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Original article

Prevalence and molecular characterization of plasmid-mediated colistin resistance among multidrug-resistant Gram-negative bacilli at Ain Shams University Hospitals

*Nahed M. El-Ghannam, Shereen E. Taha, Nesma A. Hassanin**

Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University

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ABSTRACT

Background: Colistin has become a critical last-resort option for treating severe infections that remain unresponsive to other antibiotics, especially carbapenems. The rise in colistin resistance, along with the discovery of mobile colistin resistance (*mcr*) genes, significantly complicates the management of these infections. Determining colistin susceptibility can be challenging due to the drug's unique chemical properties and the various resistance mechanisms employed by bacteria. **Aim:** We aimed to assess the prevalence of colistin resistance among multidrug-resistant (MDR) Gram-negative bacilli (GNB) isolated from different clinical samples at Ain Shams University Hospitals and for the molecular detection of different plasmid genes mediating such resistance (*mcr-1* to *mcr-10*). **Methods:** We used the colistin broth disk elution method (CBDE) to assess colistin susceptibility. Resistant strains were further subjected to a conventional polymerase chain reaction (PCR) assay to identify the genes mediating such resistance. **Results:** In total, we identified 12 isolates resistant to colistin by the CBDE test, with *Klebsiella pneumoniae* being the most resistant bacterial species. These resistant isolates were examined using conventional PCR which revealed the presence of multiple *mcr* genes, with *mcr*-2 being the most commonly detected. **Conclusion:** Our study highlights the concerning prevalence of colistin resistance among MDR GNB at Ain Shams University Hospitals. The detection of multiple *mcr* genes in these isolates underscores the importance of continuous surveillance and molecular characterization of colistin resistance mechanisms.

Introduction

The increasing prevalence of infections by multidrug-resistant (MDR) Gram-negative bacilli (GNB), especially the carbapenem-resistant strains, is a serious public health concern since they are susceptible to only a few antibiotics. The patients admitted to the intensive care units (ICUs) are more prone to such serious infections which is the prime cause of mortality [1]. The World Health Organization (WHO) has listed carbapenem-resistant bacterial strains among the critical priority pathogen group as it poses a great threat to human health and classified colistin as an important drug for human medicine [2].

Colistin (also known as polymyxin E) is the last resort drug of choice for the treatment of lethal infections caused by carbapenem-resistant GNB. It was originally isolated from *Paenibacillus*

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* *Corresponding author:* Nesma A. Hassanin
E-mail address: nesma@med.asu.edu.eg

polymyxa in the 1940s. Colistin and polymyxin B are the only drugs from the polymyxin class of antibiotics approved to treat such infections in humans. This antibiotic has been used in veterinary science since the mid-twentieth century but its use in humans has been restricted for a decade due to its known many unresolved issues like pharmacokinetics in critically ill subjects, dosing, nephrotoxicity, paucity of susceptibility data, and development of resistance. Hence, there is a lack of consensus on the optimum use of this antibiotic to date [3]. Colistin is a narrow-spectrum cationic polypeptide antibiotic. Although the exact mechanism of antibacterial activity of colistin has not been fully elucidated, the main target has clearly been established to be lipid A of the lipopolysaccharide of the outer membrane of GNB [4]. Colistin binds to the negatively charged phosphate group of the lipopolysaccharide which results in disarrangement of cell membrane. Ultimately, there is a loss of cell membrane integrity resulting in increased permeability of the cell, leakage of cell contents, and finally cell lysis. Whereas colistin's major mode of action is on the bacterial cell membrane, a secondary mode of action by inhibition of respiratory enzymes, namely type II NADH quinone oxidoreductases, has also been proposed [5].

The re-introduction of colistin use in clinical practice has resulted in its increased reports of resistance among GNB. Resistance to colistin is usually acquired, either chromosomal or plasmid mediated. Chromosomal colistin resistance in the bacterium is thought to be due to chromosomal mutations in the regulatory genes mgrB, phoPQ, and pmrAB that are associated with lipopolysaccharide alteration [6]. Mobile colistin resistant (*mcr*) genes located on plasmids encodes phosphoethanolamine transferase that modifies the phosphoethanolamine moiety of lipid A, conferring resistance to colistin. Since the first report of mcr-1 in late 2015, ten different *mcr* variants (*mcr*-1 to *mcr*-10) have been reported [7]. There is a lack of systematic studies to find out the prevalence of colistin resistance in MDR GNB in clinical samples collected from different clinical settings. A few published reports about this issue were a part of the global antimicrobial surveillance plan where the strains were randomly selected. However, these reports were not representative of the whole population due to the small sample size [3]. According to a study from China, the overall prevalence of colistin resistance among GNB was 9.6% which was much higher than the reported prevalence in Western countries [8]. Another study in India reported the overall rate of colistin resistance to be 19.6% [3]. In all MDR GNB isolates from clinical samples, colistin is not regularly checked and that may lead to unawareness of the actual resistance [9].

This study aimed to assess the prevalence of colistin resistance among MDR GNB isolated from different clinical samples at Ain Shams University Hospitals and for the molecular detection of different plasmid genes mediating such resistance (*mcr*-1 to *mcr*-10).

Materials and methods

This observational cross-sectional study was performed at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University in the period between July 2023 to May 2024, and approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University (FWA000017585).

Bacterial strains

In this study, sixty MDR GNB isolates were obtained from different clinical samples collected from inpatients. The antimicrobial susceptibility reports of all bacterial isolates and patient data (age, gender, site of admission, & type of sample) were provided by the Central Laboratories of Ain Shams University Hospitals. The isolates were identified as MDR if non-susceptible to at least one agent in three or more classes of antibiotics according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [10]. Confirmatory identification of collected isolates to the species level was performed by conventional microbiological techniques [11].

Phenotypic detection of colistin resistance

All MDR isolates were subjected to (CBDE) test to determine colistin susceptibility according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) & the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [10, 12]. The CBDE method was conducted using four 10-ml cation-adjusted Mueller-Hinton broth (CA-MHB; Remel, Lenexa, KS) tubes for each isolate, with 0, 1, 2, and 4 colistin disks (10 µg; BD, Sparks MD) added, resulting in final concentrations of 0 (growth control), 1, 2, and 4 µg/ml, respectively **(Figure 2).** The tubes were incubated at room temperature for

30 minutes to allow the colistin to diffuse from the disks. Bacterial Inocula were created by suspending fresh colonies from an overnight sheep's blood agar plate in normal saline, adjusting the turbidity to align with a McFarland 0.5 standard. A 50-µl aliquot of this standardized suspension was added to each tube, followed by gentle vortexing to achieve a final inoculum of 7.5 × 105 CFU/ml. After a 16- to 20-hour incubation at 35°C in ambient air, colistin MIC values were determined visually. The MIC was read at the lowest concentration which completely inhibited the growth of the tested isolate. Following CLSI guidelines, colistin susceptibility was to be interpreted for *Enterobacteriaceae* & *Pseudomonas aeruginosa* as intermediate when MIC was ≤ 2 µg/mL and as resistant when MIC was ≥ 4 µg/mL, while susceptibility was to be reported as sensitive when MIC was ≤ 2 µg/mL & as resistant when MIC was ≥ 4 µg/mL, following EUCAST guidelines.

Bacterial DNA isolation and purification

Pure colonies from colistin-resistant isolates were cultured & incubated overnight in Luria–Bertani (LB) media at 37°C & then DNA extraction procedure was performed using easy pure bacteria genomic DNA kit (lot #R10206). The isolated bacterial DNA was stored at -20°C.

Molecular characterization of colistin resistance genes

All isolates confirmed to be colistin-resistant by CBDE test were subjected to conventional PCR assay following the protocol provided by **Singh et al.** [13] for molecular detection of plasmid-mediated *mcr* genes (*mcr*-1 to *mcr*-10) using specific primers as listed in **(table 1).** In the PCR tubes, 5 µl of the master mix, 1 µl of the forward primer, 1 µl of its reverse primer, 2 µl of free nuclease water, & 1 µl of tested DNA sample were added. The previous steps were repeated to each gene separately. The thermal cycling conditions for *mcr*-1 gene were as follows: pre-denaturation at 95°C for 5 minutes, denaturation at 94°C for 25 seconds, annealing at 52°C for 25 seconds, & extension at 72°C For 25 seconds. The thermal cycling conditions for *mcr*-2, 3, 4, 9, &10 genes were as follows: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 53°C for 30 seconds, & extension at 72°C For 30 seconds. The thermal cycling conditions for *mcr*-5, 6, 7, &8 genes were as follows: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, & extension at 72°C for 30 seconds. The reaction was endpoint conventional PCR, and the amplified products were visualized using agarose gel electrophoresis stained with ethidium bromide and visualized using UV LUT-300D trans-illuminator (LABNICS, UK).

Analysis of the results

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 27. The quantitative data were presented as means, standard deviations, and ranges for parametric data, and as medians and inter-quartile ranges (IQR) for non-parametric data. Also, qualitative variables were presented as numbers and percentages. The one-sample Kolmogorov-Smirnov test can be used to test that a variable is normally distributed. The comparison between groups regarding qualitative data was done by using the Chi-square test and/or Fisher exact test when the expected count in any cell was found less than 5. The comparison between two independent groups with quantitative data and non-parametric distribution was done by using the Mann-Whitney test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *p*-value was considered significant as the following: *p* -value > 0.05 as non-significant (NS). *p* -value < 0.05 as significant (S), & *p* -value < 0.01 as highly significant (HS).

Results

Patient data & isolated pathogens

This study included sixty patients, 34 males & 26 females, from which different clinical samples were collected & the MDR bacterial strains were isolated. The median age of patients was 27.5 years old (ranging from one month to 88 years). Most of the bacterial strains were obtained from blood (29/60, 48.3%) followed by wound (14/60, 23.3%), sputum (7/60, 11.7%), urine (6/60, 10%), and bronchoalveolar lavage (4/60, 6.7%). Most patients were intensive care unit (ICU) residents (45/60, 75%) as illustrated in **figure (1).** *Klebsiella pneumoniae* was the most isolated Gram-negative bacilli (36/60, 60%), followed by *Pseudomonas aeruginosa* (16/60, 26.7%), & *Escherichia coli* (8/60, 13.3%).

Antimicrobial susceptibility profile of isolated pathogens

According to the susceptibility reports provided by the Central Laboratories of Ain Shams University Hospitals, all isolated pathogens were found to be resistant to multiple tested antibiotics (ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin, piperacillin-tazobactam, cefoxitin, cefotaxime, ceftazidime, ceftazidime-avibactam, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, doxycycline, trimethoprim-sulfamethoxazole, & nitrofurantoin). Due to resistance to more than three different antimicrobial classes, the isolates tested were identified as multidrug-resistant **(Table 2).**

Results of CBDE test

Of the 60 isolates tested, 48 (80%) were reported as intermediate or sensitive following the guidelines of CLSI & EUCAST, respectively, & 12 (20%) were reported as resistant as illustrated in **table (3)**, with *Klebsiella pneumoniae* being the most resistant (7/12, 58.3%), followed by *Pseudomonas aeruginosa* (4/12, 33.3%), & *Escherichia coli* (1/12, 8.3%).

There was no statistically significant difference between sensitive/intermediate and resistant isolates as regards gender, age, and site of admission of the study patients **(Table 4). Table 5** also reveals no statistical significance between colistin susceptibility & type of isolated pathogen.

Genomic characterization of colistin resistance among tested isolates

Various *mcr* genes were detected using conventional PCR assay. As illustrated in **figure (3)**, the most frequently detected gene was *mcr*-2, which tested positive in 7 resistant isolates. In contrast, the *mcr-5* gene was not detected in any resistant isolates. Since all resistant isolates were positive for one or more *mcr* genes, further examination of chromosomal mutations was not conducted. **Table 6** illustrates the relation of different *mcr* gene variants with type of isolated organism, where no statistical significance was detected.

Table 1. Sequences of primers used to detect *mcr* genes.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
***mcr*-1**	CGGTCAGTCCGTTTGTTC	CTTGGTCGGTCTGTAGGG	[14]
***mcr*-2**	TGTTGCTTGTGCCGATTGGA	AGATGGTATTGTTGGTTGCTG	[14]
***mcr*-3**	TTGGCACTGTATTTTGCATTT	TTAACGAAATTGGCTGGAACA	[14]
***mcr*-4**	ATTGGGATAGTCGCCTTTTT	TTACAGCCAGAATCATTATCA	[14]
***mcr*-5**	ATGCGGTTGTCTGCATTTATC	TCATTGTGGTTGTCCTTTTCTG	[13]
***mcr*-6**	GTCCGGTCAATCCCTATCTGT	ATCACGGGATTGACATAGCTAC	[13]
***mcr*-7**	TGCTCAAGCCCTTCTTTTCGT	TTCATCTGCGCCACCTCGT	[13]
***mcr*-8**	AACCGCCAGAGCACAGAATT	TTCCCCCAGCGATTCTCCAT	[13]
***mcr*-9**	TCAGGGTGAAAGTTATTCCG	GTCAGGATTATAGACGCTGG	[7]
***mcr*-10**	GGGTAATCCCCTTGGTTTTA	TATCGTGGGAATATGTCCTG	[7]

Table 2. Antimicrobial susceptibility testing for the tested isolates.

	Total number of tested isolates	Susceptible	Resistant
Ampicillin	38	0 (0%)	38 (100%)
Ampicillin-Sulbactam	38	0 (0%)	38 (100%)
Amoxicillin-Clavulanate	38	0 (0%)	38 (100%)
Piperacillin	16	0 (0%)	16 (100%)
Piperacillin-Tazobactam	16	0 (0%)	16 (100%)
Cefoxitin	38	0 (0%)	38 (100%)
Cefotaxime	38	0 (0%)	38 (100%)
Ceftazidime	54	3 (5.6%)	51 (94.4%)
Ceftazidime-Avibactam	16	0 (0%)	16 (100%)
Ceftriaxone	38	0 (0%)	38 (100%)
Cefepime	54	0 (0%)	54 (100%)
Aztreonam	16	4 (25%)	12 (75%)
Imipenem	60	2 (3.3%)	58 (96.7%)
Meropenem	60	0 (0%)	60 (100%)
Amikacin	6	2 (33.3%)	4 (66.7%)
Gentamicin	38	3 (7.9%)	35 (92.1%)
Tobramycin	38	0 (0%)	38 (100%)
Ciprofloxacin	60	0 (0%)	60 (100%)
Levofloxacin	60	8 (13.3%)	52 (86.7%)
Doxycycline	38	9 (23.7%)	29 (76.3%)
Trimethoprim-Sulfamethoxazole	44	6 (13.6%)	38 (86.4%)
Nitrofurantoin	6	1 (16.7%)	5 (83.3%)

Table 3. Assessment of colistin susceptibility of the tested isolates by CBDE test & interpretation according to CLSI & EUCAST guidelines.

		Total no. of isolates = 60
CBDE Test MIC (µg/mL)	≤1	41 (68.3%)
	2	7 (11.7%)
	4	4 (6.7%)
	>4	8 (13.3%)
Colistin sensitivity interpretation according to CLSI	Intermediate	48 (80%)
	Resistant	12 (20%)
Colistin sensitivity interpretation according to EUCAST	Sensitive	48 (80%)
	Resistant	12 (20%)

CBDE: Colistin broth disk elution, CLSI: The Clinical & Laboratory Standards Institute, EUCAST: The European Committee on Antimicrobial Susceptibility Testing, MIC: minimum inhibitory concentration.

Table 4. Relation of colistin susceptibility among ICU versus non-ICU patients and their demographic characteristics.

		Colistin sensitivity interpretation according to CLSI/EUCAST		***P*-value**
		Intermediate/Sensitive	**Resistant**	
		No.= 48	**No.= 12**	
Gender	**Female**	21 (43.8%)	5 (41.7%)	0.896
	Male	27 (56.2%)	7 (58.3%)	
Age (Years)	**Median (IQR)**	29 (3 – 62)	16.5 (2.5 – 59)	0.460
	Range	0.07 – 88	0.06– 66	
Admission	**ICU**	35 (58.3%)	10 (16.7%)	0.456
	Non-ICU	13 (21.7%)	2 (3.3%)	

P-value > 0.05: Non-significant; *P*-value < 0.05: Significant; *P*-value < 0.01: Highly significant. *: Chi-square test, ≠: Mann-Whitney test. CLSI: The Clinical & Laboratory Standards Institute, EUCAST: The European Committee on Antimicrobial Susceptibility Testing, ICU: intensive care unit, IQR: interquartile range.

Table 5. Colistin susceptibility interpretation among different species of tested isolates.

		Colistin sensitivity interpretation according to CLSI/EUCAST		***P*-value**
		Intermediate/Sensitive	**Resistant**	
		No.= 48	**No.= 12**	
Isolated organism	***K. pneumonia***	29 (60.4%)	7 (58.3%)	0.764
	P. aeruginosa	12 (25%)	4 (33.3%)	
	E. coli	7 (14.6%)	1 (8.3%)	

P-value > 0.05: Non-significant; *P*-value < 0.05: Significant; *P*-value < 0.01: Highly significant. *: Chi-square test, ≠: Mann-Whitney test.
CLSI: The Clinical & Laboratory Standards Institute, EUCAST: The European Committee on Antimicrobial Susceptibility Testing.

Table 6. The relation of different colistin-resistance encoding genes with the studied colistin-resistant species.

		Isolated organism			***P*-value**
		K. pneumoniae	***P. aeruginosa***	***E. coli***	
		No.= 7	**No.= 4**	**No.= 1**	
MCR 1	**Negative**	4 (57.1%)	2 (50%)	0 (0%)	0.565
	Positive	3 (42.9%)	2 (50%)	1 (100%)	
MCR 2	**Negative**	2 (28.6%)	2 (50%)	1 (100%)	0.366
	Positive	5 (71.4%)	2 (50%)	0 (0%)	
MCR 3	**Negative**	5 (71.4%)	4 (100%)	1 (100%)	0.424
	Positive	2 (28.6%)	0 (0%)	0 (0%)	
MCR 4	**Negative**	3 (42.9%)	4 (100%)	1 (100%)	0.117
	Positive	4 (57.1%)	0 (0%)	0 (0%)	
MCR 5	**Negative**	7 (100%)	4 (100%)	1 (100%)	_
	Positive	0 (0%)	0 (0%)	0 (0%)	
MCR 6	**Negative**	6 (85.7%)	4 (100%)	1 (100%)	0.677
	Positive	1 (14.3%)	0 (0%)	0 (0%)	
MCR 7	**Negative**	4 (57.1%)	4 (100%)	1 (100%)	0.240
	Positive	3 (42.9%)	0 (0%)	0 (0%)	
MCR 8	**Negative**	5 (71.4%)	4 (100%)	1 (100%)	0.424
	Positive	2 (28.6%)	0 (0%)	0 (0%)	
MCR 9	**Negative**	5 (71.4%)	4 (100%)	1 (100%)	0.424
	Positive	2 (28.6%)	0 (0%)	0 (0%)	
MCR 10	**Negative**	5 (71.4%)	4 (100%)	1 (100%)	0.424
	Positive	2 (28.6%)	0 (0%)	0 (0%)	

P-value > 0.05: Non-significant; *P*-value < 0.05: significant; *P*-value < 0.01: highly significant. *: Chi-square test

Figure 1. Distribution of studied patients among hospital departments.

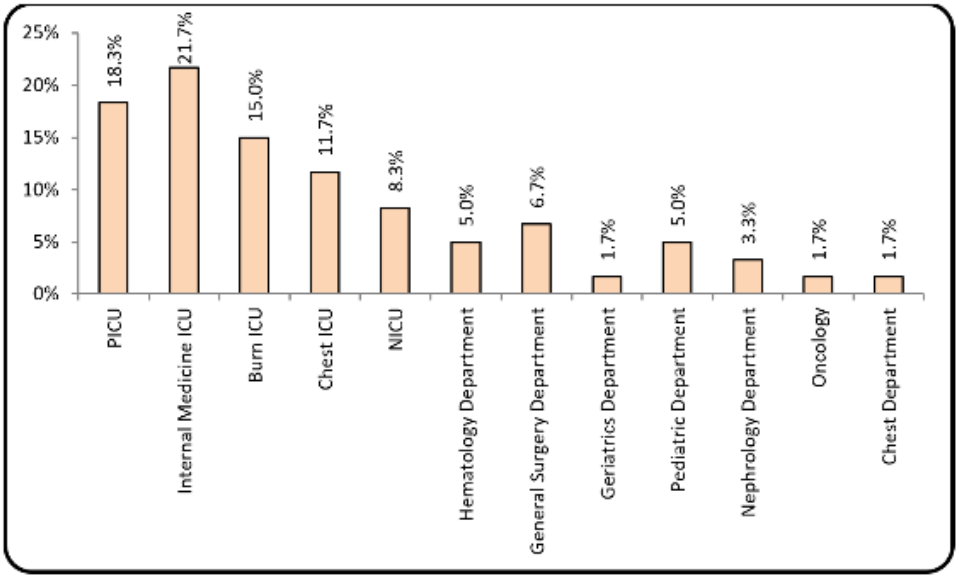


Figure 2. Colistin broth disk elution (CBDE) test. (a) Tubes for an *Escherichia coli* isolate with a colistin MIC of <1 µg/ml, reported as colistin intermediate/sensitive according to CLSI/EUCAST guidelines. (b) Tubes for a *Klebsiella pneumoniae* isolate with a colistin MIC of 4 µg/ml, reported as colistin-resistant according to CLSI/EUCAST guidelines. (c) Tubes for a *Pseudomonas aeruginosa* isolate with a colistin MIC of >4 µg/ml, reported as colistin-resistant according to CLSI/EUCAST guidelines.

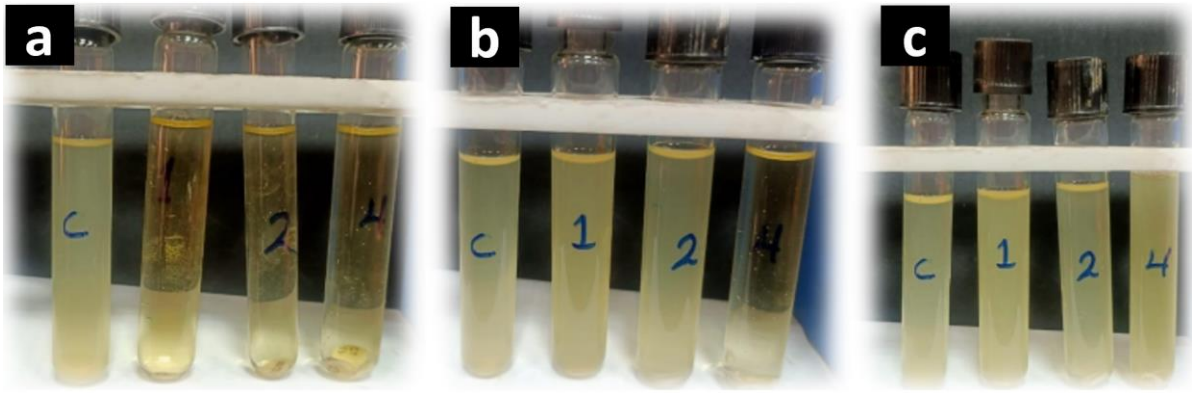


Figure 3. Distribution of colistin-resistance encoding genes among the studied colistin-resistant species.

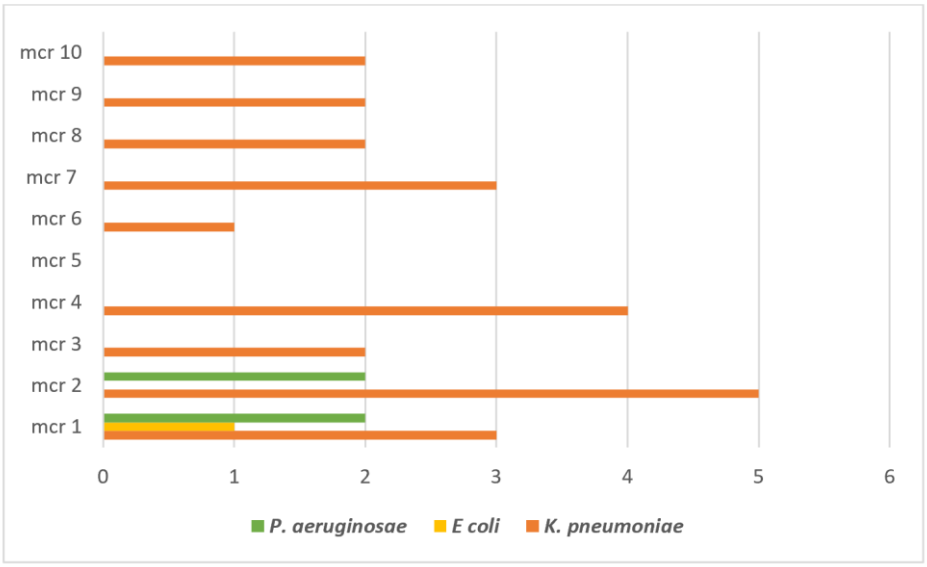


Figure 4. Agarose gel electrophoresis of the amplified products of the PCR assay. (LT) *mcr-1* gene target sequence (300bp) was detected in lanes 1,4,6,7,8,&12. (RT) *mcr-2* gene target sequence (500bp) was detected in lanes 2,3,5,7,8,9,&10.

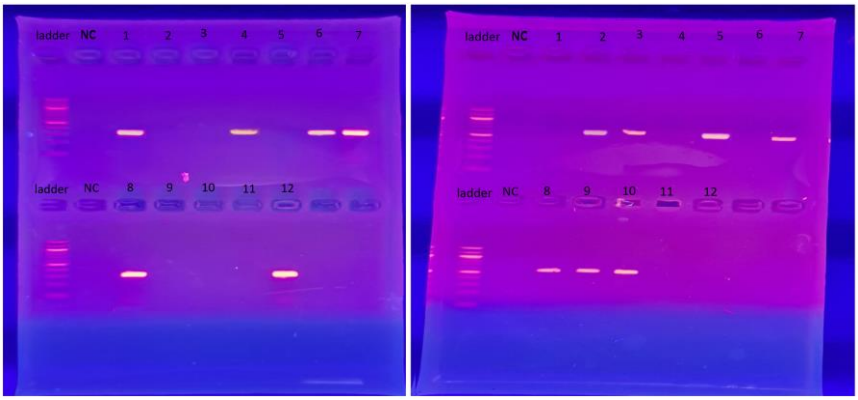
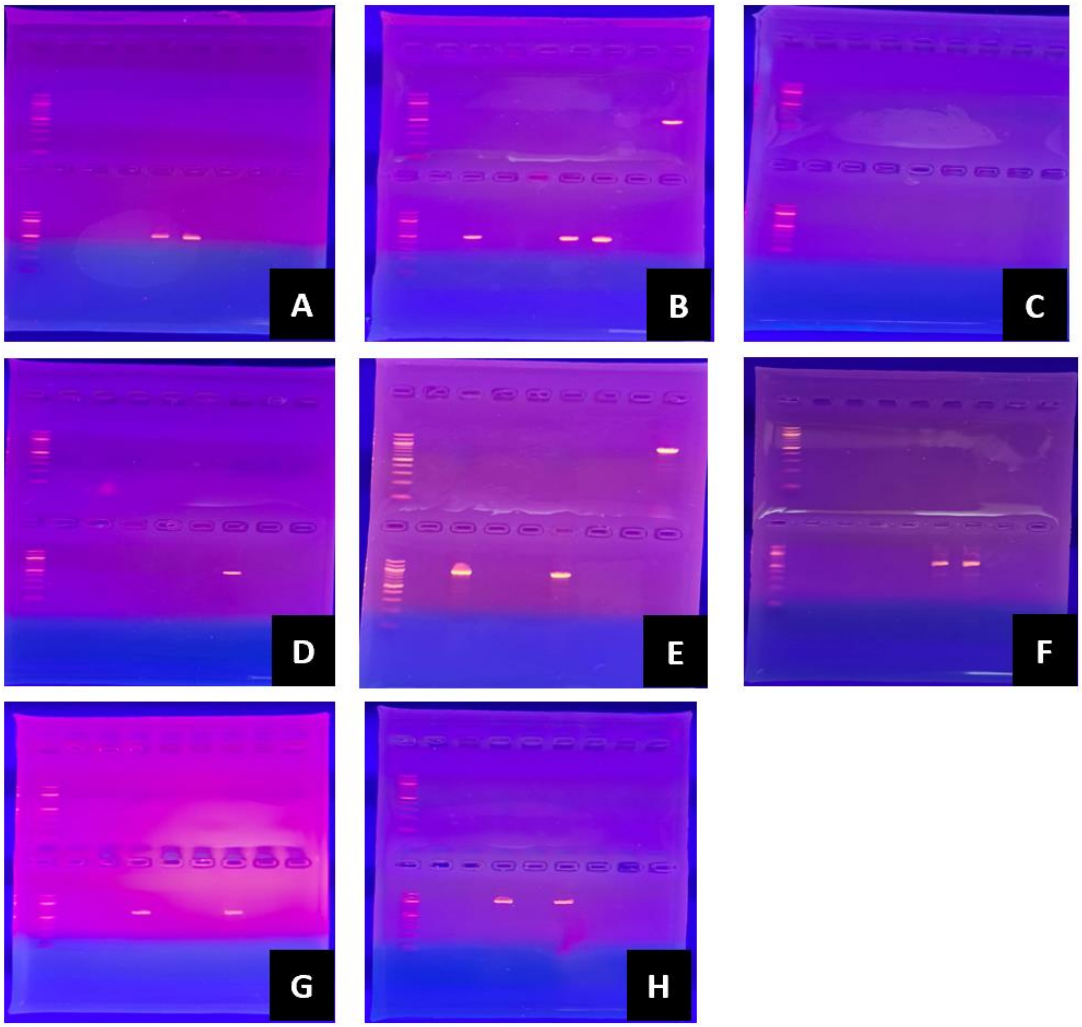


Figure 5. Agarose gel electrophoresis of the amplified products of the PCR assay. (A) *mcr-3* gene target sequence (500bp) was detected in lanes 10&11. (B) *mcr-4* gene target sequence (500bp) was detected in lanes 7,8,11,&12. (C) *mcr-5* gene target sequence was not detected in any lane. (D) *mcr-6* gene target sequence (400 bp) was detected in lane 12. (E) *mcr-7* gene target sequence (800 bp) was detected in lanes 7,8,&11. (F) *mcr-8* gene target sequence (500 bp) was detected in lanes 11&12. (G) *mcr-9* gene target sequence (600bp) was detected in lanes 9&12. (H) *mcr-10* gene target sequence (900bp) was detected in lanes 9&11.



Discussion

Colistin is considered the last line of defense against many MDR GNB infections. However, its effectiveness is diminished by the widespread occurrence of colistin resistance, which significantly restricts available treatment options. Identifying resistance genes is a key to managing the spread of resistance, with horizontal gene transfer being the main method of transmission among bacteria [15]. This cross-sectional study was conducted to evaluate the prevalence of colistin resistance among MDR GNB isolates at Ain Shams University Hospitals, employing both phenotypic & genotypic testing methods.

In our study, sixty MDR GNB isolates were obtained from different clinical samples collected from inpatients, 36 males & 24 females. Patients ranged in age from neonates to 88 years old; the median age was 27.5 years. Most patients were ICU residents (45/60, 75%). In concordance with our findings, **Tosi et al.** & **Wu et al.** reported in their studies that the prevalence of ICU infections with MDR GNB isolates was 40-60% [16, 17]. This could be attributed to antibiotic use at a higher frequency, higher dose, and for longer duration in ICU settings [18].

Klebsiella pneumoniae was the most isolated GNB (36/60, 60%), followed by *Pseudomonas aeruginosa* (16/60, 26.7%), & *Escherichia coli (E. coli)* (8/60, 13.3%). This

coincides with previous Egyptian investigations by **Ibrahim et al.** who found MDR K. pneumoniae to be the most isolated GNB at Ain Shams University Hospitals, followed by *Escherichia coli* (18%) & *Pseudomonas aeruginosa* (15%) [19]. On the other hand, the United States National Healthcare Safety Network has indicated a rising incidence of MDR GNB, including *E. coli, Klebsiella pneumoniae*, and *Enterobacter* spp, with over 60% identified as *Acinetobacter* species. Similarly, the European Antimicrobial Resistance Surveillance Network has documented notable resistance patterns among GNB, reporting the highest levels of resistance in Acinetobacter spp, followed by *E. coli* and *Klebsiella pneumoniae* [20]. Various studies from hospitals in India reveal that the prevalence of MDR GNB ranges from 19% to 60%, with the prevalence of drug-resistant *Enterobacterales* is approximately 18.5% [21].

All MDR GNB isolates in the current study were subjected to colistin broth disk elution test (CBDE) to determine colistin susceptibility according to the guidelines of CLSI & EUCAST [10, 12]. Of the 60 isolates tested, 48 (80%) were reported as intermediate/sensitive, & 12 (20%) were reported as resistant. This agrees with a study performed in Kasr Al-Ainy University Hospitals by **Abdel-Aty et al.** who reported that the colistin resistance percentage between the studied isolates was 22.8% [22]. Another study conducted in Egypt by **El-Mahallawy et al.** at the National Cancer Institute in 2022 reported a remarkably high level of colistin resistance among the studied MDR *Enterobacterales* isolates with a prevalence of 19.9%, which was much higher than a previous rate of 8.8% reported in the same hospital in 2019 & was attributed to the widespread use of colistin in high-risk patients due to lack of other treatment options. Unpublished data on the use of colistin at the National Cancer Institute showed that 35% of infectious episodes necessitated the addition of colistin [23]. Similarly, the present findings are consistent with Panigrahi et al. who reported a high prevalence of colistin resistance (19.6%) in MDR GNB infections in ICU patients [3].

Another study in Egypt by **Ghandour et al.** found that 54.7% of isolates were resistant to colistin [24]. In Iran, **Moosavian & Emam** reported a colistin resistance rate of 13.6% [25]. **Balkhair et al.** stated the prevalence of colistin resistance among the carbapenem-resistant blood culture isolates as 13.4% which showed a startling 70% increase in colistin resistance when compared with a prevalence of 7.9% reported by a previous study from the same setting [26].

The high incidence of colistin resistance among MDR isolates could be attributed to multiple factors: colistin is not strictly regulated, and it is often administered in inappropriate dosages for illnesses that could be treated with lower antibiotics. Colistin is commonly used in agriculture, pisciculture, and farm and dairy animals. Consequently, small amounts of colistin leak into the environment leading to the development of colistin-resistant microorganisms, which subsequently enter the human body in various ways [27].

Our study revealed *Klebsiella pneumoniae* as the most colistin-resistant isolate (58.3%), followed by *Pseudomonas aeruginosa* (33.3), & *E. coli* (8.3%). Our findings coincide with **Elkhatib et al.** who found that among the eleven colistin resistant isolates, 8 (72.7%) were *K. pneumoniae* and 3 (27.3%) were *Pseudomonas aeruginosa* [28]. Furthermore, **Panigrahi et al.** reported that the rate of colistin resistance was found to be higher in *Klebsiella pneumoniae* as compared to other species [3]. Conversely, **Ibrahim et al.** reported the prevalence of colistin resistance among Gram-negative isolates as 14%, with *Pseudomonas aeruginosa* being the most resistant (20%), followed by *K. pneumoniae* (16.6%), & *E. coli* (5.5%). In Hungary, the rates of colistin resistance were 0.6% for *Enterobacterales* & 1.3% for *Pseudomonas* spp. [19]. Interestingly, our study revealed no statistically significant difference between sensitive/intermediate and resistant isolates as regards gender, age, site of admission of the study patients, & type of isolated pathogen in our study.

Various *mcr* genes conveying colistin resistance were detected in the current study using conventional PCR assay. Interestingly, the most detected gene was mcr-2 which was positive in 7 resistant isolates (58.3%) & 5 of these isolates were *K. pneumoniae*, followed by *mcr*-1 (50%), *mcr* 4 (33.3%), *mcr* 7 (25%). *mcr*-3,8,9, &10 had each a detection rate of 16.7%, while *mcr*-5 gene was not detected in any resistant isolate. This comes in agreement with a study by **El-Khatib et al.** who tested 11 colistin-resistant isolates for mcr-1 to mcr-5 genes by PCR and found that three were positive for mcr-2: two *K. pneumoniae* and one *Pseudomonas aeruginosa* [28]. Conversely, a study by **Shi et al.** stated *mcr*-1 being the most prevalent

one (86.1%), followed by *mcr*-9 (5.7%), *mcr*-5 (4.4%), and *mcr*-3 (3%) [29]. In Egypt, **Mahmoud et al.** reported *mcr*-1 in 94.4% of colistin-resistant isolates while *mcr*-2 was revealed in 27.8% [30]. Among 43 colistin-resistant isolates in a study by **Khattab et al.,** *mcr*-1 gene was detected in one isolate while mcr-2 was detected in two isolates [31]. The difference in detecting resistant mechanisms was most appropriately explained by the WHO which highlighted that the negative PCR test results cannot reliably predict susceptibility to colistin, as they may not identify chromosomal mechanisms or new mcr genes not included in testing protocols [32].

In the current study, 50% of colistin-resistant isolates carried multiple genes mediating such resistance. This goes in agreement with a large-scale study in Vietnam by **Le et al.** who investigated colistin resistance in patients and healthy individuals, finding that 25.9% of patients carried at least one colistin-resistance gene, while 9.4% had multiple such genes. The most detected gene was *mcr*-1 (16.5%), followed by *mcr*-9 (11.8%), *mcr*-6 (10.6%), *mcr*-4 (9.4%), & *mcr*-2 (5.9%). None of the patients tested positive for the *mcr*-3, *mcr*-7, or *mcr*-8 genes. Among healthy individuals, 24% were positive for at least one colistin-resistance gene, with *mcr*-10 being the most frequent (27.0%), followed by *mcr*-1 (24.3%), *mcr*-8 (21.6%), and *mcr*-9 (13.5%). No healthy individuals tested positive for the *mcr*-2 or *mcr*-5 genes [33]. The presence of multiple colistin-resistance genes in some patients highlights the complexity of antimicrobial resistance and the potential for co-selection of resistance traits. This co-existence of resistance genes within single bacterial isolates emphasizes the need for thorough surveillance to monitor the emergence and spread of resistance determinants [34].

The findings of several studies prove the global distribution of the mcr genes, having been detected in 57 countries on all continents except Antarctica, & the most frequent *mcr*-positive isolates among these countries have been from livestock sources, followed by humans, meat and food products [35]. The *mcr*-mediated transmission of colistin resistance poses significant global health implications, making immediate intervention crucial. As international travel becomes increasingly accessible, the failure to contain the spread of mcr could lead to worldwide outbreaks of hard-to-treat diseases in both humans and animals, with potentially disastrous consequences [9].

Colistin, historically used for several decades, has recently gained importance as a treatment option due to the rise of multidrug-resistant bacteria, which left few alternatives. However, its effectiveness is now significantly threatened by the global surge in colistin-resistant strains [36]. A deep understanding of colistin resistance is crucial for developing strategies to mitigate its growing impact on public health. While research has often focused on clinical settings and healthcare-related infections, there is increasing recognition of the need to examine the prevalence of colistin-resistant genes among seemingly healthy individuals, as these asymptomatic carriers play a crucial role in the spread of resistance [37]. The interaction between human carriers and environmental reservoirs, including water sources and agricultural spaces, further accelerates the spread and persistence of resistance genes [38].

In response to the rise of plasmid-borne colistin resistance, the World Health Organization (WHO) has recommended reducing the use of antibiotics, including colistin, in animal feed, particularly those critical for human medicine. The WHO's One Health approach emphasizes the need to address both human and animal health to combat antimicrobial resistance [39]. Nevertheless, the use of colistin in livestock remains widespread in many low- and middle-income countries, highlighting disparities in global efforts to combat resistance [40].

The current study reveals several limitations that must be considered when interpreting the results. Firstly, the reliance on a specific phenotypic testing method for colistin susceptibility, such as the CBDE test, may not capture all resistance mechanisms, particularly those related to plasmid-mediated resistance, which can vary between bacterial strains. Limited financial resources restricted our ability to implement more comprehensive genomic testing methodologies, which could have provided a deeper understanding of resistance mechanisms. Additionally, the budget limitations affected the sample size, as we were only able to analyze a smaller number of bacterial isolates, potentially impacting the generalizability of our findings. The cross-sectional nature of the study means that it only provides a snapshot of resistance at one point in time, leaving open the

possibility that resistance patterns may evolve or change, especially with the increasing use of colistin.

Conclusions

The current research highlights the alarming prevalence of colistin resistance among MDR GNB at Ain Shams University Hospitals. Through molecular characterization, the presence of resistance genes emphasizes the urgent need for continuous surveillance and strict antibiotic stewardship to curb the spread of resistant strains. The findings underscore the critical importance of implementing more effective infection control measures and exploring alternative therapeutic options to manage infections caused by MDR GNB. The results of this study also highlighted the regional & global differences in the prevalence of colistin resistance associated with nosocomial infections worldwide. Further research into colistin resistance is crucial to address this growing public health concern.

Ethical approval

This observational cross-sectional study was approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University (FWA000017585).

Author contributions

All authors conceptualized the study & developed the methodology. Nesma A. Hassanin & Nahed M. El-Ghannam conducted the investigation and presented the results. Nesma A. Hassanin & Nahed M. El-Ghannam wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. All authors provided funding.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The authors declare that no data was used for the research described in the article.

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