5% were sensitive to chloramphenicol, and 75% were sensitive to tigecycline. In our study, none of the colistin-resistant *Pseudomonadaceae* isolates were susceptible to ceftazidime or fosfomycin. Among the isolates of colistin-resistant *Pseudomonadaceae*, only 16.7% were sensitive to aztreonam, 33.3% were sensitive to imipenem, 50% were sensitive to gentamycin and ciprofloxacin, and 66.7% were sensitive to piperacillin-tazobactam. Sorour et al. [42] found that 75% of the colistin-resistant *Pseudomonadaceae* isolates were not susceptible to ceftazidime and cefepime, and 50% of them were sensitive to gentamicin and ciprofloxacin. However, El-Din et al. [49] recorded that 83.33% of the isolates were resistant to ceftazidime and cefepime, 66.6% of the isolates were resistant to gentamicin and ciprofloxacin, and 50% of the isolates were resistant to piperacillin, aztreonam, carbapenem, and amikacin. The high rate of antibiotic resistance in our investigation could be clarified by the fact that the majority of the clinical specimens were taken from hospitalized patients and ICUs. Other resistance risk factors include the use of mechanical breathing or invasive equipment, comorbid diseases, prior usage of antibiotics, and prolonged hospital admissions [42]. Although chromosomal mutations are the primary cause of colistin resistance, transferable plasmid-mediated colistin resistance genes have also been reported. The most common gene responsible for colistin resistance in humans is the *mcr-1* gene [50]. According to PCR data, 2 of 18 (11.1%) colistin-resistant isolates in our investigation were positive for the *mcr-1* gene, including one isolate out of the 11 (9%) colistin-resistant isolates *Klebsiella* spp. and the only *E.coli* colistin-resistant isolate. In agreement with our findings, Rabie et al. [47] reported that *mcr-1* was in only 2 of 24 (8.3%) colistin-resistant isolates, including one out of eight (12.5%) colistin-resistant *E.coli* isolates, and the other one was *K. pneumoniae* out of the 16 (6.25%) colistin-resistant *K. pneumoniae* isolates at Zagazig University Hospitals, Egypt. Similarly, Zaferet al. [51] detected *mcr*-1 only in two of 40 (5%) colistin-resistant isolates, including one of 22 (4.5%) *K. pneumoniae* isolates and one of 18 (5.6%) *E. coli isolates* at Cairo University, Egypt. Furthermore, El-Mokhtar et al. [52] tested for the presence of *mcr-1* in 10 *E.coli* colistin-resistant isolates from Assuit University Hospital and 12 *E. coli* colistin-resistant isolates from Minia University Hospital and found that the *mcr-1* gene was present in all of the colistin-resistant *E. coli* isolates. On the other hand, Emara et al. [33] stated that none of their phenotypically colistin-

resistant isolates had the *mcr*-1 gene at Tanta University, Egypt. However, Abozahra et al. [29] found that the *mcr-1* gene was present in 84.4% of the colistin-resistant *K. pneumoniae* isolates from Damanhour, Egypt. One possible explanation for the elevated rates of *mcr*-1 carriage in some regions in Egypt could be the abundance of poultry and livestock [29]. Globally, Quan et al. [45] found that 19 of 22 (86.4%) colistin-resistant *E.coli* carry *mcr*-1, whereas only 1 isolate from 4 (25%) *K. pneumoniae* colistin-resistant isolates carries *mcr-1*. Luo et al. [10] found that 52.5% of colistin-resistant *E. coli* isolates harbored the *mcr-1* gene, explaining their elevated *mcr*-1 carriage rates as a result of China's high meat and cattle consumption. Our investigation revealed that all the colistin-resistant *Pseudomonas* spp. tested negative for the *mcr-1* gene. This is consistent with El-khatib et al. and Emara et al [33, 43], who reported that *mcr-1* was negative for the tested colistin-resistant *P. aeruginosa* isolates. This disagreed with El-Din et al. and Abd El-Baky et al. [49, 53], who found that *mcr-1* was present in 44.4% and 50% of the *P. aeruginosa* isolates that were resistant to colistin, respectively. None of the isolates tested were positive for the *mcr-2* gene. Our results are supported by research on *K. pneumoniae* and *E. coli* isolates from patients at Zagazig University and the National Cancer Institute, Cairo University in Egypt, which showed that none of the examined isolates carried *mcr-2* [47, 51]. Furthermore, Abd El-Baky et al. [53] noted that no isolates tested positive for the *mcr-2* gene in research on *P. aeruginosa* isolates from patients at Minia University Hospital, Minia, Egypt. However, El-khatib et al. [43] detected *mcr-2* in 3 of 11(27.3%) colistin-resistant Gram-negative isolates including one *P. aeruginosa* isolate and 2 isolates of *K. pneumoniae* in a study included some hospitals in Cairo, Egypt. Also, El-Din et al. [49] recorded that the percentage of colistin-resistant *P. aeruginosa* with *mcr-2* genes was 16.67% at Sohag University Hospitals, Egypt. Variations in sample size, host genetic variables, and geographic distribution may be the cause of this disparity in the distribution of *mcr-1* and *mcr-2* in isolates that are resistant to colistin. Notably, the World Health Organization (2018) explained this disparity in *mcr* genes distribution in colistin-resistant isolates by concluding that it is not possible to predict sensitivity to colistin from negative PCR data since the test cannot rule out the existence of additional *mcr* genes or even chromosomal mechanisms of resistance that are not covered by the test.

Conclusion

The development of colistin resistance among XDR and MDR Gram-negative bacteria in our clinical setting is alarming and necessitates close adherence to infection control procedures as well as rigorous antimicrobial stewardship programs. The present investigation confirms earlier reports of the detection of the plasmid-mediated gene *mcr-1*. Additional research including looking into other *mcr* genes and chromosomal mutations as alternative determinants of resistance is also imperative to completely comprehend other molecular mechanisms underlying colistin resistance among clinical Gram-negative isolates.

Abbreviations

ASUHs: Ain Shams University Hospitals

CLSI: Clinical and Laboratory Standards Institute

E. coli: *Escherichia coli*

ETT: Endotracheal tube

EUCAST: European Committee on Antimicrobial Susceptibility Testing

ICUs: Intensive care units

K. pneumoniae: *Klebsiella pneumoniae*

LIA: Lysine Iron Agar

MDR: Multidrug-resistant

MIC: Minimum inhibitory concentration

MIO: Motility, Indole, Ornithine

NCI: National Cancer Institute

P. aeruginosa: *Pseudomonas aeruginosa*

TSI: Triple sugar iron agar

UTIs: Urinary tract infections

XDR: Extensively drug-resistant

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Conflict of interest

Non declared

Ethical approval

This study was approved by the Research Ethics Committee of a university. The committee detail and the assigned approval code was hidden for the double-blind review process.

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