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Detection of *mcr-1* to *mcr-5* Genes-Mediated Colistin-Resistance in Gram-Negative Clinical Isolates

By

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ABSTRACT

Background: Bacteria especially Gram-negative (GN) are worldwide critical public health trouble, owing to their high antibiotic resistance, along with a shortfall of new active antimicrobial agents. This led to reconsideration of Colistin, as a drug of last hope for these resistant infections. Colistin re-use has provoked the development of Colistin resistance. Mobile-Colistin-resistance (*mcr*) genes mediated by plasmid have been reported. Screening of these Colistin resistant isolates (CRIs) and their resistant genes will assist in exploring the cause and level of Colistin resistance in Egypt.

Objectives: Isolation and identification of the causative Gram-negative bacteria (GNB) from different infections in hospitals, determination of their antibiotic susceptibility pattern as well as Colistin susceptibility, and examination of the existence of *mcr-1* to *mcr-5* genes in acquired CRIs.

Methodology: Specimens were collected from 284 immunocompromised patients from urine, sputum, blood, and wound swaps. Isolation and identification of the causative isolates were made by different microbiological procedures. Antibiotic susceptibility pattern was made by the Kirby-Bauer method, while broth microdilution method was used to determine Colistin susceptibility. Lastly, *mcr* genes were detected in acquired CRIs using multiplex polymerase chain reaction (PCR).

Results: 270 GN isolates were isolated from 284 clinical specimens, *Klebsiella pneumoniae* was the highest frequent GN isolate with 147 (54.44%) isolates. The highest pattern of resistance was detected toward ceftazidime. Acquired CRIs was detected in 11 (4%) of the isolates, these isolates were identified as *K. pneumoniae* 8 (72.7%) isolates, and *Pseudomonas aeruginosa* 3 (27.3%) isolates. The *mcr-2* gene was detected in 3 out of 11 (27.3%) CRIs. These isolates were *P. aeruginosa* (one isolate), and *K. pneumoniae* (2 isolates).

Conclusion: The prevalence of Colistin-resistance in our study still has minimum levels and has not spread to the public yet. Other resistant mechanisms are acquired to be investigated

Key words: Colistin-resistance, mcr genes, Gram-negative bacteria, multiplex PCR, Egypt

Introduction

Our world is encountering a massive and rising threat due to the high rate of antimicrobial resistance *(Sherry and Howden, 2018).*
Resistance to antibiotics has been stated as "the silent Tsunami facing modern medicine". The emergence of multidrug resistant (MDR), extended drug-resistant (XDR), and increasingly pan-drug-resistant (PDR) GNB have affected practice in every field of medicine *(Exner et al., 2017).*

Polymyxin E (Colistin) was considered "a miracle" antibiotic at its first commercial in the 1950s, with a low resistance level and a bactericidal effect toward Gram negative bacteria (GNB) *(Baron et al., 2016)*. Lately, Colistin was considered one of the last choices for curing MDR GNB. Colistin molecule is a cyclic poly-cationic peptide. It binds to the anionic lipopolysaccharide (LPS) part of the bacterial outer membrane (OM) and competes with Ca^{2+} and Mg^{2+} cations causing disruption of bacterial OM. These effects increase the OM permeability and consequently cell death *(Luo et al., 2017)***.**

Colistin misuse, in addition to the overuse, was the reason for development of Colistin resistance. However, Colistin-acquired resistance was recognized as a result of chromosomal mutations only. The provoke of mobile-Colistin-resistance (*mcr*) genes, which was discovered by Liu *et al* as *mcr-1* gene at the end of 2015 in China participated in the resistance to Colistin *(Liu et al., 2016)*. The enzyme produced by the *mcr-1* gene alters the lipid A part of LPS of the OM lipopolysaccharides *(Hinchliffe et al., 2017)*.

Subsequently, *the mcr-2* gene was originally discovered in *Escherichia coli* in Belgium *(Xavier et al., 2016)*. The *mcr-3* gene was originally identified in *E. coli* in China *(Yin et al., 2017)*. The *mcr-4* gene was originally identified in *S. enterica* in Italy *(Carattoli et al., 2017)*, and the *mcr-5* was originally detected in Germany in *S. paratyphi (Borowiak et al., 2017)***.**

The resistance conveyed by plasmids has mainly two hazards, first, multiple antibiotic resistance genes can be transferred, and second, plasmids have a greater resistance spread degree. This bacterial resistance threat may rapidly become prevalent coupled with absence of new antibiotics against MDR bacteria *(Alonso et al., 2005)*.

The objective of this study was to isolate and identify the causative GNB from different hospital infections, determine their antibiotic susceptibility pattern, detect CRIs, and investigate the existence of *mcr-1* to *mcr5* genes in the CRIs.

METHODOLOGY

Specimens:

The current study was carried out in the Microbiology and Immunology Department, Faculty of Pharmacy, Egyptian Russian University, Egypt on 284 specimens taken from immunocompromised patients, from July 2020 to July 2022 from some hospitals in Cairo, Egypt. The specimens were collected from urine, sputum, blood, and wound swaps, under complete aseptic precautions with the help of specialized clinicians.

Gram-negative bacterial isolation and identification:

The specimens collected were examined using the Gram staining technique and cultivated on MacConkey's and blood agar plates. The plates were incubated aerobically at 37°C for 24 hours, and any bacterial growth was identified by the traditional biochemical methods.

Antibiotic susceptibility testing:

The antimicrobial susceptibility pattern of the isolates was detected by the Kirby-Bauer disc-diffusion method on Mueller-Hinton agar plates *(CLSI, 2012)*.

GN isolates were tested against the following antimicrobials: meropenem (10 μg), gentamicin (10 μg), ceftazidime (30 μg), cefepime (30 μg), tigecycline (15 μg), aztreonam (30 μg), amoxicillin/ clavulanic acid (20/10 μg) and ciprofloxacin (5 μg). The interpretation of the results was done according to the clinical and laboratory standard institute (CLSI) guidelines *(CLSI, 2021)*.

Phenotypic detection of Colistin resistance:

Colistin susceptibility was tested with the broth microdilution method. The interpretation of the results was done according to the CLSI recommendations, isolates with minimum inhibitory concentration (MIC) ≥ 2 μg/ml were considered resistant and MIC < 2 μg/ml were considered sensitive *(CLSI, 2021)*.

Detection of *mcr* genes:

Plasmid was first extracted from acquired CRIs with the QIAprep Spin Miniprep kit supplied by Qiagen, Hilden, Germany. Amplification of *mcr* genes was performed by multiplex PCR, using OnePCR™ Master Mix (GeneDireX, Inc., Taiwan) with a set of primers listed in (Table 1) described by *(Rebelo et al., 2018)*, the primers were supplied from (Thermo Fisher Scientific Inc, U.S.A.).

The PCR setup was 1 cycle of initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30s, annealing at 50°C for 90s, extension at 72°C for 2 min, and A final cycle of extension at 72°C for 10 min. The amplification output was analyzed by electrophoresis with a suitable DNA ladder.

Statistical analysis:

To analyze collected data, SPSS program version 26 Inc. Chicago, USA was used.

RESULTS

Table (1): Primers sequence of *mcr-1* to *mcr-5* genes used in this study *(Rebelo et al., 2018)***.**

Gene primers	Sequences (5′→ 3′)	Amplicon size (bp)
*mcr1*_F *mcr1*_R	AGTCCGTTTGTTCTTGTGGC AGATCCTTGGTCTCGGCTTG	320 bp
*mcr2*_F *mcr2*_R	CAAGTGTGTTGGTCGCAGTT TCTAGCCCGACAAGCATACC	715 bp
*mcr3*_F *mcr3*_R	AAATAAAAATTGTTCCGCTTATG AATGGAGATCCCCGTTTTT	929 bp
*mcr4*_F *mcr4*_R	TCACTTTCATCACTGCGTTG TTGGTCCATGACTACCAATG	1,116 bp
*mcr5*_F *mcr5*_R	ATGCGGTTGTCTGCATTTATC TCATTGTGGTTGTCCTTTTCTG	1,644 bp

Collection of clinical isolates:

GN isolates were obtained from 284 different clinical specimens, including 96 (33.8%) urine specimens, 71 (25%) blood specimens, 65 (22.89%) sputum specimens, and 52 (18.31%) wound swap

specimens (Table 2). Patients in the current study were of both gender, 159 (56 %) specimens were collected from male patients while 125 (44 %) specimens were collected from female patients (Table 2).

Type of growth:

A total of 264 (93%) specimens out of 284 specimens showed positive culture, conversely, 20 (7%) of total specimens showed no growth. 258 (97.7%) isolates out of 264 positive culture isolates were pure cultures that showed a single bacterial growth and 6 (2.3%) were mixed cultures that showed at least more than one type of bacterial growth (Table 2).

Table (2): Demographic characteristics of the study:

Characteristics	Total number of specimens (n=284)
Male **Female**	159 (56 %) 125 (44 %)
Urine **Blood** **Wound** **Sputum**	96 (33.8%) 71 (25%) 65 (22.89%) 52 (18.31%)
Positive culture **Mono-microbial** **Poly-microbial** **Negative culture**	264 (93%) 258 (97.7%) 6 (2.3%) 20 (7%)

Gram-negative bacterial outcome:

Klebsiella pneumoniae was the highest frequent Gram-negative isolate representing 54.44% followed by *Pseudomonas aeruginosa* 14.81% then *Escherichia coli* 5.55% (Table 3).

Table (3): Distribution of Gram-negative bacterial isolates:

Organisms	Total number of isolates (n=270)
Klebsiella pneumoniae	147 (54.44%)
Pseudomonas aeruginosa	48 (17.78%)
Escherichia coli	40 (14.81%)
Acinetobacter baumannii	15 (5.56%)
Proteus mirabilis	15 (5.56%)
Providencia rettgeri	2 (0.74 %)
Serratia marcescens	2 (0.74 %)
Providencia heimbachae	1 (0.37%)

Drug resistance pattern in Gram-negative isolates:

The antimicrobial susceptibility test interpretation was made according to CLSI guidelines *(CLSI, 2021)*. The antimicrobial susceptibility tests showed that the highest pattern of resistance was toward ceftazidime 257 (95.2%) followed by gentamicin 251 (93%), cefepime 236 (87.4%), amoxicillin/clavulanic acid 223 (82.6%), ciprofloxacin 211 (78.2%), aztreonam 199 (73.7%), meropenem 155 (57.4%) and tigecycline 155 (57.4%) (Fig. 1) and (Table 4). The results revealed that 233 (86.3%) isolates out of 270 Gram-negative isolates, were MDR, this finding was statistically significant (P=<0.001) **(Fig. 2)**

Table (4): Resistance pattern of the Gram-negative bacterial isolates.

Isolate / Antimicrobial	*K. pneumoniae* (N= 147)	*P. aeruginosa* (N= 48)	*E. coli* (N= 40)	*A. baumannii* (N= 15)	*P. mirabilis* (N= 15)	*P. rettgeri* (N= 2)	*S. marcescens* (N= 2)	*P. heimbachae* (N= 1)
	Number of resistant isolates							
Amoxicillin/ Clavulanic acid	129 (87.76%)	48 (100%)	22 (55 %)	15 (100%)	4 (26.67%)	2 (100%)	2 (100%)	1 (100%)
Aztreonam	119 (80.95 %)	33 (68.75%)	31 (77.5%)	15 (100%)	1 (6.67%)	0 (0%)	0 (0%)	0 (0%)
Cefepime	141 (95.92%)	44 (91.67%)	31 (77.5%)	13 (86.67%)	4 (26.67%)	1 (50%)	2 (100%)	0 (0%)
Ceftazidime	145 (98.64%)	46 (95.83%)	40 (100%)	15 (100%)	7 (46.67%)	2 (100%)	1 (50%)	1 (100%)
Ciprofloxacin	134 (91.16%)	32 (66.67%)	32 (80%)	9 (60%)	3 (20%)	0 (0%)	1 (50%)	0 (0%)
Gentamicin	144 (97.96%)	45 (93.75%)	35 (87.5%)	11 (73.33%)	13 (86.67%)	1 (50%)	2 (100%)	0 (0%)
Meropenem	103 (70.07%)	22 (45.83%)	20 (50%)	9 (60%)	1 (6.67%)	0 (0%)	0 (0%)	0 (0%)
Tigecycline	62 (42.18 %)	48 (100%)	11 (27.5%)	15 (100%)	15 (100%)	2 (100%)	2 (100%)	0 (0%)

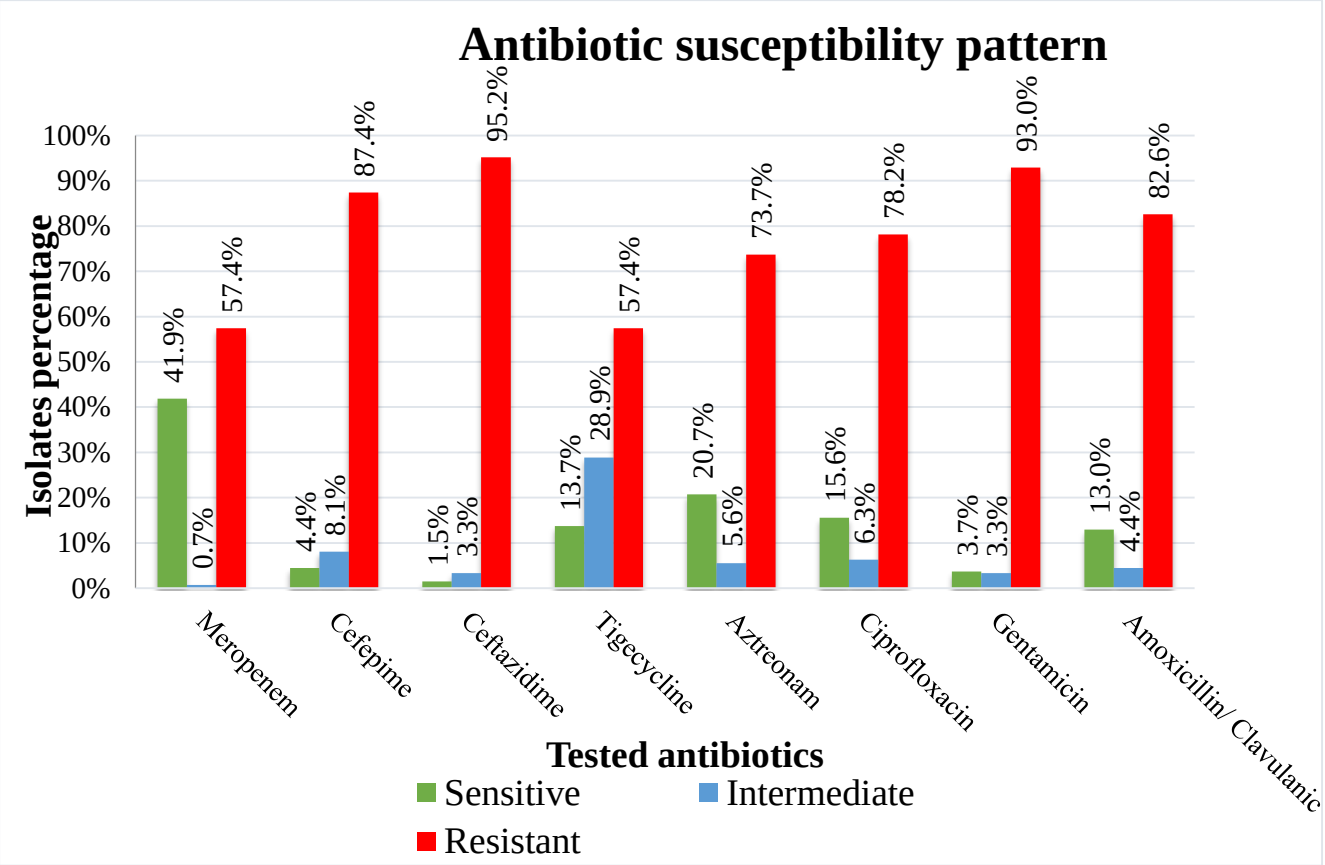

Figure (1): Antibiotic susceptibility test results of isolates

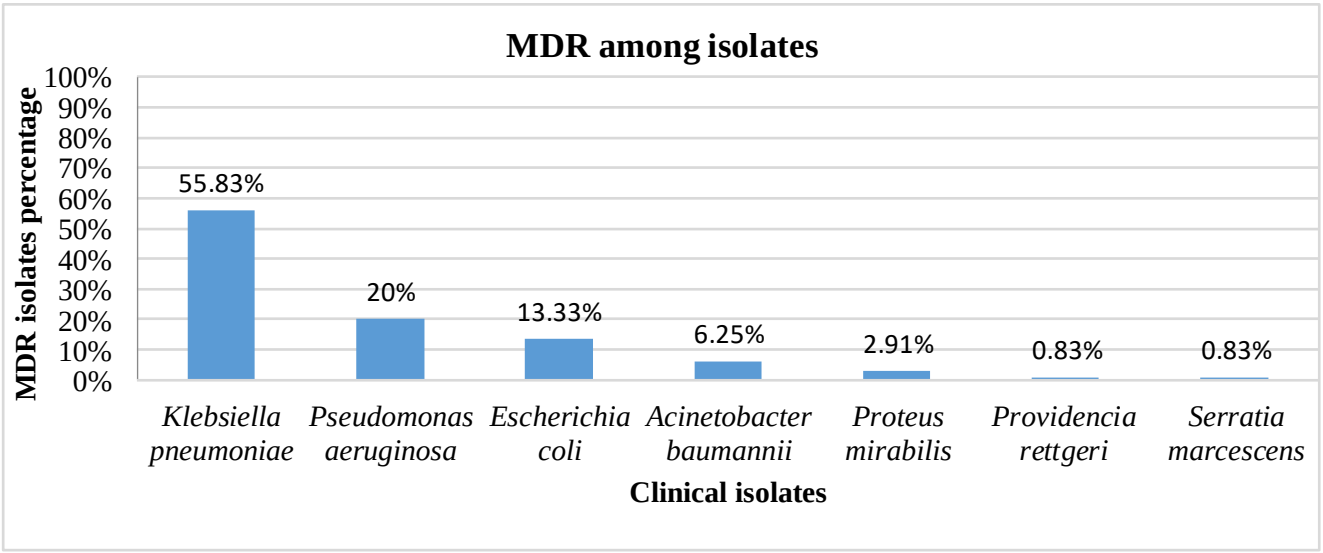

Figure (2): Frequency of MDR among the tested isolates.

Proteus mirabilis, Providencia rettgeri, Serratia marcescens, and *Providencia heimbachae* were intrinsically resistant to Colistin, so they were excluded from the Colistin susceptibility test. Eleven (4.4%) isolates out of 250 isolates, were acquired Colistin-resistant. Among these

Phenotypic detection of Colistin-resistance:

resistant isolates, 8 (72.7%) isolates were *K. pneumoniae*, and 3 (27.3%) isolates were *P. aeruginosa,* this was found to be statistically non-significant (P=0.347) (table 5). MICs for Colistin were found to be 32 µg/ml in 5 isolates (45.5%), 16 µg/ml in 2 isolates (18.1%), 8 µg/ml in 2 isolates (18.1%), and 4 µg/ml in 2 isolates (18.1%)

Table (5): Colistin susceptibility distribution.

Isolate	Susceptible N=239	Resistant N=11	Total N=250	X^2	P
Klebsiella pneumoniae	139(94.6%)	8(5.4%)	147	3.301	0.347
Pseudomonas aeruginosa	45(93.8%)	3(6.3%)	48		
Escherichia coli	40(100%)	0(0.0%)	40		
Acinetobacter baumannii	15(100%)	0(0.0%)	15		

The clinicomicrobiological profile of the 11 CRIs:

The clinicomicrobiological profile correlates patient details such as gender and specimen type with microbiology test findings, such as The eleven CRIs were resistant to cefepime, ceftazidime, and amoxicillin/clavulanic acid. Most of these CRIs were collected from wound swabs specimens (45.5%), followed by blood specimens (27.2%), sputum specimens (18.1%), and urine specimens (9%) (Table 6)

the type of pathogen and its antibiotic susceptibility.

Five out of the eight Colistin-resistant *K. pneumoniae* isolates were found to be resistant to all tested antimicrobial agents.

Table (6): Clinicomicrobiological profile of the 11 CRIs.

Isolate number	Patient gender	Clinical specimen	Identified pathogen	Effective antibiotics
1	Male	Blood	*Klebsiella pneumoniae*	-
2	Male	Wound	*Klebsiella pneumoniae*	-
3	Male	Wound	*Klebsiella pneumoniae*	Meropenem Tigecycline
4	Female	Sputum	*Pseudomonas aeruginosa*	Meropenem Tigecycline
5	Male	Blood	*Pseudomonas aeruginosa*	Meropenem Gentamicin Ciprofloxacin
6	Female	Urine	*Klebsiella pneumoniae*	-
7	Male	Sputum	*Klebsiella pneumoniae*	Meropenem Tigecycline
8	Male	Blood	*Klebsiella pneumoniae*	-
9	Male	Wound	*Klebsiella pneumoniae*	-
10	Female	Wound	*Pseudomonas aeruginosa*	Meropenem Aztreonam
11	Male	Wound	*Klebsiella pneumoniae*	Meropenem Tigecycline

Genotypic detection of Colistin-resistance genes:

The *mcr* genes presence had been tested using multiplex PCR technique, as a step in the detection of the cause of Colistin resistance. The results indicated that out of 11 acquired CRIs only 3 (7.6%) isolates had *mcr-2* gene (Fig. 3). These isolates were *P. aeruginosa* (one isolate) with MIC 8 μg/ml that had been taken from wound infection of a female patient, and *K. pneumoniae* (2 isolates) with MIC 32 μg/ml that had been taken from wound infection of male patients (Table 6).

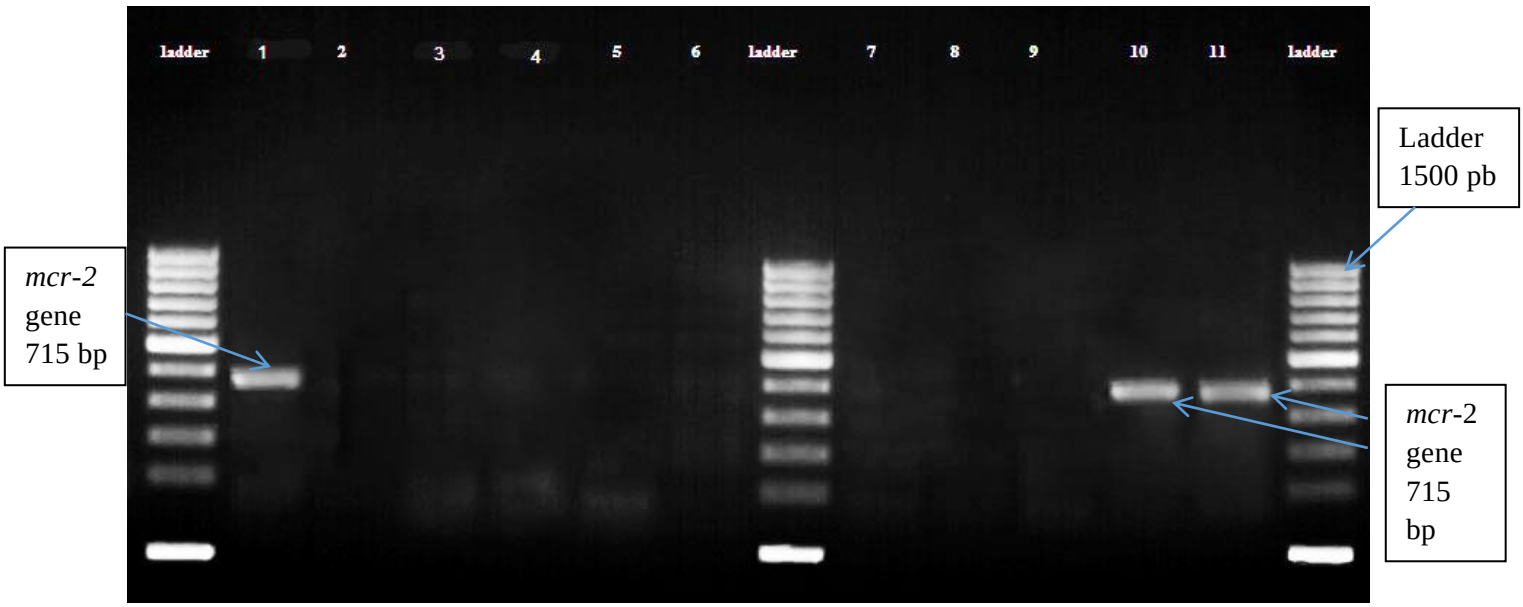

Figure (3): Agarose gel electrophoresis of amplified *mcr*-2 gene.

DISCUSSION

The world is encountering a critical disaster since the beginning of the growing antibiotic resistance in the 1970s between GNB. The primary concern is the diminishing of alternative antimicrobials that cure fatal pathogens *(Falagas et al., 2005)*.

Colistin has been restored for curing infections caused by MDR-GNB, including *A. baumannii, E. coli, P. aeruginosa, and K. pneumoniae,* as Colistin has low bacterial resistance level *(Bialvaei and Samadi Kafil, 2015)***.** Unfortunately, Colistin misuse and overuse among people in addition to veterinary use had led to the appearance of pathogens resistant to Colistin *(Liu et al., 2016)*.

The current study was conducted on 270 bacterial isolates that recovered from 284 immunocompromised patients of different ages who suffered from various bacterial infections. The predominant isolate was *K. pneumoniae* followed by *P. aeruginosa* and *E. coli*, while the least common isolates were *A. baumannii*, *P.* mirabilis, *P. rettgeri*, *S. marcescens* and *P. heimbachae*. The number of bacterial isolates is in agreement with Emara *et al* and Bhuyan *et al*. results showed that *K. pneumoniae* was the highest frequently isolated bacteria, followed by *E. coli* and *P. aeruginosa*, and the least common isolates were *Proteus* spp., *Morganella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp. *(Bhuyan et al., 2018; Emara et al., 2019)*. On the contrary, *E.coli* was the highest frequent GN isolates in Abrar *et al*. and Kaur and Singh studies, followed by *K. pneumoniae, Pseudomonas spp., Acinetobacter spp., Citrobacter spp., Proteus* spp. and *Enterobacter spp. (Abrar et al., 2018; Kaur and Singh, 2018)*. This results variation may be attributed to the difference in the specimens type, cases number, difference between countries, the overall health status of the patients, and the sticking to infection control measures *(Emara et al., 2019)***.**

Regarding the antimicrobial susceptibility results, our study showed that the greatest resistance pattern was observed against the third-generation cephalosporin ceftazidime followed by gentamicin, cefepime, amoxicillin/clavulanic acid, ciprofloxacin and aztreonam. These findings suggested that these antimicrobial agents maybe not suitable for the initiation of empirical therapy for GNB infections in Egypt. Conversely, a lower rate of resistance was noticed toward meropenem and tigecycline. This relatively lower resistance rate could be credited to the restricted utilization of these antimicrobial agents in Egypt. The results of this study

were more or less similar to the study of Emara *et al.* who found that the maximum rate of resistance was against ampicillin followed by amoxicillin/clavulanate, aztreonam and ceftriaxone while the low resistance rate was noticed toward amikacin, imipenem, gentamicin and meropenem *(Emara et al., 2019)*. Also, Tharbendra *et al.* found that the GN isolates were most effectively treated with gentamicin, imipenem, and meropenem *(Tharbendra et al., 2018)*. Conversely, Sarmah *et al.* found that amoxicillin/clavulanic showed the highest rate of sensitivity followed by piperacillin/tazobactam *(Sarmah et al., 2016)***.** The difference in susceptibility could be attributed to the diversity in antibiotic treatment protocols across various geographical areas.

MDR has been described as acquired antimicrobial resistance to one agent in a minimum of three antimicrobial categories *(Emara et al., 2019)*. In this study regarding drug resistance among GN isolates, 233 (86.3 %) MDR isolates out of 270 isolates were detected, and this was statistically significant (P=<0.001). Supportive to these results, Emara *et al.* found that out of 244 GN isolates, 150 (61.5%) isolates were considered MDR *(Emara et al., 2019)*. In addition, Nepal *et al.* report that out of 177 GN isolates 96 (54.2%) isolates were MDR *(Nepal et al., 2017)***.** Conversely, Elsherif *et al.* report that out of 50 GN isolates 12 (24%) isolates were MDR *(Elsherif et al., 2015)*. In the present study, the high rate of resistance could be explained by the irresponsible use of antibiotics, the ease of access to antibiotics, and the discontinuation of treatment due to the negligence of patients *(Chander, 2016)*.

Regarding the Colistin susceptibility test, the disc diffusion method is not reliable to detect Colistin resistance, because of inadequate diffusion of Colistin into the medium as it is a large molecule. CLSI and the European Committee on antimicrobial susceptibility testing (EUCAST) collaborated and established a polymyxin breakpoints working group to asses Colistin susceptibility testing, this group recommended that the broth microdilution technique is the most reliable method for testing the susceptibility of Colistin *(CLSI, 2017; EUCAST, 2017)*.

In this study, 31 (11.48%) isolates out of 270 GN isolates were Colistin-resistant, these 31 resistant isolates include 20 (7.4%) intrinsic-resistant isolates and 11 (4%) acquired resistant isolates and this acquired resistance was statistically non-significant (P=0.347). The eleven CRIs were 8 (72.7%) isolates *of K. pneumoniae* and 3 (27.3%) isolates of *P. aeruginosa*. The CRIs have MICs within the range of 4 to 32 µg/ml. In agreement with these results, Emara *et al*. detected 10 (4.3%) acquired CRIs out of 244 isolates, these isolates include 8 *K. pneumoniae* isolates, one *E. coli* isolates and one *P. aeruginosa* isolates *(Emara et al., 2019)*. In addition, Nitz *et al.* reported that out of 99 *P. aeruginosa* isolates only 1 (1 %) isolate was resistant to Colistin *(Nitz et al., 2021)*. Moreover, Papadopoulos *et al.* also report that out of 131 MDR GNB, only 1 isolate 0.8% was resistant to Colistin *(Papadopoulos et al., 2019)*. The bacterial resistance toward Colistin is relatively low, possibly due to its little and limited use, especially for XDR infections *(Gales et al., 2006)***.** Conversely, Moubareck *et al.* and Monaco *et al.* found that out of 89 carbapenem-resistant Enterobacteriaceae (CRE) isolates 28 (31.4%) of the isolates were Colistin-resistant and among 197 CRE, Colistin resistance was detected in 85 (43%) of the isolates respectively. This great resistance rate was attributed to a consequence of the huge spread of carbapenemase-producing organisms that led to increased Colistin consumption to treat this threat *(Monaco et al., 2014; Moubareck et al., 2018)*. The variation in Colistin-resistant results observed in different studies can be attributed to the type of specimens, sample sizes, the overall health status of patients, geographical locations, different antibiotic policies, and adherence to infection control measures.

Concerning the clinicomicrobiological specifics of the 11 CRIs in our study. The CRIs were more prevalent in wound swabs specimens, followed by blood specimens, sputum specimens and urine specimens. This differed from Arjun *et al*., study which stated that the urine specimens were the predominant source of the isolates, followed by blood specimens, respiratory specimens, pus specimens and cerebrospinal fluid specimens *(Arjun et al., 2017)*. However, Emara *et al.* reported that the most prevalent specimens were respiratory specimens, followed by urine specimens and wound swabs specimens *(Emara et al., 2019)*. These variations in the clinicomicrobiological specifics between studies could be explained by the variance in the disease of the patients and their comorbidity, subsequently the variation in the taken specimens and types of the used antibiotics.

In this study, the 11 acquired CRIs were tested for the presence of *mcr-1* to *mcr-5* genes by PCR. Three isolates were found to be positive for the *mcr-2* gene, with two of these isolates being *K. pneumoniae* and the third being *P. aeruginosa* isolate. The other 8 isolates tested negative for the presence of *mcr* genes. This low availability of *mcr* genes is consistent with Khattab *et al.* who showed that 43 CRIs11 out of 280 Colistin resistance isolates, were held 2 *mcr-2* gene and 1 *mcr-1* gene, the *mcr* genes identified in *K. pneumoniae* isolates *(Khattab et al., 2021)*. In addition, Meheissen *et al.* didn't find *mcr* genes in 70 isolates resistant to Colistin *(Meheissen et al., 2022)*. Conversely, Newton-Foot *et al.* report that out of 18 CRIs, 15 isolates harbored the *mcr-1* gene *(Newton-Foot et al., 2017)***.** In addition, Luo *et al.*, study identified the presence of the *mcr-1* gene in 21 (52.5%) out of 40 Colistin-resistant *E. coli* isolates in China. The high prevalence of *mcr-1* carriage in China was attributed to the country's significant livestock and meat production, which is linked to the elevated rates of CRIs in the region *(Luo et al., 2017)*.

In our study, the 11 CRIs are composed of 8 *K. pneumoniae* isolates and 3 *P. aeruginosa* isolates. Since the 11 CRIs were also resistant to most of the examined antimicrobial agents, they pose a significant threat to public health and have the potential to cause a crisis. In our study, *K. pneumoniae* was found to be the most prevalent CRI. *K. pneumoniae is considered to be one of the clinically significant organisms that have raised significant public health concerns*, additionally, *K. pneumoniae* is recognized as an opportunistic pathogen capable of causing many different diseases. Moreover, *K. pneumoniae* is displaying an alarming pattern in the acquisition of antibiotics resistance. Infections caused by K. pneumoniae are often linked to high mortality rates and extended high costs of hospitalization *(Giske et al., 2008)*.

The difference in detecting resistant mechanisms was most appropriately explained by the world health organization (WHO), which stated that negative results in PCR cannot reliably predict susceptibility to Colistin, because the test may not rule out the presence of chromosomal mechanisms or even newly discovered *mcr* genes that have not been incorporated into the testing protocol *(WHO, 2018)*. In our study, the absence of the *mcr* genes among the remaining CRIs could be explained by the presence of other resistant mechanisms that are either plasmid or chromosomal-mediated, such as drug efflux, decreased permeability of bacterial cell membrane, or inactivation by enzymes.

CONCLUSION

The greatest resistance pattern was observed against ceftazidime, with 86.3% of the tested isolates were found to be MDR bacteria. The prevalence of Colistin resistance in study cases is still low and has not been extended to the community yet. The low prevalence of Colistin resistance in our study may be attributed to its limited use in Egypt. Out of 250 isolates 11 isolates were acquired CRIs, 8 isolates of them were *Klebsiella pneumoniae* and 3 isolates were *Pseudomonas aeruginosa*. *Klebsiella pneumoniae* has to be considered a public

health threat as in our study, *Klebsiella pneumoniae* was the most commonly isolated bacteria, with 91.1% of the isolates were MDR. Furthermore, 8 isolates out of the 11 CRIs were *K. pneumoniae*. Regarding the *mcr* genes detection, the *mcr-2* genes were detected in 3 acquired CRIs. Other mechanisms of Colistin resistance, including other *mcr* genes, should be investigated. The identified antibiotic resistance genes were found to be located on plasmids, which play a crucial role in the dissemination of resistance among bacteria.

Recommendations

Further testing to detect other potential *mcr* genes should be conducted to determine the underlying cause of Colistin resistance. In CRIs, combination therapy with other antibiotics should be tested. Hospitals should establish guidelines and systems for monitoring bacterial resistance and antibiotic usage. Strict adherence to effective infection control measures in hospitals and antimicrobial stewardship is crucial to prevent the spread of antibiotic-resistant bacteria.

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الكشف عن وجود *جينات mcr* المقاومة للكوليستن بين عزلات سريرية سالبة الجرام

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خلفية البحث: تشكل البكتريا وخاصة السالبة الجرام تهديداً خطيراً للصحة العامة وذلك نتيجة لمقاومتها العالية للمضادات الحيوية المتوفرة بالاضافة الى نقص وجود مضادات حيوية فعالة جديدة تجاه هذه الانواع من البكتريا. ادى هذا الي إعادة استخدام المضاد الحيوي الكوليستن كاخر امل في علاج هذة العدوى المقاومة. ولكن أدى الاستخدام الواسع للكوليستين إلى ظهوربكتريا مقاومة للكوليستين، والتي اكتسبت هذة المقاومة من تحولات في الصبغي الوراثي للبكتيريا، وحديثا تم اكتشاف جينات مقاومة للكوليستين توجد علي البلازميد الخاص بهذة البكتريا (mcr) و في هذا البحث سيساعد فحص هذه العزلات المقاومة للكوليستن والجينات المسئولة عن المقاومة في التعرف علي سبب ومستوى مقاومة الكوليستن.

الهدف من البحث: يهدف هذا البحث الى فصل العزلات السالبة الجرام المسببة للامراض المختلفة والتعرف عليها والتعرف ايضا علي خصائصها الخاصة بمقاومة المضادات الحيوية المختبرة بالبحث بالاضافة الي مقاومتها للكوليستن. و في النهاية البحث عن وجود بعض الجينات المسؤلة عن مقاومة هذا النوع من الميكروبات للكوليستن (cr-1m الى mcr-5).

طرق البحث: تم جمع العينات السريرية من 284 مريض من المرضى منقوصي المناعة من عينات بولية وعينات بلغم وعينات دم وعينات جروح. وقد تم التعرف علي العزلات عن طريق الاختبارات الميكروبيولوجية القياسية ثم تحديد نمط حساسية المضادات الحيوية باستخدام طريقة كيربي باور، بينما تم استخدام طريقة التخفيف الدقيق لاختبار حساسية الكوليستين. وأخيراً، تم كشف جينات mcr في العزلات المقاومة للكوليستن باستخدام تقنية تفاعل البلمرة المتعددة (Multiplex PCR).

النتائج: تم عزل 270 عزلة سالبة الجرام من 284 عينة سريرية، وكانت عزلات الكليبسيلا الرئوية الأكثر انتشارًا بعدد 147 عزلة بنسبة (54.44%). وقد كان أعلى نمط لمقاومة العزلات للمضادات الحيوية المختبرة في البحث تجاه المضاد الحيوي السيفتازيديم. وقد تم اكتشاف وجود عزلات مقاومة مكتسبة للكوليستن في عدد 11 عزلة بنسبة (4%) من العزلات، وتم التعرف هذه العزلات على أنها كليبسيلا نيومونيا بعدد 8 عزلات بنسبة (72.7%)، وسودوموناس أيروجينوزا بعدد 3 عزلات بنسبة (27.3%). وقد تم الكشف عن وجود جين mcr-2 في عدد 3 عزلات من اصل 11 عزلة مقاومة للكوليستن،وكانت هذه العزلات عبارة عن عدد 2 عزلة من كليبسيلا نيومونيا وعزلة واحدة من سودوموناس أيروجينوز

الاستنتاج: مستوى انتشار البكتريا المقاومة للكوليستين في دراستنا لا يزال من المستويات المنخفضة و التي لم تنتشر بعد إلى الجمهور. هذا ويجب الاستمرار في البحث عن آليات الاخرى للبكتريا لمقاومة الكوليستن.

الكلمات الدالة: مقاومة الكوليستن- جينات ال mcr- البكتريا السالبة الجرام- تفاعل البلمرة المتعددة- مصر