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Original article

Molecular mechanisms of colistin resistance among multi-drug resistant (MDR) *Klebsiella pneumoniae* and *Escherichia coli* isolated from ICU patients and their susceptibility towards eravacycline

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ABSTRACT

The evolution of colistin-resistant strains is considered a great threat for patients admitted to intensive care unit (ICU). This study focused on screening the existence of *mcr-1, mcr-2* and mutation in *pmrA* gene in *Klebsiella pneumoniae (K. pneumoniae)* and *Escherichia coli (E. coli)* isolates collected from patients admitted to ICU in Ain Shams University hospitals. Also, this study evaluated the susceptibility of colistin resistant microorganisms to eravacycline antibiotic. **Methods:** Isolation and identification of *K. pneumonia* and *E. coli* were performed then antimicrobial susceptibility test and VITIC -2 compact system were used. Colistin susceptibility and minimum inhibitory concentrations were determined. The *mcr-1, mcr-2* and *pmrA* genes were detected and then *pmrA* gene was sequenced. Eravacycline susceptibility against colistin resistant strains was determined via E-test. **Results:** Colistin resistance appeared in 42.9% (36 out of 84) isolates. Mobilized colistin resistance (*mcr-1)* revealed in 94.4% (34 out of 36), Mcr-2 revealed in 27.8% (10 out of 36) and *pmrA* gene revealed in 61.1% (22 out of 36). Sequencing of *pmrA* gene in eight selected isolates revealed two-point mutation in all isolates. All colistin resistant strains showed sensitivity to eravacycline except two *K. pneumoniae* isolates. **Conclusion:** This study revealed a high rate of *mcr-1, mcr-2* and *pmrA* genes among MDR *K. pneumoniae* and *E. coli* isolated from ICU. Plasmid mediated *mcr* is a source of acquired resistance to colistin. So, there was a recommendation for broader surveillance of this resistance pattern. Eravacycline could be used for treatment of infections caused by colistin resistant *K. pneumonia* and *E. coli.*

Introduction

The horrible rise in antimicrobial resistance is becoming a critical catastrophe worldwide. The main problem is that there are no other potential options that may be an alternative to treat these resistant strains, especially those lead to health care associated infections, and spread to cause community acquired infections, denoting a global crisis of antimicrobial resistance that can't be stopped [1].

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Enterobacteriaceae are ubiquitous organisms that reside in nature and could cause variety of infections in humans. Unfortunately, some Enterobacteriaceae such as *K. pneumonia* and *E. coli* are generally found to have several resistance mechanisms against multiple antibiotics, including carbapenems [2,3]. Therefore, old abandoned antibiotics such as colistin was recalled as a life-saving and last treatment resort against these resistant pathogens [4].

Colistin is one of the five polymyxin antibiotics initially isolated in 1947 from the soil bacterium *Paenibacillus polymyxa* subsp. *Colistinus*. It disrupts cell membrane integrity, causing leakage of valuable cellular components and eventually death of the bacterial cell. It also acts by inhibiting type II NADH-quinone oxidoreductases (NDH-2) which is key respiratory enzyme. Colistin is an effective bactericidal agent against aerobic Gram-negative bacteria that is frequently considered the mainstay of lethal infections, such as carbapenem-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter baumannii* (*A. baumanni*), *K. pneumoniae* and *E. coli* [5].

Colistin resistance has been increased due to different mechanisms including chromosomal mutation in the *PmrA/PmrB* and *PhoP/PhoQ* two-component regulatory systems, or through transferable plasmid encoding colistin resistance (the mobilized colistin resistance or *mcr-1*) [6]. Mobilized colistin resistance (*mcr)* genes have been globally disseminated and increasingly reported worldwide where nine other *mcr* genes families have been discovered from various hosts and pathogens [7]. So, novel therapeutic options are definitely needed for the treatment of colistin resistant infections. Since antimicrobial discovery and emergence of resistance to these new drugs are nearly simultaneous. Noteworthy drugs with novel mechanisms of action that will overcome current resistance mechanisms are required [8].

Eravacycline (TP-434), is a new fluorocycline of the tetracycline family, that are active against a wide range of pathogens, including multi-drug resistant (MDR) and extensively-drug resistant (XDR) Gram-negative, Gram-positive, and anaerobic pathogens. Eravacycline is also effective against colistin-resistant and ceftazidime-avibactam-resistant strains [9].

We therefore aim to evaluate prevalence of colistin resistance among MDR *E.coli* and *K.pneumoniae* isolated from ICU patients in Egypt, in vitro susceptibility of these strains toward eravacycline antibiotic, and to assess presence of *mcr-1, mcr-2* and mutated *pmrA* gene.

Materials and methods

This is an observational cross-sectional study that was conducted on patients admitted to ICU at Ain Shams University (ASU) hospital during the period from January 2021 till February 2022. Inclusion criteria involved male and female patients admitted to different critical units at ASU hospital with signs of infections including clinical pulmonary infection score ≥ 7, urine analysis showing significant pyuria, surgical wound showing hyperemia, pyogenic membrane, suspected systemic septicemia accepted to participate in the study. Exclusion criteria included refusal to participate in the study or receiving broad spectrum antimicrobial within 48 hours. This study was approved by research ethics committee of faculty of medicine ASU (IRB NO: FWA 000017585).

Bacterial identification and antimicrobial susceptibility

Eighty four (84) non-repetitive MDR Enterobacteriaceae strains were collected from different clinical samples including blood, urine, sputum, and pus. Multi-drug resistant defined as resistance to at least one agent in three or more antimicrobial categories. The Enterobacteriaceae strains were identified by conventional methods according to Clinical and Laboratory Standards Institute (CLSI) 2022 [10] including colonial morphology, staining reaction, biochemical tests and API 20E. Susceptibility to different antimicrobials was performed using disc diffusion method and VITEK-2 compact system (bioMérieux, France) according to CLSI 2022 breakpoints [10]. Microbroth dilution method was done to detect minimal inhibitory concentration (MIC) of colistin according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [11]. Susceptibility to eravacycline among colistin resistant strains was performed using E test according to Food and Drug Administration (FDA) 2018 breakpoints [12].

Detection of colistin resistance genes (*mcr1, 2* and *pmr A*) by polymerase chain reaction (PCR)

DNA extraction: DNA of the samples was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to manufacturer's instructions.

PCR amplification: Primers used were provided from (Metabion, Germany) are mentioned in **table (1).** They were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler. The initial denaturation step was performed at 94°C for 5 minutes. After the initial denaturation, subsequent PCR cycles (35 cycles) began with a separate denaturation step that lasts 30 seconds at 94°C. Then, primers annealing was done at 60°C for 40 seconds. Following primers annealing, primers extension was done at 72°C for 45 seconds. The final extension step was done at 72°C for 10 minutes [13, 14].

Analysis of the PCR Products: PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gene ruler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed by a computer software (automatic image capture protein simple formerly cell bioscience, USA)

Analysis of *pmrA* gene by sequencing

PCR products were purified using QIAquick pcr product extraction kit (Qiagen, Valencia). Big dye terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using centrisep spin column. DNA sequences were obtained by applied biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) [15] was initially performed to establish sequence identity to GenBank accessions. Phylogenetic analyses were done using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 [16].

Results

Eighty-four MDR Enterobacteriaceae isolates were obtained from hospitalized ICU patients in ASU hospital between January 2021 and February 2022. Half of them were isolated from males and the other half were isolated from females with age ranged from 9 months to 48 years **(Table 2)**

Sixty-two (73.8%) of patients had received antibiotics before isolating MDR isolates, but none of them had been given colistin. The isolates were retrieved from different clinical samples including blood, urine, sputum, wound, pus, blood from central line, cerebrospinal fluid (CSF) and nephrostomy tube discharge. The majority of them were isolated from blood 24 (28.6%) and urine 18 (21.4%). The isolates included 72(85.7%) *K. pneumonia* and 12(14.3%) *E. coli*. **(Figure 1)**

All isolates were resistant to ampicillin-sulbactam, piperacillin-tazobactam, meropenem, cefepime, ceftriaxone, cefoperazone, ceftazidime, cefotaxime, cefoxitin, and tobramycin. Amikacin showed the least resistance (54/84; 64.3%) **(Table 3).**

Colistin MIC ranged between 0.5 and 32 mg/L as tested by broth microdilution. Resistance appeared in (36/84; 42.9%) **(Table 4).** Eravacycline MIC ranged between 0.09 and 0.75 mg/L as tested by E test. All colistin resistant isolates were sensitive to eravacycline except two *K. pneumonia* isolated from infected central line **(Table 4).**

Regarding colistin resistance among MDR *K. pneumoniae* and *E. coli* isolates, it was found that (30/72; 41.7%) of *K. pneumoniae* and (6/12; 50%) of *E. coli* were resistant **(Table 5).**

Investigating certain mechanisms of colistin resistance by pcr revealed that plasmid-encoded *mcr-1 was* the most common mechanism (34/36; 94.4%) harbored by *Klebsiella spp.* (28 /30, 93.3%) and *E. coli* (6/6:100%) respectively.

The second gene revealed was *pmrA gene* (22/36; 61.1%) that was harbored by *Klebsiella spp.* (16/30; 53.3%) and *E. coli* (6 /6; 100%) respectively. The least gene was plasmid-encoded *mcr-2* (10/36; 27.8%) that was harbored by *Klebsiella spp*. (10/30; 33.3 %) **(Table 6) (Figure2).**

More than one resistance genes were detected in the same isolate: four isolates harbored the three studied genes (4/36; 11.1%), six isolates harbored both *mcr-1* and *mcr-2* (6/36; 16.7%) and sixteen isolates harbored both *mcr-1* and *pmrA* (16/36; 44.4%). **(Table 6)**

Sequencing of the *pmrA* gene in 8 selected isolates (6 *K. pneumoniae* and 2 *E. coli*) revealed two point mutations in all selected isolates. First mutation was (TCA>CCA) mutates proline 190 to serine. The second mutation were (ATC>ACT) mutates threonine 192 to isoleucine **(Table 7)**.

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
pmrA	F (5′-ATGACAAAAATCTTGATGATTGAAGAT -3′)	675	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Beceiro et al.** [13]
	R(5′-TTATGATTGCCCCAAACGGTAG-3′)							
Mcr-1	F(5′-AGTCCGTTTGTTCTTGTGGC-3′)		94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Liu et al.** [14]
	R(5′-AGATCCTTGGTCTCGGCTTG-3′)							
Mcr-2	F(5′-CAAGTGTGTTGGTCGCAGTT-3′)		94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Liu et al.** [14]
	R(5′-TCTAGCCCGACAAGCATACC-3′)							

-F, forward oligonucleotide; R, reverse oligonucleotide.

Table 2. Demographic data of included patients and type of isolates (84 isolates)

Gender	Female	42 (50.0%)
	Male	42 (50.0%)
Age (years)	Median (IQR)	14 (0.75 – 48)
	Range	0.02 – 77
Organism	*K. pneumonia*	72 (85.7%)
	E- coli	12 (14.3%)

- IQR (interquartile range)

Table 3. Antimicrobial susceptibility among isolates.

Antibiotics	Resistant	Sensitive	Intermediate
	No. (%)	No. (%)	No. (%)
SXT(Sulpha-Trimethoprim)	74 (90.2%)	8 (9.8%)	0 (0.0%)
DO (doxycycline)	64 (78.0%)	14 (17.1%)	4 (4.9%)
LEV(Levoofloxacin)	60 (71.4%)	12 (14.3%)	12 (14.3%)
CIP(Ciprofloxacin)	82 (97.6%)	0 (0.0%)	2 (2.4%)
TOB(Tobramycin)	84 (100.0%)	0 (0.0%)	0 (0.0%)
GN(Gentamycin)	72 (85.7%)	2 (2.4%)	10 (11.9%)
AK(Amikacin)	54 (64.3%)	20 (23.8%)	10 (11.9%)
MEM(Meropenem)	84 (100.0%)	0 (0.0%)	0 (0.0%)
FEP(Cefepime)	84 (100.0%)	0 (0.0%)	0 (0.0%)
CRO(Ceftriaxone)	84 (100.0%)	0 (0.0%)	0 (0.0%)
CFP(Cefoperazone)	84 (100.0%)	0 (0.0%)	0 (0.0%)
CAZ(Ceftazidime)	82 (97.6%)	2 (2.4%)	0 (0.0%)
CTX(Cefotaxime)	84 (100.0%)	0 (0.0%)	0 (0.0%)
FOX(Cefoxitin)	84 (100.0%)	0 (0.0%)	0 (0.0%)
TZP(Pipracillin-Tazobactam)	84 (100.0%)	0 (0.0%)	0 (0.0%)
SAM (Ampicillin - Sulbactam)	84 (100.0%)	0 (0.0%)	0 (0.0%)
AMP(Ampicillin)	84 (100.0%)	0 (0.0%)	0 (0.0%)

Table 4. Colistin and eravacycline susceptibility among isolates.

Colistin MIC (84 MDR isolates)	Resistant	36 (42.9%)
	Sensitive	48 (57.1%)
	Intermediate	0 (0.0%)
	Median (IQR)	2 (1 – 8)
	Range	0.5 – 32
Eravacycline MIC (36 colistin resistant isolates	Resistant	2 (5.6%)
	Sensitive	34 (94.4%)
	Intermediate	0 (0.0%)
	Median (IQR)	0.32 (0.13 – 0.5)
	Range	0.09 – 0.75

Table 5. Colistin susceptibility among MDR *K. pneumoniae* and *E. coli.*

		Organism	
		Klebsiella	***E. coli***
		No.= 72	**No.= 12**
Colistin MIC	Median (IQR)	1.5 (1 – 8)	3 (2 – 16)
	Range	0.5 – 16	1 – 32
	Resistant	30 (41.7%)	6 (50.0%)
	Sensitive	42 (58.3%)	6 (50.0%)
	Intermediate	0 (0.0%)	0 (0.0%)

Table 6. Colistin resistance genes among colistin resistant *K. pneumoniae* and *E. coli*

		Organism		
		K. pneumoniae **(30)**	***E. coli*** **(6)**	**Total (36)**
MCR 2 gene	Negative	20 (66.7%)	6 (100%)	26 (72.2%)
	Positive	10(33.3%)	0 (0.0%)	10 (27.8%)
MCR 1 gene	Negative	2 (6.7%)	0 (0.0%)	2 (5.6%)
	Positive	28 (93.3%)	6(100%)	34 (94.4%)
pmrA gene	Negative	14 (46.7%)	0 (0.0%)	14 (38.9%)
	Positive	16 (53.3%)	6 (100%)	22 (61.1%)
More than one gene	*-MCR1 +MCR2+pmrA*	4 (13.3%)	0 (0.0%)	4 (11.1%)
	MCR1+ pmrA-	10 (33.3%)	6 (100.0%)	16(44.4%)
	-MCR1+MCR2	6 (20%)	0 (0.0%)	6 (16.7%)

Table 7. Accession number and source of mutated *pmrA* gene among colistin resistant *K. pneumoniae* and *E. coli.*

Accession No.	**Strain ID**	**Sample type**	**Nucleotide change at *pmrA***	
			P190S CCA [Pro]	**T192I ACT [Thr]**
OM362234	Ec4	Sputum	TCA [Ser]	ATC [Ile]
OM362235	Kp5	Sputum	TCA [Ser]	ATC [Ile]
OM362236	Kp7	Blood	TCA [Ser]	ATC [Ile]
OM362237	Kp10	Blood	TCA [Ser]	ATC [Ile]
OM362238	Ec11	Central line	TCA [Ser]	ATC [Ile]
OM362239	Kp12	Wound	TCA [Ser]	ATC [Ile]
OM362240	Kp13	Blood	TCA [Ser]	ATC [Ile]
OM362241	Kp16	Blood	TCA [Ser]	ATC [Ile]

Figure 1. Frequency of clinical samples included in the study.

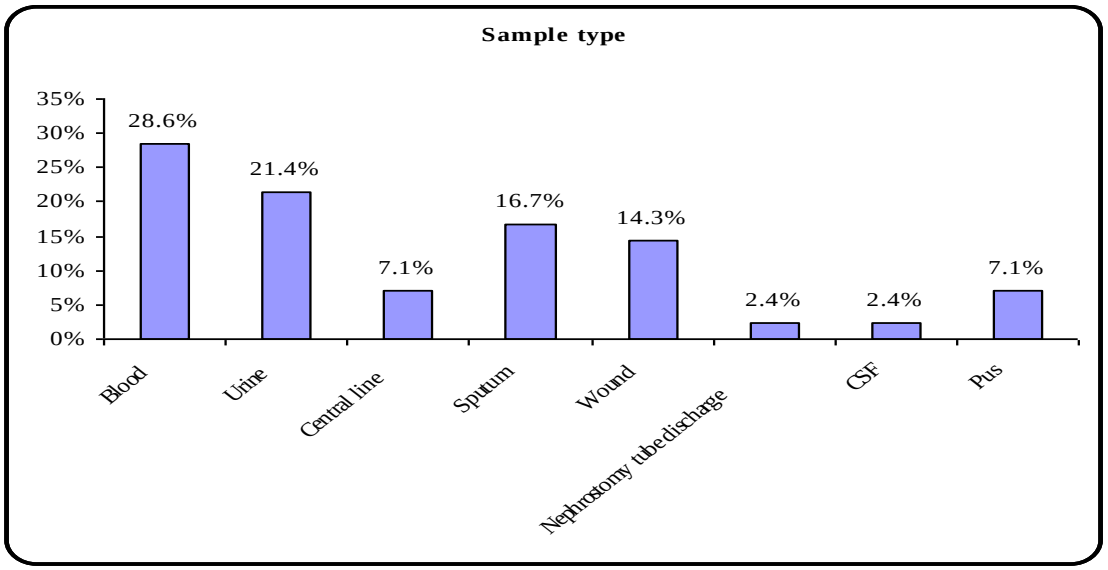
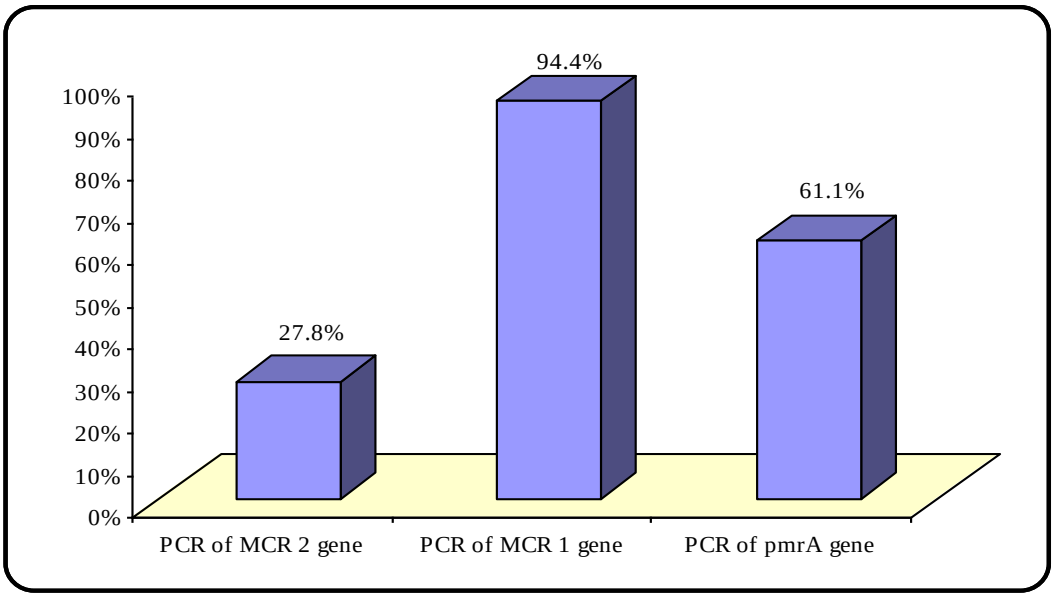


Figure 2. Frequency of colistin resistance genes among colistin resistant isolate.



Discussion

Multidrug-resistant bacteria causing serious infections are commonly reported in patients admitted in ICU, and leads to increasing death rates. Colistin has become one of the few available antimicrobials against life threatening infections caused by these bacteria. However, the uncontrolled use of colistin explains why colistin resistance acquisition is added to the problem of MDR [17].

Resistance to colistin has many molecular mechanisms given the fact that the mechanism underlying resistance is unknown. It's been claimed that resistance to this antibiotic is linked to several forms of lipopolysaccharides modification and the rise of plasmid carrying genes responsible for colistin resistance [18].

This study aimed to detect resistance to colistin antibiotic among MDR *E. coli* and *Klebsiella* strains isolated from ICU patients in Ain Shams University hospital, Cairo, Egypt. Some molecular mechanisms causing resistance to colistin were studied by detecting presence of and sequencing *pmrA* gene, chromosomal gene, and detecting *mcr-1* and *mcr-2* genes , plasmid-encoded genes.

In the current study, a total of 84 MDR *K. pneumoniae* and *E. coli* isolates were recovered from patients admitted ICU during the study period. We revealed that 42.9 % (36/84) of isolates were colistin-resistant, 50% (6/12) were MDR *E. coli* and 41.7% (30/72) were *K. pneumoniae* isolates as assessed by broth microdilution. This is nearly similar to which declared by **Zaki et al.** who detected colistin resistance of a rate 44% in *K. pneumoniae* and 42% in *E. coli* [19]. Also, **Moosavian et al.** detected colistin resistance in 59.4% of *E. coli* and 40.6% of *K. pneumoniae* [20]. Different results detected by **Rabie et al.** who reported that colistin resistance rate was 12% among MDR and XDR isolates, 33.3%, 66.7% among MDR *E. coli* and *K. pneumoniae* isolates respectively [21]. **Zafer et al.** reported that percentage of colistin resistance among MDR *E. coli* and *K. pneumoniae* isolates was 8.8% [22]. Meanwhile **Luo et al.** and **Buchler et al.** found colistin resistance in a rate of 3% and 3.8% respectively [23,24**]**.

This inconsistency of colistin resistance prevalence amongst previous studies could be as a result of getting different specimens from diverse wards all over the hospitals with different sample size. Intensive care unit patients are frequently subjected to unregulated use of empirical combined antibiotic therapy this could leads to rise the prevalence of antimicrobials resistance.

By investigating plasmid mediated *mcr-1*, we detected it in 94.4% of colistin resistant strains (all colistin resistant *E.coli* and 93.3% of colistin resistant *K. pneumoniae*) which is a very high percentage in comparison with other studies done in Egypt and worldwide. In Egypt, **Ghandour et al.** reported 35.78% of *E. coli* isolates were positive for *mcr-1*[25]. **Zafer et al.** declared that only 5% of isolates obtained *mcr-1* (*E. coli* and *K. pneumoniae*)[22]. **Rabie et al.** reported that *mcr-1*

was detected 2 isolates (8.4%); one *E. coli* isolate (4.2%), the other *K. pneumoniae* isolate (4.2%), while **Zaki et al.** detected *mcr-1* gene in 2 out of 50 (4%) colistin resistance [19,21]

Also, the gene was detected at lower level worldwide; **Luo et al.** found the gene in 21 colistin resistance *E. coli* out of 40 (52.5%) [23]. **Moosavian et al.** detected *mcr-1* in *E. coli* isolates with a rate of 1.2% and in *K. pneumoniae* isolates (0.4%) [20]. Moreover, **Emara et al.** and **Tanfous et al.** reported that colistin *mcr-1* gene was not detected among their phenotypically resistant isolates [26, 27]. High rate of *mcr-1* carriage in the present study may be due to isolation of these strains from patients admitted to ICU. So, plasmid-encoded *mcr-1* gene is of special alarm to public health, as it can transmit more easily than colistin resistance genes encoded chromosomally.

Regarding *mcr-2*, It was detected in 27.8% of colistin resistant strains (33% of colistin resistant *K. pneumoniae* and not present in *E.coli* isolates). Different previous studies reported absence of *mcr-2* among colistin resistant *K .pneumoniae* and *E.coli* [19, 21, 22, 23]. The *mcr-2* gene was only reported in Belgium by Sun and his colleagues which posed a hypothesis that *mcr-2* dissemination occurs by a different mechanism [28]. Inconsistencies between assays may be due to obstruct detection of isolates with *mcr-1* and *mcr-2* [29]. No previous studies in Egypt report detection of *mcr-2*. This could raise attention toward investigating the application of infection control measures and adherence to antimicrobial stewardship especially in ICU.

Analysis of *pmrA* gene in colistin-resistant isolates revealed that the gene presents in 61.1% of colistin resistant strains (all colistin resistant *E. coli* and 53.3% of colistin resistant *K. pneumoniae*). Sequencing of eight selected isolates revealed two-point mutations in all these isolates. First mutation was (TCA>CCA) mutates proline 190 to serine (P190S). The second mutation was (ATC>ACT) mutates threonine 192 to isoleucine(T192I). So, there is a major role of *pmr A* mutations in colistin resistance among our isolates.

Different mutations were detected by few previous studies. **Azam et al.** showed that there was a deletion mutation in *pmrA* gene (G53S) among *mcr* negative colistin resistant *K. pneumoniae* isolates [30]. **Pragasam et al.** declared point mutation (A170G) in the *pmrA* gene in two *K. pneumoniae* isolates. This mutation leaded to change of amino acid (E57G) [31]. **Sato et al.** demonstrated substitution mutation L105P (CTG ….CCG) in *pmrA* gene in four *mcr* negative colistin resistant *E.coli* [32]. These studies reported mutation of chromosomal genes responsible for colistin resistance without detection of *mcr* gene in the same isolates. However, we detected more than one resistance mechanism in the same isolate, four *K. pneumoniae* isolates carried *mcr-1, mcr-2* and mutated *pmr A* genes. Another 6 isolates carried *mcr-1* and *mcr-2* genes. Also, 10 *K. pneumoniae* and 6 *E. coli* isolates carried *mcr-1* as well as *pmrA*.

The main molecular mechanism of resistance to colistin arises by variation of two-component *pmrA*B and PhoPQ systems that are chromosomally encoded, leading to alteration of the bacterial outer membrane**.** The rise of *mcr*-1 gene that is plasmid-mediated and encodes resistance to colistin in different bacteria can transfer horizontally from one bacterium to another one. Also, it can disseminate among animals, humans, and the environment [33]. Presence of more than resistance mechanism in the same isolate is alarming threat.

Identification of new *mcr* variants such as *mcr*-3, *mcr*-4, mcr-5, *mcr*-6, *mcr*-7, *mcr*-8, *mcr*-9, and *mcr*-10 is mandatory and of great importance to health. There is a strong warning of fast spread of plasmid-mediated resistance variants. This requires more studies to assess the causes involved, the acquisition mechanisms and dissemination mechanisms amongst different bacteria**.**

New antibiotics development against colistin resistant bacteria is mandatory. Eravacycline, fully synthetic tetracycline, inhibits bacterial protein synthesis (i.e., acyl-tRNA transfer) by binding to the 30S ribosomal subunit [34]. In our study all colistin resistant isolates were sensitive to eravacyclin, MIC ranged (0. 13 to 0.5), except two *K. pneumoniae* isolated from infected central line. This result was near that reported by **Fyfe et al.** as they concluded that eravacycline had potent in vitro activity against naturally occurring *mcr-1*positive bacterial isolates, MIC ranged (0.031 to 0.5), and unaffected by the overexpression of *mcr-1* in Enterobacteriaceae [35]. **Falagas et al.** revealed that eravacycline demonstrates potent broad-spectrum activity against Gram-negative bacilli (with the exception of *P. aeruginosa* and *Burkholderia* spp.)[36].

Conclusion

The horrible increase in antimicrobial resistance is becoming a critical catastrophe worldwide and become more prevalent especially in vulnerable patients. In Egypt, prevalence of resistance to colistin antibiotic is increasing at worrying rates. Actually, the presence of *mcr-1* gene and *pmr A* gene mutations seem to have a role in causing colistin resistance in our study. Therefore, strict surveillance of colistin resistance must continue to limit further spread. In addition, all authorized individuals should address this problem by prohibiting the uncontrolled use of colistin especially in agriculture.

Limitations

Limited detection of all the possible colistin resistance genes as there is no fund for this study was one of the major challenges. We could not use gene expression studies to study the actual effect of mutations in detail.

Recommendations

Whole genome sequencing of multidrug resistant organisms should be done in further research studies to give a detailed pattern of resistance genes and virulence factors that will help us to overcome resistance and develop more potent and less toxic treatment options. Moreover, Detection of the dissemination rate of resistance genes should be done regularly through prospective surveillance and epidemiological studies. There is an urgent need to implement the antibiotic stewardship and exert more efforts to maintain the efficacy of colistin as the last treatment option.

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