



# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

### Detection of plasmid-mediated colistin resistance in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in Suez Canal University Hospitals

Sally Maged Khattab<sup>1\*</sup>, Mohamed Aly El-Sweify<sup>1</sup>, Lobna Ahmed Metwally<sup>1,2</sup>, Sahar Zakria El-azab<sup>1</sup>, Asmaa Abd Elkreem Hashem<sup>1</sup>

<sup>1</sup> Microbiology and Immunology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

<sup>2</sup> Microbiology Department, College of Medicine, Taif University, Taif, Saudi Arabia.

#### ARTICLE INFO

##### Article history:

Received 2 June 2021

Received in revised form 29 June 2021

Accepted 4 July 2021

##### Keywords:

PCR

*Enterobacteriaceae*

Colistin

Resistance

#### ABSTRACT

**Background:** The polymyxins including colistin are the “last resort” antibiotics for treatment of infections with carbapenem resistant *Enterobacteriaceae*. Recently, transferable colistin resistance mediated by the *mcr*-genes has been described in *Enterobacteriaceae*. The testing of colistin susceptibility is challenging. The aim of the work is to detect the occurrence of *mcr-1* and *mcr-2* genes in phenotypically colistin-resistant carbapenem resistant *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) isolates using conventional polymerase chain reaction (PCR). **Methods:** One hundred and sixteen carbapenem-resistant *E.coli* and *K.pneumoniae* isolates were collected from patients admitted to different wards of Suez Canal University Hospitals (SCUHs) in Ismailia. Urine, endotracheal aspirates, blood, pus and sputum specimens were collected from different patients. Minimal inhibitory concentration (MIC) by broth microdilution method was done to assess phenotypic colistin resistance. The colistin resistant *E.coli* and *K.pneumoniae* isolates were tested by conventional PCR to detect plasmid mediated *mcr-1* and *mcr-2* genes. Chi-square test was applied and *p*-value < 0.05 was considered statistically significant. **Results:** Forty three isolates out of 116 carbapenem resistant isolates (37.1%) were colistin resistant as shown by MIC by broth microdilution method. The 43 colistin resistant *E.coli* and *K.pneumoniae* isolates were tested by conventional PCR to detect plasmid mediated *mcr-1* and *mcr-2* genes. Two *mcr-2* genes and one *mcr-1* gene were detected. **Conclusion:** Results revealed that the prevalence of colistin resistance among carbapenem-resistant *E.coli* and *K.pneumoniae* in SCUHs is concerning; further limiting potential therapeutic options. Plasmid mediated colistin resistance genes *mcr-1* and *mcr-2* is emerging in SCUHs which refer to a problem in the hospital as by horizontal transfer of this plasmid, the resistance can spread to many isolates in the hospital.

#### Introduction

The polymyxins including colistin are considered the last choice of antibiotics for treatment of infections with carbapenem resistant *Enterobacteriaceae* (CRE). In 2012 colistin was reclassified by the World Health Organization as critically important for human medicine [1].

Colistin interacts with the bacterial outer membrane by removing divalent cations from the negatively-charged phosphate groups of the Lipid A of the lipopolysaccharide membrane which leads to cell lysis [2].

DOI: 10.21608/MID.2021.78200.1162

\* Corresponding author: Sally Maged Khattab

E-mail address: [sally.maged@med.suez.edu.eg](mailto:sally.maged@med.suez.edu.eg)

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4 license <https://creativecommons.org/licenses/by/4.0/>.

Previously, colistin resistance was considered only due to chromosomal mutations in the genes encoding the PmrA/PmrB and PhoP/PhoQ signalling systems or the negative regulator MgrB. These mutations cause modifications to the Lipid A molecule [2]. Recently, transferable resistance of the polymyxins mediated by the *mcr*- genes has been detected. These *mcr*- genes were located on the plasmid which raises the concern that the resistance may be transferred to Gram-negative bacteria. The *mcr-1* gene firstly discovered in *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in China [3], after that several studies across the world reported the presence of the *mcr-1* gene in various bacteria from many other countries such as United States, European Union, Turkey, South Africa, Malaysia, Greece, Italy, Algeria, Tunisia and Kuwait [4]. The first study carried out in Egypt in 2016 reported the presence of *mcr-1* gene in *E. coli* strain taken from an intensive care unit (ICU) patient [5].

In 2016, the *mcr-2* plasmid-mediated colistin resistance was detected in *E. coli* first in Belgium from pigs and then from patients [6] *Mcr-2* gene which is a phospho-ethanolamine transferase, modifies the lipopolysaccharide and shares 80.6% identity with *mcr-1*. After that, transferable colistin resistance gene *mcr-3* has been identified in *E. coli* and *K. pneumoniae* from Asia and the United States and then some studies found *mcr 4, 5, 6, 7, 8* and *9* [7].

The transferring of plasmid-borne *mcr* genes will diminishes the clinical choice of the last-resort colistin, which may lead to more treatment failures and increased morbidity and mortality. As a result, the European Medicines Agency, is currently updating the guidelines of using colistin. They considered upgrading of colistin as an antibiotic category 2 used for critical medicine and only if no other options actually found [8].

Colistin susceptibility testing is considered challenging due to recommendation updates by Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) which were declared in 2016 [9] that stated standard broth microdilution (BMD) method to test colistin. As a result, colistin susceptibility is probably neither tested routinely nor correctly against CRE isolates in most diagnostic laboratories in Egypt, and the frequency with which colistin resistance occurs is difficult to estimate from local data.

The purpose of the present study is to evaluate the frequency of colistin resistance among *E. coli* and *Klebsiella* carbapenem resistant isolates obtained from patients in Suez Canal University Hospitals (SCUHs), and to determine how often *mcr-1* and *mcr-2* genes are detected among the colistin-resistant subset.

### Patients and Methods

A cross-sectional descriptive study was conducted on patients admitted to SCUHs in Ismailia. One hundred and sixteen carbapenem resistant isolates of *E. coli* and *K. pneumoniae* were collected from those patients. The study was conducted on patients admitted to the urology, internal medicine, surgery wards, intensive care unit (ICU), and neonatal intensive care unit.

#### Inclusion criteria:

- Both sexes.
- All age groups.
- Patients showing signs of sepsis or pyogenic infections.
- Patients having risk factors for acquisition of infection as:
  - Patients having high-risk procedures (such as surgery, urinary tract instrumentation, indwelling urinary or intravenous catheters and patients admitted to the burn unit).
  - Immunocompromised and debilitated patients and patients having serious underlying disease.

#### Exclusion criteria:

- Patients refusing to participate in the study.

#### Specimens:

Different microbial specimens were collected on clinical suspicion of infection according to standard definitions. Specimens included urine from catheterized and non-catheterized patients, respiratory specimens (from both intubated and non-intubated patients), blood and swab specimens.

#### Methods:

##### 1) Collection of specimens:

Specimens were collected under aseptic conditions and transported in suitable transport media (when needed) to be processed in the Microbiology laboratory Department, Faculty of Medicine, Suez Canal University for the isolation and identification of *E. coli* and *K. pneumoniae*.

## 2) Processing of specimens:

Collected specimens were inoculated onto blood agar plates and MacConkey agar plates. The plates were incubated aerobically at  $35 \pm 2^\circ\text{C}$  for 24 hours.

### A. Identification of *E.coli* and *K. pneumoniae* [10]:

They were non-spore forming Gram negative rods. On blood agar: they appeared as moist smooth and greyish-white colonies. On MacConkey agar: they appeared as pink colored colonies due to lactose fermentation. *K.pneumoniae* strains produced mucoid colonies. They were catalase positive, oxidase negative, ferment glucose with acid production and reduce nitrate to nitrite.

### B. Suspected isolates were screened for genus and species by biochemical reactions based on metabolic characteristics [11]

\*IMVC tests (Indole test, Methyl red, Voges-Proskauer test (VP), and Citrate test), ornithine decarboxylation and motility tests were done for lactose fermenting colonies.

### 3) Antimicrobial susceptibility testing:

Disk diffusion susceptibility test was performed on each isolate according to CLSI guidelines (2018) [12]. Antibiotic disks were purchased from (Oxoid, UK). The following antimicrobial agents were included:

- Amoxicillin-clavulanate (20/10  $\mu\text{g}$ ), Cefotaxime (30 $\mu\text{g}$ ), Ceftazidime (30 $\mu\text{g}$ ), Cefepime (30 $\mu\text{g}$ ), Imipenem (10 $\mu\text{g}$ ), Meropenem (10 $\mu\text{g}$ ).
- Ciprofloxacin (5 $\mu\text{g}$ ), Levofloxacin (5 $\mu\text{g}$ ).
- Gentamycin (10 $\mu\text{g}$ ), Amikacin (30 $\mu\text{g}$ ).
- Trimethoprim-sulfamethoxazole (1.25/3.75 $\mu\text{g}$ ).

### 4) Screening & confirmatory tests for suspected carbapenemase production in Enterobacteriaceae isolates [12]:

*E.coli* and *K.pneumoniae* isolates that showed inhibition zone diameter  $\leq 22$  mm for either meropenem or imipenem were considered carbapenem nonsusceptible (intermediate or resistant) and were subjected to the following:

#### ❖ Modified carbapenem inactivation method (mCIM).

One microlitre loopful of the test isolate was suspended in 2 ml of trypticase soy broth (TSB), and

meropenem disk was added into the suspension. The culture was incubated for 4 h at  $35^\circ\text{C}$ . *Escherichia coli* ATCC 25922 (a carbapenem-sensitive type strain) was adjusted using McFarland standard no. 0.5 in sterile saline and streaked in three directions on the Mueller-Hinton Agar (MHA) plates to ensure even and standardized cell lawn. The disk was transferred from TSB suspension onto MHA plate, and the plate was incubated overnight at  $35^\circ\text{C}$ . After incubation, the diameter of the inhibition-zone was measured. Carbapenemase positive test strain: if it showed inhibition zone 6-15 mm or presence of colonies within a 16-18 mm zone. Carbapenemase negative test strain: if it showed inhibition zone  $\geq 19$  mm. Intermediate test strain: if it showed inhibition zone 16-18mm.

### 5) Phenotypic detection of colistin resistance:

#### Minimum inhibitory concentration method:

Colistin susceptibility was performed by broth microdilution method according to CLSI 2012 [13]. Antibiotic stock solution was prepared from pure colistin sulfate powder (Sigma-Aldrich, Germany). The amount needed and the diluents in which it was dissolved (distilled water) was calculated by using the following formula to determine the amount of antimicrobial powder.

$$\text{weight}(mg) = \frac{\text{volume}(ml) \times \text{concentration}(\frac{\mu g}{ml})}{\text{potency}(\frac{\mu g}{mg})}$$

Preparation of antibiotic dilution range was done according to CLSI 2018 [12]. The inoculum was prepared by making a direct broth suspension (brain heart broth) of isolated colonies selected from an 18- to 24-hour blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This resulted in a suspension containing approximately  $1$  to  $2 \times 10^8$  colony forming units (CFU)/mL for *Escherichia coli* ATCC®a 25922. The adjusted inoculum suspension in broth was diluted to obtain a final concentration after inoculation approximately  $5 \times 10^5$  CFU/mL. Within 15 minutes after the inoculum has been standardized as described above, 100  $\mu\text{l}$  of the adjusted inoculum was added to each well containing 100  $\mu\text{l}$  of antimicrobial agent in the dilution series (and a positive control tube containing only broth), and mixed. The inoculated microtiter plate was incubated at  $35 \pm 2^\circ\text{C}$  for 16 to 20 hours in an ambient air incubator. The amount of growth in the wells containing the antimicrobial

agent was compared with the amount of growth in the growth-control wells (no antimicrobial agent). For a test to be considered valid, acceptable growth ( $\geq 2$  mm button or definite turbidity) must occur in the growth-control well.

**The lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC.**

#### **6) Genotypic detection of plasmid-mediated *mcr-1* and *mcr-2* colistin resistance genes.**

Plasmid DNA was extracted from the test isolates by using QIAprep Mini-Prep Kit (QIAGEN, Germany). Polymerase chain reaction was performed and the product was analyzed according to the laboratory protocol optimized by National Food Institute, Denmark, for detection of plasmid-mediated colistin resistance genes, *mcr-1* and *mcr-2* [14], using previously published primers. The reaction mixture was prepared in a total volume of 25  $\mu$ l including 3  $\mu$ l of template DNA, 12.5  $\mu$ l of 2X ABT Red master mix (Sigma-aldrich, Egypt), and 2  $\mu$ l of both forward and reverse primers. The volume completed with distilled water up to 25  $\mu$ l. Reaction mixtures without a DNA template served as negative controls. Amplification was carried out in a thermal cycler (Peltier Thermal cycler, MJ Research, U.S.A) with the following thermal cycling: 95°C 5 mins + 25X (95°C 30 sec + 58°C 60 sec + 72°C 60 sec) + 72°C 10min. Amplicons obtained from PCR reactions were analyzed by gel electrophoresis (Major Science, Taiwan) and finally visualized with ultraviolet light. Amplicon size (bp) of the tested genes was identified and compared to a 100 bp molecular size standard DNA ladder (Sigma-aldrich, Egypt).

## **Results**

### **Results of demographic and clinical data**

The study was carried out during the period from 2018 till 2020. Specimens collected from patients admitted in different wards in SCUHs in Ismailia. Number of specimens collected was 536, included endotracheal aspirate, blood, urine, pus and sputum specimens. Number of *K.pneumoniae* isolates was 189 and *E.coli* was 91 isolates. Among them carbapenem-resistant *K.pneumoniae* were 90 isolates and carbapenem-resistant *E.coli* were 26 isolates.

From the 536 examined clinical specimens, *E.coli* and *K. pneumoniae* were isolated at a rate of 52.2%, while other organisms were isolated at a rate of

45.4%. No growth was detected in 2.4% of the cases (Figure 1).

Number of *E.coli* and *K.pneumoniae* strains isolated was 280. Among them 116 (26 *E.coli* & 90 *K.pneumoniae*) were carbapenem resistant isolates (41.4%). The 116 carbapenem resistant *E.coli* and *K.pneumoniae* isolates were collected from 64 male patients (55.2%) and 52 female patients (44.8%).

Table 1 shows that 46.6% of the studied carbapenem-resistant strains were isolated from endotracheal aspirates. Other carbapenem-resistant strains were isolated from blood (21.6%), urine (9.4%), sputum (8.6%) and pus (13.8%) specimens.

### **Results of antibiotic susceptibility testing**

Antibiotic susceptibility test was done to the carbapenem resistant *K.pneumoniae* (90, 77.6 %) and *E.coli* (26, 22.4 %) isolates by disk diffusion method (Kirby Bauer method). It showed 100% extended spectrum beta-lactamase as all isolates were resistant to both amoxicillin-clavulanic and ceftazidime antibiotics. While gentamycin, amikacin, levofloxacin and cefipime showed 51.3%, 63%, 79.5% and 87.7% resistance respectively. (Figure 2).

Modified carbapenem inactivation method was done to assess carbapenemases activity among the resistant isolates. Eighty two isolates were carbapenemases positive (70.7%) (Figure 3). Among them, 14 out of 26 *E.coli* isolates were carbapenemase positive (53.8%). While 68 isolates out of the 90 carbapenem-resistant *K.pneumoniae* isolates were carbapenemase positive (75.6%).

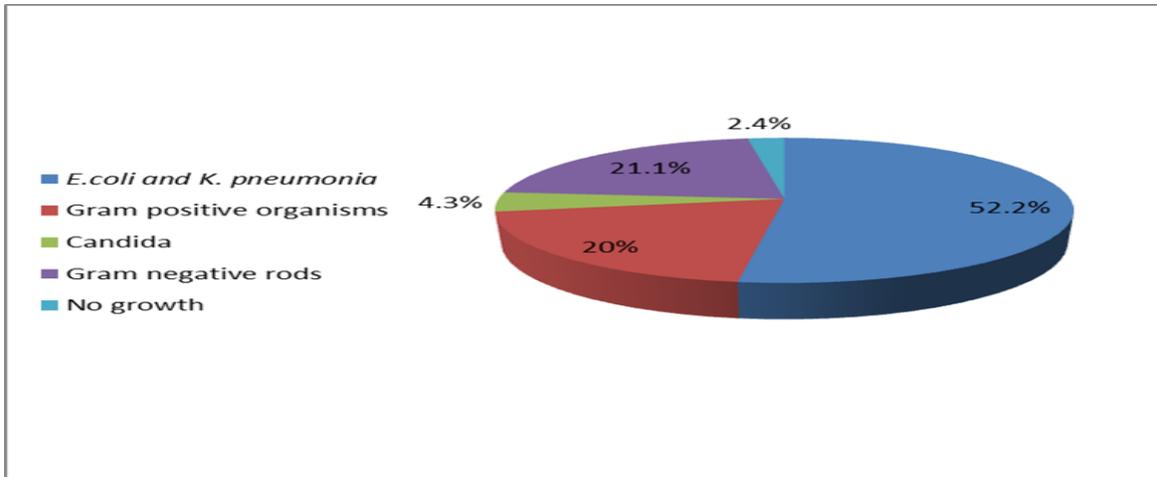
Phenotypic colistin resistance detection was done to the 116 CR isolates by minimal inhibitory concentration method using broth microdilution method (Figure 4). Forty three isolates (37.1%) were colistin resistant (Non-wild type). Thirty nine colistin resistant isolates were collected from the ICU, while two isolate were from the internal medicine department and two from the surgery department. Out of the 43 isolates, 29 isolates were obtained from endotracheal aspirate specimens, 6 isolates were obtained from blood, 4 from urine and 4 from pus.

The 43 colistin resistant *E.coli* and *K.pneumoniae* isolates were tested by conventional PCR and agarose gel electrophoresis for detection of plasmid mediated colistin resistance genes *mcr-1* and *mcr-2* (Figure 5). Two *mcr-2* genes and one *mcr-1* gene were detected among the 43 isolates (Table 2).

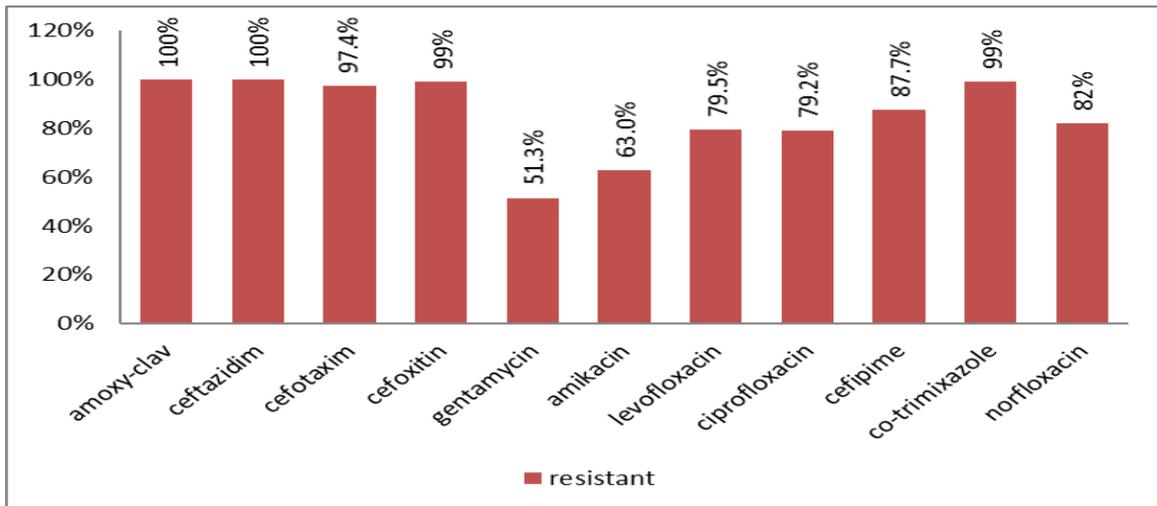
Regarding the three positive *mcr* isolates; all of them were *K.pneumoniae* isolates, two were collected from endotracheal tube and all of them were from

ICU. They showed high level of antibiotic resistance and they were phenotypically colistin resistant (Table 3).

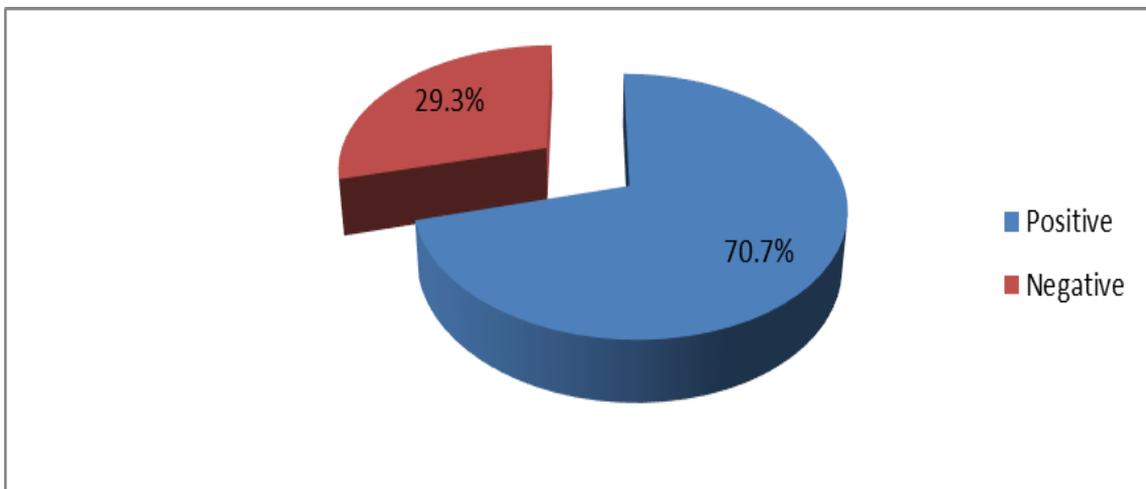
**Figure 1.** Frequency distribution of *E.coli* and *K. pneumoniae* in relation to other organisms (N = 536).

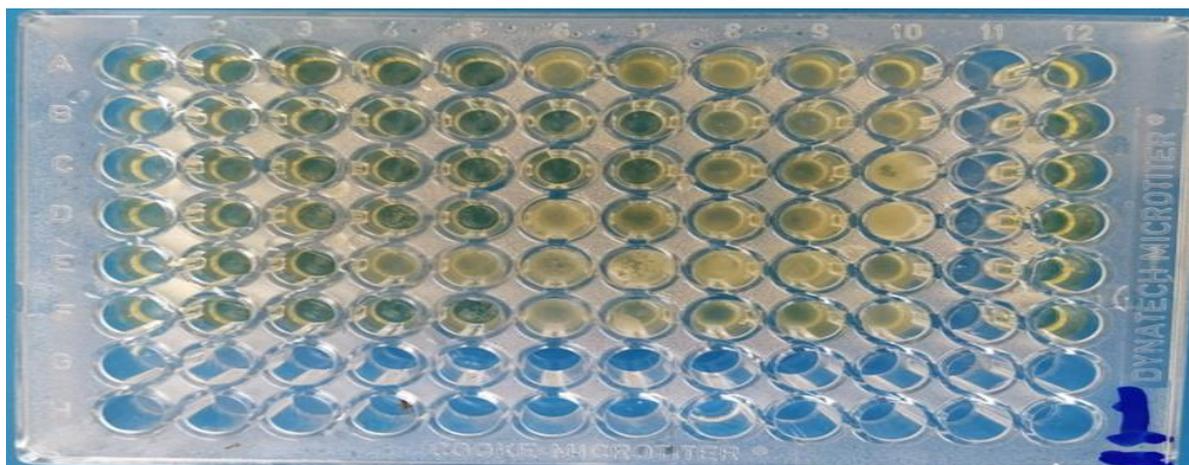


**Figure 2.** Frequency of antibiotic resistance pattern of the studied carbapenem resistant *E.coli* and *K.pneumoniae* isolates (N =116).

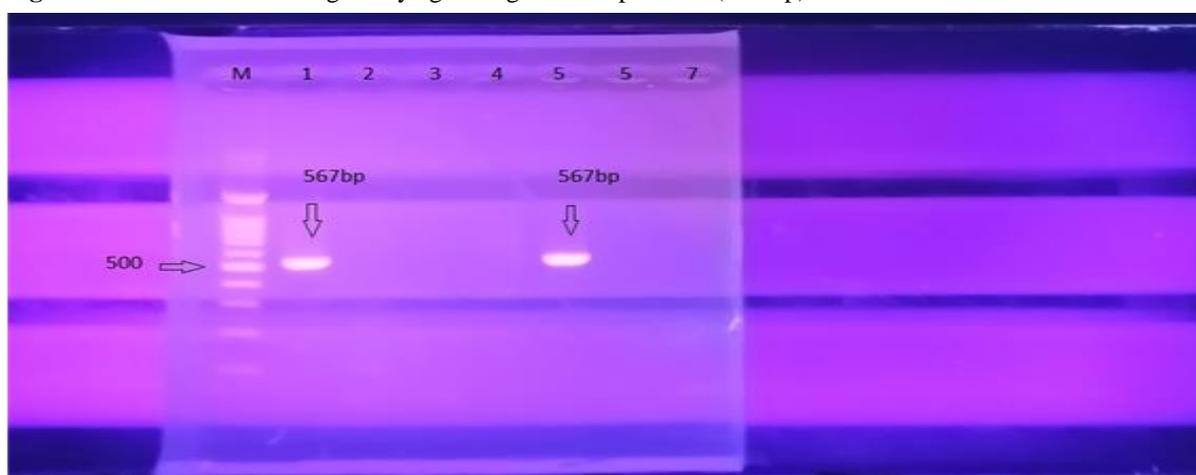


**Figure 3.** Frequency distribution of carbapenemases activity among *E.coli* and *K.pneumoniae* isolates by mCIM test (N = 116).



**Figure 4 .** MIC (BMD) of Colistin antibiotic among *E.coli* and *K.pneumoniae* isolates according to CLSI 2019.

Row A shows MIC result=8, Row B MIC result=2, Row C MIC result=2, Row D MIC=8, Row E MIC=32, Row F MIC=16.

**Figure 5.** Detection of *mcr-2* gene by agarose gel electrophoresis (567 bp).

Lane M shows 100 bp molecular size standard DNA ladder. Lane 1 shows a positive control strain, lane 2 shows a negative control strain. Lanes 5 shows a positive isolate.

**Table 1.** Distribution of the isolated carbapenem-resistant *E.coli* and *K.pneumoniae* isolates according to the type of the specimen.

| No. of specimens<br>(N=280) | No. CR. <sup>1</sup> isolates<br>(N=116) | Percent of CR.<br>specimens | Percent to all CR. strains. |
|-----------------------------|------------------------------------------|-----------------------------|-----------------------------|
| <b>ETT<sup>2</sup>(80)</b>  | 54                                       | 67.5% (54/80)               | 46.6% (54/116)              |
| <b>Blood (45)</b>           | 25                                       | 55.5% (25/45)               | 21.6% (25/116)              |
| <b>Urine(89)</b>            | 11                                       | 12.3%(11/89)                | 9.4% (11/116)               |
| <b>Sputum(24)</b>           | 10                                       | 41.6%(10/24)                | 8.6% (10/116)               |
| <b>Pus(42)</b>              | 16                                       | 38%(16/42)                  | 13.8% (16/116)              |

<sup>1</sup>Carbapenem resistant <sup>2</sup>Endotracheal tube

**Table 2.** Frequency of *mcr-1* & *mcr-2* genes among phenotypic colistin resistant isolates (N=43).

|                   | Number | Percentage | P value |
|-------------------|--------|------------|---------|
| <i>MCR-1</i> gene | 1/43   | 2.3%       | >0.05   |
| <i>MCR-2</i> gene | 2/43   | 4.6%       | >0.05   |

**Table 3.** Data about the three colistin resistant isolates by PCR.

|                              | Specimen           | Department | Organism             | Antibiotic susceptibility                                        | Carbapenemase result | MIC result |
|------------------------------|--------------------|------------|----------------------|------------------------------------------------------------------|----------------------|------------|
| <b><i>Mcr-1</i> positive</b> | Endo-tracheal tube | ICU        | <i>K. pneumoniae</i> | Resistant to all antibiotics used except gentamycin and amikacin | Negative             | 16         |
| <b><i>Mcr-2</i> Positive</b> | Pus                | ICU        | <i>K. pneumoniae</i> | Resistant to all except gentamycin, amikacin and ciprofloxacin   | Negative             | 128        |
| <b><i>Mcr-2</i> Positive</b> | Endo-tracheal tube | ICU        | <i>K. pneumoniae</i> | Resistant to all antibiotics used                                | Positive             | 16         |

### Discussion

With the increased spread of carbapenem resistance, treatment options are decreasing. Colistin is considered as a drug of choice in the treatment of carbapenem resistant isolates. Unfortunately, colistin resistance has emerged and became a big problem [3].

*Klebsiella pneumoniae* carbapenem resistant isolates were 90 out of 189 *K.pneumoniae* isolates (47.6%) while *E.coli* carbapenem resistant isolates were 26 out of 91 *E.coli* isolates (28.6%). This result shows accordance with **El-Sweify et al.** [15] who reported carbapenem resistance in 44.3% of *K. pneumoniae* isolates in SCUHs. Higher results were detected by **Melake et al.** [16] and **Amer et al.** [17] as they reported 62.5% and 62.7% carbapenem-resistance among *K.pneumoniae* and *Enterobacteriaceae* isolates. On the other hand, lower results of carbapenem resistance were also reported in different studies as **Khare et al.** [18] and **Okoche et al.** [19] reported 37.9% and 28.6% carbapenem resistance. **Khalifa et al.** [20] reported 15.7% *E.coli* carbapenem resistant isolates. The difference of prevalence among different studies may be due to the difference in application of infection control measures and antibiotic policies in different hospitals. Also the variety of specimens obtained from different wards all over the hospitals may contribute to the difference of these results. The high

trend of carbapenem resistant *K.pneumoniae* in our study could be attributed to the frequent use of carbapenems as an empiric therapy in our hospital, as well as lack of implementation of antimicrobial stewardship program.

The current study showed that 46.6% of the collected carbapenem-resistant isolates were in endotracheal aspirates obtained from the ICU. That shows accordance with the study of **Saeed et al.** [21] as they collected most of the carbapenem resistant isolates from endotracheal aspirates in ICU. The high prevalence of carbapenem-resistant isolates found in the ICU with highest prevalence among endo-tracheal aspirates may be related to the low immunity status of patients and the respiratory tract invasive procedures done in ICU. It may also reflect cross-infection with multi-resistant clones or long-term exposure of respiratory tract microbiota to antibiotics, causing accumulation of resistance determinants in strains causing respiratory tract infection. Also, the presence of the tube for long period may cause inflammation and edema of the respiratory tract which aids in repeated infections.

Antibiotic susceptibility testing was done to the carbapenem resistant *K.pneumoniae* and *E.coli* isolates. It showed 100% resistance to  $\beta$ -lactam antibiotics due to ESBL production, while gentamycin, amikacin, levofloxacin and cefipime showed 51.3%, 63%, 79.5% and 87.7% resistance

respectively. In a study done by **Lin et al.** [22] antibiotic susceptibility to carbapenem-resistant isolates showed resistance to amikacin 55.3%, ciprofloxacin 84.2%, amoxicillin/Clavulanic acid 97.4% and cefipime 97.4% which showed accordance with our results. Also a study done in Egypt in El-Mansoura University by **Moemen and Masallat** [23] reported high resistance among the carbapenem-resistant isolates as it showed ESBL production in 97.6%, amikacin 52.4%, ciprofloxacin 95.2%. Another study done in Egypt by **Shawky et al.** [24] reported 100% ESBL production among their isolates, 77.7% resistance to amikacin and 87.1% resistance to ciprofloxacin. The abuse of antibiotics is the basis of appearance of the high level resistance among different antibiotics, also self-medication by patients and lack of implementation of antibiotic policies in hospitals may contribute.

Modified carbapenem inactivation method was done in our study to assess the presence of carbapenamases enzymes among the resistant isolates. Among the 26 carbapenem-resistant *E.coli* isolates, 14 isolates were carbapenemase positive (53.8%). While 68 isolates out of the 90 carbapenem-resistant *K.pneumoniae* isolates were carbapenemase positive (75.6%). Similar levels of carbapenamases detection was found in a study by **Laolerd et al.** [25] and a study done by **Qadri et al.** [26] as they detected 77.7% and 67.3% carbapenamases among carbapenem resistant isolates respectively. Decreased rates of carbapenemase production among the carbapenem-resistant isolates were detected in another study conducted by **Kandeel** [27] who reported 18.5% carbapenamases among his isolates. In the current study many of potential risk factors for carbapenamases acquisition were observed. This is similar to previous reports describing these risk factors, including prolonged hospital stay, antimicrobials exposure, mechanical ventilation, pulmonary disease, current stay in the ICU and use of indwelling device as the most prominent ones. Also the horizontal transmission of plasmid mediated carbapenem resistance among different isolates may contribute to the high prevalence of carbapenem resistance in the hospital.

The presence of carbapenem-resistant isolates with negative carbapenamases results may be attributed to other causes as efflux pumps, drug impermeability or production of carbapenamases Class-D that can't be detected by mCIM method

(**Benmahmod et al.**) [28]. With the increased emergence and spread of carbapenem resistance, treatment options are decreasing.

Minimum inhibitory concentration for colistin was done by broth microdilution method. Forty three isolates out of 116 (37.1%) carbapenem-resistant isolates were colistin resistant (Non-wild type). A study done by **Capone et al.** [29] reported 36.1% colistin resistance among *K.pneumoniae* carbapenem-resistant isolates. Lower levels of colistin resistance were found in other studies as **Rojas et al.** [30] and **Bhaskar et al.** [31] detected 13% and 27.7% colistin resistant isolates among carbapenem-resistant *K.pneumoniae* isolates respectively. Higher result of colistin resistance was detected in a study by **Monaco et al.** [32] as they reported 43% colistin resistant isolates. In Alexandria, Egypt, a study done by **Shawky et al.** [28] reported 14% colistin resistance among carbapenem resistant isolates. Many risk factors were identified for colistin resistance in GNRs, including recent prior hospitalization, prior carbapenem resistance, prior treatment with colistin, exposure to chlorhexidine, the presence of multiple comorbidities in the patients, increasing age, male sex, length of hospitalization, and the presence of indwelling urinary catheters and all of these factors present in our hospital.

In this study the 43 colistin and carbapenem resistant *E.coli* and *K.pneumoniae* isolates were tested by conventional PCR for detection of plasmid mediated colistin resistance genes *mcr-1* and *mcr-2*. Two isolates harbor *mcr-2* gene (4.6%), meanwhile, only one isolate has *mcr-1* gene (2.3%) were detected among the 43 isolates. A study done by **Moosavian et al.** [33] reported 1.7% *mcr-1* gene. Higher prevalence of *mcr-1* gene detection were found in a study done in Egypt by **Zaki et al.** [34] as they reported 4% *mcr-1* genes. Regarding *mcr-2* gene **Zhang et al.** [35] and **Jayol et al.** [36] reported *mcr-2* gene with prevalence of 1.5% and 1% respectively. The presence of the plasmid mediated colistin resistant gene in the current study may be due to previous antibiotic therapy (e.g. colistin, beta-lactam/beta-lactamase inhibitors, carbapenems, glycopeptides), previous hospitalisation and previous colonisation with multidrug-resistant bacteria (e.g. KPC). Additional risk factors may be immunosuppression and the use of fluoroquinolones.

To the best of our knowledge by reviewing previous studies, plasmids that are harboring *mcr*

genes are different from plasmids carrying other antibiotic resistance genes. This may explain the high rate of antibiotic resistance found in our strains with the low rate of *mcr* genes detected.

Low rates of horizontal transmission of plasmid-mediated colistin resistance genes *mcr-1* and *mcr-2* is still noticed in the hospital as the dissemination of *mcr* in *K.pneumoniae* is contingent on colistin selection pressures, and by extension, that by limiting colistin usage, further dissemination of the gene in *K. pneumoniae* could be stopped. In SCUH the usage of colistin in treatment of patients is still limited.

In conclusion, this study revealed that the presence of colistin resistance among carbapenem resistant isolates in SCUHs is concerning because further limiting potential therapeutic options and inevitably leading to higher mortality rates. In addition the detection of the plasmid mediated *mcr-1* and *mcr-2* raises the concern and the necessity for paying attention to the horizontal transmission of this resistance which may eventually lead to pan-drug resistant isolates. Strict application of standard infection control practices in the hospitals especially in the critical areas are highly recommended to prevent the emergence and the spread of the resistant organisms. Also, we recommend the wise use of carbapenems, colistin and other antibiotics.

**Conflict of interest:** The authors have no conflict of interest to declare.

#### Financial Disclosures

The authors declared that this study has received no financial support and was totally funded by the authors.

#### References

- 1-Newton-Foot M, Snyman Y, Maloba MR, Whitelaw AC. Plasmid-mediated *mcr-1* colistin resistance in *Escherichia coli* and *Klebsiella spp.* clinical isolates from the Western Cape region of South Africa. *Antimicrobial Resistance & Infection Control* 2017; 6(1):78.
- 2-Olaitan A, Morand S, Rolain J. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Frontiers in Microbiology* 2014; 5: 643.

- 3-Srijan A, Margulieux KR, Ruekit S, Snesrud E, Maybank R, Serichantalergs O, et al. Genomic characterization of nonclonal *mcr-1*-positive multidrug-resistant *Klebsiella pneumoniae* from clinical samples in Thailand. *Microbial Drug Resistance* 2018; 24(4): 403-10.
- 4-Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, et al. The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. *Nature Communications* 2018; 9(1): 1179.
- 5-Elnahriry S, Khalifa H, Soliman A, Ahmed A, Hussein A, Shimamoto T. Emergence of plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate from Egypt. *Antimicrobial Agents and Chemotherapy* 2016; 60(5): 3249-3250.
- 6-Xavier BB, Lammens C, Ruhai R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *EuroSurveillance Monthly* 2016; 21(27): 30280.
- 7-Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBio* 2017; 8(3): 00543-17.
- 8-Caniaux I, Van Belkum A, Zambardi G, Poirel L, Gros MF. MCR: modern colistin resistance. *European Journal of Clinical Microbiology & Infectious Diseases* 2017; 36(3): 415-20.
- 9-European Committee on Antimicrobial Susceptibility Testing (EUCAST). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. European Committee on Antimicrobial Susceptibility Testing. 2016.

- 10-**Holt J, Kieg N, Sneath P, Staley J.** Bergey Manual of Determinative Bacteriology, Williams and Wilkins publishers, Baltimore. 1994.
- 11-**Mahon C, Lehman D, Manuselis G.** Textbook of diagnostic microbiology. 5<sup>th</sup> edition. Missouri: Saunders- Elsevier 2014; 420-454.
- 12-**Clinical and Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Clinical and Laboratory Standards Institute, Wayne. 2018
- 13-**Clinical and Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Disk Susceptibility Testing. Approved Standard M02-A11. Clinical and Laboratory Standards Institute, Wayne. 2012.
- 14-**Cavaco L, Mordhorst H and Hendriksen R.** PCR for plasmid-mediated colistin resistance genes, *mcr-1* and *mcr-2* (multiplex). protocol optimized at National Food Institute, Denmark. 2016; 2.
- 15-**El-Sweify M, Gomaa N, El-Maraghy N, Mohamed H.** Phenotypic Detection of Carbapenem Resistance among *Klebsiella pneumoniae* in Suez Canal University Hospitals, Ismailiya, Egypt. International Journal of Current Microbiology and Applied Sciences 2015; 4: 10-18.
- 16-**Melake N, Mahmoud A, Elraghy N, Labib A, Hassan D, Elbrolosy AM.** Detection of *Klebsiella pneumoniae* carbapenemases and metallo- $\beta$ -lactamases among *Klebsiella pneumoniae* isolates from hospitalized patients at Menoufia University Hospitals, Egypt. Menoufia Medical Journal 2016; 29(4): 801.
- 17-**Amer W, Khalil H, Abd EL Wahab M.** Risk factors, phenotypic and genotypic characterization of carbapenem resistant *Enterobacteriaceae* in Tanta University Hospitals, Egypt. International Journal of Infection Control 2016; 12(2): 2.
- 18-**Khare V, Gupta P, Haider F, Begum R.** Study on MICs of tigecycline in clinical isolates of carbapenem resistant *Enterobacteriaceae* (CRE) at a tertiary care centre in North India. Journal of Clinical and Diagnosis Research 2017; 11(3): 18-21.
- 19-**Okoche D, Asiimwe B, Katabazi F, Kato L, Najjuka CF.** Prevalence and characterization of carbapenem-resistant *Enterobacteriaceae* isolated from Mulago National Referral Hospital, Uganda. PLoS One 2015; 10(8): 013574.
- 20-**Khalifa H, Soliman A, Ahmed A, Shimamoto T, Hara T, Ikeda M, et al.** High carbapenem resistance in clinical Gram-negative pathogens isolated in Egypt. Microbial Drug Resistance 2017; 23(7): 838-844.
- 21-**Saeed N, Alkhawaja S, Azam N, Alaradi K, Al-Biltagi M.** Epidemiology of carbapenem-resistant *Enterobacteriaceae* in a Tertiary Care Center in the Kingdom of Bahrain. Journal of Laboratory Physicians 2019; 11(2): 111.
- 22-**Lin D, Chen J, Yang Y, Cheng J, Sun C.** Epidemiological study of carbapenem-resistant *Klebsiella pneumoniae*. Open Medicine 2018; 13(1): 460-466.
- 23-**Moemen D, Masallat D.** Prevalence and characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from intensive care units of Mansoura University hospitals. Egyptian Journal of Basic and Applied Sciences 2017; 4(1): 37-41.
- 24-**Shawky S, Abdallah A, Khouly M.** Antimicrobial activity of colistin and tigecycline against carbapenem-resistant *Klebsiella pneumoniae* clinical isolates in Alexandria, Egypt. International Journal of

- Current Microbiology and Applied Sciences 2015; 4: 731-742.
- 25-**Laolerd W, Akeda Y, Preeyanon L, Ratthawongjirakul P, Santanirand P.** Carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* from Bangkok, Thailand, and their detection by the Carba NP and modified carbapenem inactivation method tests. *Microbial Drug Resistance* 2018; 24(7): 1006-1011.
- 26-**Qadri R, Ahmed S, Kakru D, Bashir G, Fomda B.** Detection of carbapenem resistant *Enterobacteriaceae* and the comparison between phenotypic methods and multiplex PCR. *Indian Journal of Microbiology Research* 2019; 6(2): 126-130.
- 27-**Kandeel A.** Epidemiology of carbapenemase producing *Enterobacteriaceae* in a general hospital. *Journal of Microbiology and Infectious Diseases* 2015; 5(2): 57-62.
- 28-**Benmahmod A, Said H, Ibrahim R.** Prevalence and mechanisms of carbapenem resistance among *Acinetobacter baumannii* clinical isolates in Egypt. *Microbial Drug Resistance* 2018; 25(4): 480-488.
- 29-**Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M, et al.** High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. *Clinical Microbiology and Infection* 2013; 19(1): 23-30.
- 30-**Rojas L, Salim M, Cober E, Richter S, Perez F, Salata RA, et al.** Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: laboratory detection and impact on mortality. *Clinical Infectious Diseases* 2016; 64(6): 711-718.
- 31-**Bhaskar B, Mulki S, Joshi S, Adhikari R, Venkatesh B.** Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae* strains. *Asian Journal of Pharmaceutical and Clinical Research* 2017; 10(9): 70-73.
- 32-**Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, et al.** Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Eurosurveillance* 2014; 19(42): 20939.
- 33-**Moosavian M, Emam N.** The first report of emerging mobilized colistin-resistance (*mcr*) genes and ERIC-PCR typing in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in southwest Iran. *Infection and Drug Resistance* 2019; 12:1001.
- 34-**Zaki M, ElKheir N, Mofreh M.** Molecular study of colistin resistant clinical isolates of *Enterobacteriaceae* species. *Journal of Clinical Molecular Medicine* 2018; 1(1): 1-4.
- 35-**Zhang J, Chen L, Wang J, Butaye P, Huang, K, Qiu H, et al.** Molecular detection of colistin resistance genes (*mcr-1* to *mcr-5*) in human vaginal swabs. *BMC Research Notes* 2018; 11(1): 143.
- 36-**Jayol A, Nordmann P, Lehours P, Poirel L, Dubois V.** Comparison of methods for detection of plasmid-mediated and chromosomally encoded colistin resistance in *Enterobacteriaceae*. *Clinical Microbiology and Infection* 2018; 24(2): 175-179.