

First Report of Colistin Resistance Gene *mcr-1* in Carbapenem-Resistant Clinical Isolates of *Klebsiella Pneumoniae* in Iraq

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Abstract

Background: The prevalence of the plasmid-borne colistin resistance gene *mcr-1* in bacteria poses a potential threat to patient treatment, particularly when hospitalized spreading of this gene causes great concern as it can transmit between different bacteria species. The aims of this study were to investigate the presence of the *mcr-1* gene among carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates from different clinical specimens and determine the clonal origin of strains carrying the *mcr-1* gene using Multi-locus sequence typing (MLST) method.

Method: In this study, 22 CRKP isolates from clinical specimens collected from the major four hospitals in Najaf/Iraq were examined. All isolates were identified by a standard biochemical test and confirmed by an automated Vitek®2 system. Antimicrobial susceptibility test was done on 12 antibiotics by the disk diffusion method. All isolates tested to detect the presence of the *mcr-1* gene using the PCR method. Determine the sequence typing by MLST for all *mcr-1*-positive CRKP isolates.

Results: Out of 147 *K.pneumoniae*, 22 carbapenem resistance isolates from different clinical specimens were detected. Antibiotic sensitivity test results revealed that all isolates (100 %) were resistant to ampicillin, Cefepime, Cefoxitin, Ceftazidime, and Ceftriaxone; however, most of the CRKP isolates (86.4 %) are sensitive to colistin. The *mcr-1* gene was found in three (13.6%) of the 22 isolates of CRKP. These three isolates are resistant to all classes of antibiotics. The MLST results revealed that three *mcr-1*-positive CRKP isolates were related to three different sequence types: ST147, ST1, and ST11.

Conclusion: The spread of CRKP isolates containing plasmid-borne *mcr-1* gene is worth our attention due to the consider of colistin as the last resort treatment against drug-resistant pathogens that increasingly identified in Najaf Hospitals/Iraq.

Keywords: Carbapenem-Resistance *K.pneumoniae*, Colistin, *mcr-1*, MLST, CRKP.

Introduction

Enterobacteriaceae, especially *K.pneumoniae* is considered to be the major organisms that cause

nosocomial infection^(1,2). *K.pneumoniae* causes some important infections, including bacteremia, neonatal meningitis, urinary tract infections as well as wound and soft tissue in developing countries⁽³⁾. Carbapenem antibiotics play an important role in the treatment of severe nosocomial infections caused by multidrug-resistant organisms *Enterobacteriaceae*⁽⁴⁾. Increased use of carbapenems leads to the activation of resistance genes against these antibiotics, resulting in their resistance⁽⁵⁾. Carbapenem-resistant *K.pneumoniae* (CRKP) infections remain a significant morbidity and mortality concern

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worldwide⁽⁶⁾. Colistin is now commonly used as a last resort antibiotic in the treatment of carbapenem-resistant *K.pneumoniae*⁽⁷⁾. Colistin is a cationic peptide which binds to bacterial lipopolysaccharide (LPS) leading to the leakage of intracellular components from the cell membrane⁽⁸⁾. However, increasing the use of colistin antibiotics has resulted in the emergence of colistin resistance in carbapenem resistance *K.pneumoniae* infection, and resistance rates are steadily increasing⁽⁹⁾. The common mechanism of colistin resistance is thought to change the two-component regulatory systems of PmrAB or PhoPQ due to chromosome-mediated mutations⁽¹⁰⁾. Recently, Liu *et al.*, (2016)⁽¹¹⁾ reported the emergence of plasmid-mediated colistin resistance gene *mcr-1* from clinical *E. coli* and *K.pneumoniae* isolates in China. Over a short period time, the *mcr-1* gene was recorded in a range of gram-negative bacterial isolates worldwide, indicating suggesting probable horizontal transmission of colistin resistance^(11,12). Many plasmids were involved in the spread of *mcr-1*, such as IncHI2 and IncI2⁽¹³⁾. Plasmid-mediated colistin resistance *mcr-1* was observed in clinical isolates of *K. Pneumoniae* in different countries including⁽¹⁴⁻¹⁷⁾. The aim of this study was to investigate the prevalence of the colistin resistance gene *mcr-1* in carbapenem resistance *K. pneumoniae* (CRKP) isolates from different clinical specimens in Najaf hospitals/Iraq and to determine the clonal origin of CRKP carrying the *mcr-1* gene.

Materials and Method

Bacterial Strains: 22 carbapenem-resistant *K. pneumoniae* isolates collected from different clinical specimens in Najaf hospitals/Iraq during the period from February to September 2018 were studied. Bacterial isolates were identified to the level of species by using the standard biochemical tests according to method described by^(18,19), confirmatory identification was carried out by VITEK ®2 system according to manufacturer's instructions.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing of carbapenem-resistant *K.pneumoniae* isolates performed on Mueller-Hinton agar plates using the Kirby-Bauer disk diffusion method. The isolates tested against the following antibiotics: Ampicillin (10µg), Cefazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Imipenem (10 µg), Meropenem (10 µg), Gentamicin (10 µg), Amikacin (30µg), Levofloxacin (5µg), Colistin (10µg) and Ofloxacin (5µg). The plates were incubated

at 37°C for 18 hours under aerobic conditions and inhibition zones diameter was measured and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2019)⁽²⁰⁾. *E. Coli* ATCC 25922 used as standard isolate in this test .

Molecular detection of the *mcr-1* gene : DNA extraction and purification from fresh bacterial colonies using the ABC DNA Isolation Kit (Qiagen, Candia). Polymerase chain reaction (PCR) was done to investigate the presence of *mcr-1* gene in all CRKP isolates. The primers for the PCR amplification of *mcr-1* gene were as follows:

forward primer (5'-GTGTGGTACCGACGCTCGG-3')

reverse primer (5'-CAAGCCCAATCGGCGCATC-3').

A total of 34 cycles were conducted as follows: 94°C pre denaturation for 5 minutes, 94°C denaturation for 20 seconds, 50°C annealed 20 seconds and 72°C extension for 30 seconds⁽²¹⁾. The PCR products analyzed by 1.5% agarose gel electrophoresis. The PCR amplicon size was 413 bp.

Multi-locus Sequence Type (MLST): Multi-locus sequence typing conducted on the three *mcr-1*-positive CRKP isolates. Specific primers were used to amplify fragments of these seven housekeeping genes in *K. Pneumoniae*, including (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) as described by Diancourt *et al.* (2005)⁽²²⁾. The PCR product were send for Sanger sequencing method using ABI3730XL, DNA sequences automated by Macrogen Corporation – Korea. Allelic profiles and sequence types (STs) were determined according to MLST databases website (<https://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumonia.html>).

Results

A retrospective study was conducted on 147 *K. pneumoniae* randomly collected from different clinical specimens in Najaf hospitals to identify carbapenem-resistant isolates by using the Kirby-Bauer disk diffusion method. In the present study, 22 isolates were identified as carbapenem-resistant *K.pneumoniae*. The distribution of carbapenem-resistant *K.pneumoniae* isolates according to source specimens was as follows: burn, 36.3% (n=8); urine, 31.8% (n=7); wound, 27.3% (n=6); and cerebrospinal fluid, 4.6% (n=1). It is important to mention that most of the isolates were collected from the burn specimens (Table-1).

Table (1): Distribution of Carbapenem resistant *K. pneumoniae* isolates obtained from different clinical specimens.

Clinical Sample	No. (%) of CRKP
Burn	8(36.3%)
Urine	7(31.8%)
Wound	6(27.3%)
CSF	1(4.6%)
Total	22 (100%)

CSF: cerebrospinal fluid: Based on the results of the antimicrobial susceptibility tests, all CRKP isolates (100%) showed resistance to ampicillin, Cefepime, Cefoxitin, Ceftazidime, and Ceftriaxone. However, the lowest resistance rate was observed for imipenem (50%) and colistin (13.6%). Antibiotics resistance patterns of carbapenem resistance *K. pneumoniae* isolates that presented in Figure (1). The results show that 19 (86.4%) of the isolates categorized as XDR phenotype, while 3 (13.6%) of isolates were resistant to all antibiotics tested categorized as PDR phenotype..

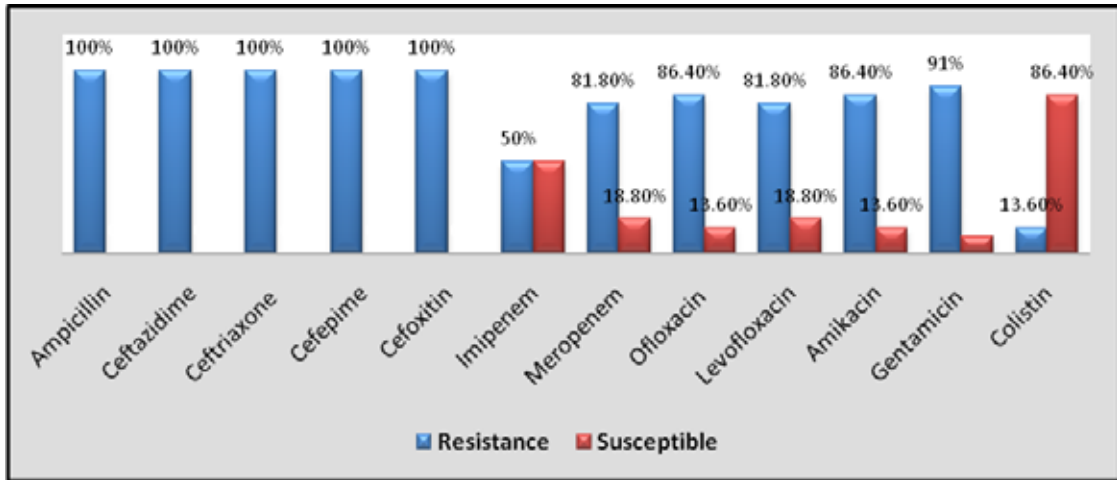


Figure (1): Antibiotic susceptibility patterns of carbapenem-resistant *K. pneumoniae* isolates .

Based on the PCR results, 3(13.6%) of carbapenem-resistant *K. pneumoniae* isolates harbor the *mcr-1* gene (Figure 2). The clinical significance of the three *mcr-1*-positive carbapenem-resistant *K. pneumoniae* isolates is summarized in Table (2). All the isolates recovered from female patients. Two of the isolates (KP4 and KP7 isolates) were identified in wound infection and one (KP19 isolate) from burn infection. All of the patients had received prior broad-spectrum antibiotics. However, none of the patients had previously received

polymyxin B or colistin. All the three *mcr-1*-positive carbapenem-resistant *K. pneumoniae*-related infections were classified as nosocomial infections (obtained from inpatients). The three isolates show resistant to all the 12 antibiotics tested, which means they could be categorized as pan-drug resistance agents. The MLST results showed that the three *mcr-1* positive isolates were assigned to three different Sequence Typing STs: ST147, ST15, and ST11.



Figure (2): Gel electrophoresis of PCR amplification has with *mcr-1* (413 bp) primer for CRKP extracted DNA. The electrophoresis was carried out for 80 min at 70 volts. M: DNA molecular marker; Lane (4 and 7) positive *mcr-1* gene.

Table (2): Clinical characteristics of colistin-resistant isolates among carbapenem-resistant *K. pneumoniae* carried the *mcr-1* gene (n= 3).

Isolate symbol	Source	Patient gender	Patient age	Hospital	Hospitalization	Type of multiple resistance	Sequence Types (STs)
KP4	Wound	Female	55 years	Al-Sader	Inpatient	Pan-drug resistance	147
KP7	Wound		36 years	Medical City			15
KP19	Burn		46 years	City			11

Discussions

In our study, 22 carbapenem-resistant *K. pneumoniae* isolates were collected from different clinical specimens in teaching hospitals in Najaf/Iraq. The results show that the prevalence rate of carbapenem-resistant *K. pneumoniae* isolates in burns was higher than that of other specimens.

In this study, the carbapenem-resistant isolates were fully resistant to the beta-lactam antibiotics as ampicillin, Cefepime, Cefoxitin, Ceftazidime, and Ceftriaxone. Half of these isolates had resistance to imipenem antibiotic. However, there was less resistance to colistin (13.6%).

Several reports documented the association of resistance to carbapenem and resistance to 3rd cephalosporins with colistin resistance among various *Enterobacteriaceae* species⁽²³⁾. It is, therefore, necessary to study the incidence of colistin resistance in isolates that produce extended-spectrum β -lactamase (ESBL) resistance⁽²⁴⁾.

The PCR result for detect *mcr-1* genes shown that all colistin-resistant *K. pneumoniae* isolates harbored the *mcr-1* gene. Hence, the prevalence of *mcr-1* among 22 carbapenem resistant *K. pneumoniae* in this investigation was 13.6% (Figure 2). This result in the present study agreement with Rolain *et al.*, (2016)⁽¹⁴⁾ that found 12.5% of *K. pneumoniae* isolates in Laos carried *mcr-1* gene. The prevalence of *mcr-1* gene in *K. pneumoniae* isolates is still rare in Europe and other countries⁽²⁵⁾. This is the first report on the dissemination of the *mcr-1* gene in carbapenem-resistant *K. pneumoniae* isolated from clinical specimens in Iraq and this result provides added insight into the mechanism of colistin resistance among *K. pneumoniae* isolates in Najaf hospitals. The main problematic for clinicians is the transfer of *mcr-1* to carbapenemase producers in a hospital environment, which could result in the production of XDR and even PDR isolates⁽²⁶⁾. Based on the study results, all *mcr-1* positive isolates exhibited a possible PDR phenotype.

Unfortunately, all these isolates were obtained from hospitalized patients with wound (KP4 and KP7 isolates) and burn (KP19 isolate) infections in Al-Sader Medical City. The occurrence of PDR resistance in Al-Sader Medical City in Najaf, a governorate with low restrictions on antibiotics admission and high burden of infectious diseases and is extremely alarming, due to the possibility of PDR transmission to other bacterial isolates and the development of impossible treated infections, which may be related with increase mortality rates. Furthermore, the present of this gene in Al-Sader Medical City, with no evident association to previously described MCR-1-producers, confirms their global emergence of *mcr-1*-harboring *K. pneumoniae* isolates. Appropriate control measures for the use of colistin in Iraqi hospitals and effective diagnosis of *mcr-1* gene in bacterial isolates, are essential to prevent future widespread of colistin resistant isolates in Iraq.

The result shows that all three *mcr-1*-positive carbapenem-resistant *K. pneumoniae* isolates recovered from female patients (Table 2). In this study, two *mcr-1*-positive carbapenem-resistant *K. pneumoniae* isolates from wound infection. *mcr-1*-positive *K. pneumoniae* isolates from wound infection have been shown in a recent report by Moosavian and Emam (2019)⁽²⁷⁾ in southwest Iran.

In the present study, the high genetic diversity among *mcr-1*-carrying isolates (KP4, KP7, KP19) where they belong to ST147, ST15, and ST11, respectively, suggesting that the distribution of the *mcr-1* gene is so far not primarily associated with any specific clonal lineage.

ST147, identified in this study in female patient with wound infection that recognized as PDR isolate. Pragasam *et al.*, (2016)⁽²⁸⁾ show (1/8) colistin-resistant *K. pneumoniae* clinical isolates belonging to ST147 isolated from patients in South India between 2013 and 2015. In another study in Tunisian by Jaidane *et al.*, (2018)⁽²⁹⁾ was recognized as one isolate of ST147 among colistin-resistant *K. pneumoniae*.

In the study, ST15 was recovered from female wound infections. ST15 defined as a globally successful international clone, which recently show associated with colistin-resistant infections⁽³⁰⁾.

Conclusion

The spread of CRKP isolates containing the plasmid-borne *mcr-1* gene is worth our attention due to their use as the last treatment line against extensively drug-resistant pathogens that increasingly recognized in Najaf hospitals/Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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