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Colistin Susceptibility among Carbapenem Resistant *Klebsiella pneumoniae* Isolated from Menoufia University Hospitals

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ABSTRACT

Background: Infections by CRKp represents a major health problem. Carbapenemases have been frequently reported in CRKp. Limited antimicrobials are still effective for CRKp like tigecycline, ceftazidime/avibactam, as well, polymyxins including colistin. With the increased use of colistin, Col R *k. pneumoniae* carbapenemase (KpC)-producing isolates are documented worldwide.

Objectives: The aim of the current study is to investigate carbapenemases and to determine colistin susceptibility and mcr genes among CRKp.

Material and methods: *k. pneumoniae* were identified by conventional methods and API20E. Carbapenem and colistin resistance were screened by disc diffusion method. Confirmatory combined mCIM/eCIM testing was used for carbapenemases detection. BMD and CBDE tests were used for colistin susceptibility detection. Multiplex PCRs were done for carbapenemases genes (*bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA48}}$, *bla*$_{\text{IMP}}$ *bla*$_{\text{VIM}}$, *bla*$_{\text{KPC}}$) and colistin resistance genes (*mcr*1, 2, 3, 4 and 5 genes) detection.

Results: Out of CRKp, 68.9% were positive carbapenemases by mCIM/eCIM testing, 33.3% of them were MBLs and 66.7% were serine carbapenemases. the most frequent carbapenemases genes were *bla*$_{\text{KPC}}$ and *bla*$_{\text{OXA-48}}$. About 16.3% of CRKp were colistin resistant by BMD test. There was fair agreement of CBDE test in relation to BMD for colistin susceptibility detection. The *mcr1* gene was detected in 9.1% of CRKp.

Conclusions: The increase detection of carbapenemases and colistin resistance among *k. pneumoniae* isolates obligate us for efficient use of prevention and control protocols to decrease the multidrug resistance in hospital and community environments.

Keywords: Carbapenemases, Colistin, BMD, CBDE and MCR, Experimental study, Menoufia University.

INTRODUCTION

Infections by carbapenem-resistant *Klebsiella pneumoniae* (CRKp) that usually shows multidrug resistance pattern to all β-lactams, fluoroquinolones, and aminoglycosides stay a major health threat globally[1,2].

The carbapenem resistance is mainly due to reduced expression and/or mutation of porins that allow carbapenem entry into the bacterial cells[3]. Moreover, the presence of carbapenemase genes on conjugative plasmids is correlated with the high incidence of carbapenem resistance[4].

Carbapenemases are either non-metallo-β-lactamases (serine carbapenemases, classes A and D) and metallo-β-lactamases (MBL, class B)[3]. MBL genes such as *bla*$_{\text{NDM}}$, *bla*$_{IMP}$, *bla*$_{\text{VIM}}$, and non-metallo-carbapenemase genes like *bla*$_{\text{OXA}}$ (class D) and *bla*$_{\text{KPC}}$ (class A), have been frequently reported in carbapenem-resistant *Klebsiella pneumoniae* (CRKp)[5].

Some limited antimicrobials are still effective for CRKp like tigecycline, and the newly approved ceftazidime/avibactam[6]. As well, polymyxins including colistin are a valuable therapeutic choice that can bind lipopolysaccharides and disrupt the outer membrane[2,6].

Although in 1970s, polymixins were largely neglected because of their toxicity. These cationic antimicrobial peptides have come back as a "final-resort" alternative therapy for numerous multidrug-resistant organisms[7]. In critical cases caused by CRKp, colistin with other antibiotics including tigecycline, meropenem, gentamicin, or fosfomycin can manage them properly[8]. With the increased use of colistin, colistin-resistant *Klebsiella pneumoniae* carbapenemase (KpC)- producing isolates are documented worldwide[8]. Which were initially considered to be chromosomally mediated only until Liu et al., 2016[9] stated the development of the first plasmid-mediated polymyxin resistance mechanism, *mcr-1* in enterobacteriaceae[2]. The occurrence of colistin-resistance (ColR) in CRKp generates a warning alarm for both clinicians and patients to return them to the pre-antibiotic era[8].

This study has aimed to investigate carbapenemases and determine colistin susceptibility and *mcr* genes among carbapenem resistant *Klebsiella pneumoniae* isolated from Menoufia University Hospitals.

MATERIAL AND METHODS

Collection of Samples:

Out of 984 patients admitted to different wards of Menoufia University Hospitals during the period of February 2021 to July 2022, 334 (33.9%) *Klebsiella pneumoniae* C.I.s were isolated as one isolate per patient in the Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University.

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Identification of the isolates: *Klebsiella pneumoniae* isolates were identified by their culture characteristics on MacConkey's agars, microscopic examination, conventional biochemical reactions, and API20E[10].

Antibiotic susceptibility testing:

The antibiotic susceptibility testing was done using Kirby-Bauer modified disc diffusion technique on Mueller-Hinton agar (Oxoid, UK) using commercially available discs (Oxoid, Basingstoke, UK) according to the CLSI guidelines[11,12].

Confirmatory carbapenemases detection by combined Modified Carbapenem Inactivation (mCIM) and EDTA Carbapenem Inactivation Method (eCIM):

The *Klebsiella pneumoniae* isolates showing resistance to at least one of four carbapenems (imipenem, meropenem, doripenem and ertapenem) were further phenotypically screened for carbapenemase production by combined mCIM/ eCIM testing[3,12].

Phenotypic detection of colistin susceptibility by Broth Microdilution (BMD) and Colistin broth disk elution (CBDE) methods:

Broth Microdilution Stock solutions of colistin from colistin sulphate powder (Sigma-Aldrich, St. Louis, MO) were reconstituted before use in sterile distilled water according to the manufacturer's instructions. Dilution methods were performed according to CLSI procedure[13].

A concentration of 0.5 MacFarland of the inoculum was prepared in Brain heart infusion broth and colistin was incorporated in the media in concentration range 0.25- 8 ug/ml, in a double fold dilution range. Colistin MIC of ≤ 2 µg /mL was considered intermediate, whereas MIC of ≥ 4 µg /mL was considered resistant[12,13,14]. Colistin broth disk elution method was done as previously described[15].

Molecular study

Detection of carbapenemases-genes:

Bacterial DNA was extracted as described before[16]. Two multiplex PCRs were done for detection of carbapenemases, (*bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA48}}$), and (*bla*$_{\text{IMP}}$ *bla*$_{\text{VIM}}$). A uniplex PCR was done for *bla*$_{\text{KPC}}$ detection using the primers in table 1 as described before[17,18].

Detection of colistin resistance genes by multiplex PCR:

The *Klebsiella pneumoniae* CIs. were tested for the presence of colistin resistance genes, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* by multiplex PCR according to ECDC protocol with PCR conditions: 33 cycles of 95 °C × 3 min, 65°C × 30 s, 72°C × 1 min, followed by 1 cycle of 72°C × 10 min using the primers in *Table 1*[12].

Table (1): Primers used in the study.

Gene	Primer Sequence (5′ –3′)	Amplicon size (bp)	T_m[b]	Reference Number
bla$_{\text{KPC}}$	F: 5'-CTGTCTTGTCTCTCATGGCC-3' R: 5'-CCTCGCTGTRCTTGTCATCC-3'	796	60	**17**
bla$_{\text{NDM-1}}$	F: 5'-TGGCAGCACACTTCCTATC-3' R: 5'-AGATTGCCGAGCGACTTG-3'	448	85	**17**
bla$_{\text{OXA-48}}$	F: 5'-TTGGTGGCATCGATTATCGG-3' R: 5'-GAGCACTTCTTTTGTGATGGC-3'	744	58	**17**
bla$_{\text{IMP}}$	F: 5'-CATGGTTTGGTGGTTCTTGT-3' R: 5'-ATAATTTGGCGGACTTTGGC-3'	488	55	**18**
bla$_{\text{VIM}}$	F: 5'-AGTGGTGAGTATCCGACAG-3' R: 5'-TCAATCTCCGCGAGAAG-3'	212	52	**17**
mcr1	F: 5'-AGTCCGTTTGTTCTTGTGGC-3' R: 5'-AGATCCTTGGTCTCGGCTTG-3'	320	58	**12**
mcr2	F: 5'-CAAGTGTGTTGGTCGCAGTT-3' R: 5'-TCTAGCCCGACAAGCATACC-3'	715	58	**12**
mcr3	F: 5'-AAATAAAAATTGTTCCGCTTATG-3' R: 5'-AATGGAGATCCCCGTTTTT-3'	929	58	**12**
mcr4	F: 5'-TCACTTTCATCACTGCGTTG-3' R: 5'-TTGGTCCATGACTACCAATG-3'	1116	58	**12**
mcr5	F: 5'-ATGCGGTTGTCTGCATTTATC-3' R: 5'-TCATTGTGGTTGTCCTTTTCTG-3'	1644	58	**12**

Ethical approval:

The study protocol was approved by the Ethical Committee of Human Right of Research at Menoufia University (IRB approval number and date 102022 MICR 23). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans and human specimens.

Statistical analysis

The data were collected, tabulated, and analysed using SPSS (Statistical Package for Social Science) version 20.0 (SPSS Inc., Chicago, IL, USA). Categorical data were described as frequency and percentage and compared between the two studied groups using Chi-square test (χ^2) and Z test (the test of proportion), sensitivity (true positive rate), specificity (true negative rate), positive predictive value, negative predictive value and accuracy were calculated for evaluation of screening tests. Kappa agreement testing was used to assess agreement between two test results. P-value was set at $\leq$0.05 for significant results.

RESULTS

Out of 984 patients admitted to different wards of Menoufia University Hospitals during the period of February 2021 to July 2022, 334 (33.9%) *Klebsiella pneumoniae* C.I.s were isolated as one isolate per patient where 230 (68.8%) isolated from male and 104 (31.2%) from female patients of all age groups.

By disc diffusion method, 135 (40.4%) of *Klebsiella pneumoniae* C.I.s that were resistant to any of carbapenems used (imipenem (10µg), ertapenem (10µg), meropenem (10µg) and doripenem (10µg)) were considered CRKp. That phenotypically confirmed for carbapenemases production by combined mCIM and eCIM test, where 93 (68.9%) were positive mCIM indicating positive carbapenemases (CPKp) and 42 (31.1%) were negative mCIM indicating negative carbapenemases as represented in ***Table 2*** *and* ***Figure 1***. In our study, 85 (62.9%) of CRKp (135) had carbapenemase genes, where 35(25.9%), 25(18.5%), 2(1.5%), 7(5.2%), 8(5.9%) and 8(5.9%) had *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{KPC, OXA-48,}$ *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ respectively as represented in ***Table 2***.

Table (2): Phenotypic and molecular detection of carbapenemases among Carbapenem resistant *Klebsiella pneumoniae*

CRKp (N=135)	Phenotypic carbapenemases testing				Carbapenemases genes 85 (62.9%)
	mCIM		eCIM		
IPM, ETP, MEM & DOP	+ve	-ve	+ve	-ve	
35 (25.9%)	35 (100%)	0 (0.0%)	0 (0.0%)	35 (100%)	*bla*$_{KPC}$ 35 (25.9%)
25 (18.5%)	25 (100%)	0 (0.0%)	0 (0.0%)	25 (100%)	*bla*$_{OXA-48}$ 25 (18.5%)
2 (1.5%)	2 (100%)	0 (0.0%)	0 (0.0%)	2 (100%)	*bla*$_{KPC}$/ *bla*$_{OXA48}$ 2 (1.5%)
7 (5.2%)	7 (100%)	0 (0.0%)	7 (100%)	0 (0.0%)	*bla*$_{NDM-1}$ 7 (5.2%)
8 (5.9%)	8 (100%)	0 (0.0%)	8 (100%)	0 (0.0%)	*bla*$_{IMP}$ 8 (5.9%)
8 (5.9%)	8 (100%	0 (0.0%)	8 (100%)	0 (0.0%)	*bla*$_{VIM}$ 8 (5.9%)
50 (37.0%)	8 (16.0%)	42 (84.0%)	8 (16.0%)	42 (84.0%)	Negative 50 (37.1%)
Total 135	93 (68.9%)	42 (31.1%)	31 (22.9%)	104 (77.0%)	Total 135

IPM, imipenem; ETP, ertapenem; MEM, meropenem; DOP, doripenem.

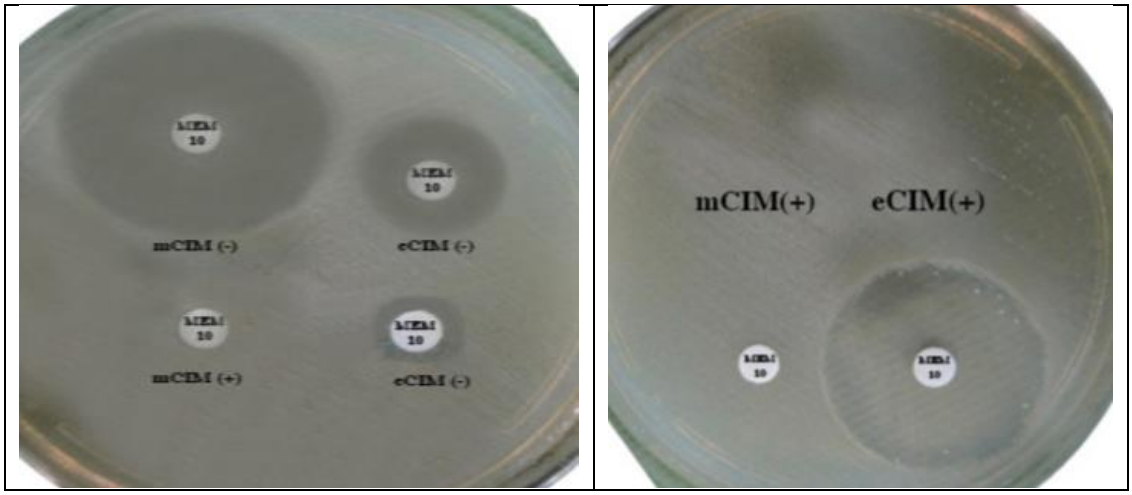

Fig. 1: **Phenotypic carbapenemases detection by combined mCIM/eCIM test.**

Out of the 93 KpCIs that showed positive mCIM test, 31(33.3%) showed positive eCIM testing indicating positive Metallo-β-lactamases (MBL), and 62(66.7%) of them showed negative eCIM testing indicating positive serine carbapenemases. In positive MBL KpCIs, 7(22.6%), 8(25.8%) and 8 (25.8%) had $bla_{\text{NDM-1}}$, bla_{IMP} and bla_{VIM} respectively with 8 (25.8%) were negative for carbapenemase genes. While in serine carbapenemases positive KpCIs, 35(56.5%), 25(40.3%) and 2(3.2%) had bla_{KPC}, $bla_{\text{OXA-48}}$ and $bla_{\text{KPC, OXA-48}}$ respectively as shown in ***Table 3***.

There was fair agreement of combined mCIM/eCIM testing in relation to genotying regarding total carbapenemases detection (K=0.87), serine carbapenemase (K=1) and MBLs (K=0.81). Where the sensitivity, specificity, PPV, NPV and accuracy were as follow 100%, 84.0%, 91.4%, 100% and 94.1% respectively for total carbapenemases detection. And 100% for all items regarding serine carbapenemases detection. For MBLs, the sensitivity, specificity, PPV, NPV and accuracy were as follow, 100%, 92.9%, 74.2, 100% and 94.1% as shown in ***Table 3*.**

Table (3): Combined mCIM/ eCIM testing in relation to carbapenemases genes among CRKp

CRKp (n=135)		**Carbapenemase genes**						
		Positive (n=85) (62.9%)						**Negative (n=50) (37.1%)**
Combined mCIM/ eCIM		**blaKPC (n=35)**	**BlaOXA-48 (n=25)**	**blaKPC blaOXA-48 (n=2)**	**blaNDM-1 (n=7)**	**blaIMP (n=8)**	**blaVIM (n=8)**	
Positive (n=93) (68.9%)	**Metallo-β-lactamases (MBLs) (+ve mCIM, +ve eCIM) (n=31) (33.3%)**	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (22.6%)	8 (25.8%)	8 (25.8%)	8 (25.8%)
	Serine carbapenemases (+ve mCIM, -ve eCIM) (n=62) (66.7%)	35 (56.5%)	25 (40.3%)	2 (3.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Negative carbapenemase -ve mCIM, -ve eCIM (n=42) (31.1%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	42 (100%)
Combined mCIM+eCIM		**Carbapenemase genes**						Total
		+ve			**-ve**			
Carbapenemases								
+ve		85 (100%)			8 (16.0%)			93 (68.9%)
-ve		0 (0.0%)			42 (84.0%)			42 (31.1%)
Total		85			50			135
Kappa = 0.87, P value <0.001, Sensitivity: (100%), Specificity: (84.0%), PPV: (91.4%), NPV: (100%), Accuracy (94.1%)								
Metallo-β-lactamases (MBL)								
+ve		23 (92.0)			8 (7.3)			31(22.9%)
-ve		2 (8.0)			102 (92.7)			104(77.1%)
Total		25			110			135
Kappa = 0.81, P value <0.001, Sensitivity: (100%), Specificity: (92.9%), PPV: (74.2%), NPV: (100%), Accuracy: (94.1%)								
Serine carbapenemases								
+ve		62 (100%)			0 (0.0%)			62 (45.9%)
-ve		0 (0.0%)			73 (100%)			73 (54.1%)
Total		62			73			135
*Kappa =1.0, P value <0.001, Sensitivity: (100%), Specificity: (100%), PPV: (100%), NPV: (100%), Accuracy (100%)								

*Kappa between 0.81 and 1.00: almost perfect agreement. NPV: negative predictive value. PPV: positive predictive value.

Our results showed that, by BMD method, 113 (83.7%) were colistin susceptible and 22(16.3%) were colistin resistant among the 135 CRKp with highly significant difference (P <0.001) between them either in carbapenemases negative or carbapenemases positive *Klebsiella pneumoniae* as shown in ***Table 4*.**

Table (4): Colistin susceptibility by BMD in relation to carbapenemases production in CRKp isolates.

Combined mCIM/ eCIM	Colistin by *BMD		Total	Z test	P value
	Susceptible MIC ≤2µg/mL	Resistant MIC ≥ 4µg/mL			
Carbapenemase negative Kp.	**37 (88.0%)**	**5 (12%)**	**42 (31.1%)**	**6.76**	**<0.001**
Carbapenemase positive Kp.	**76 (81.7%)**	**17 (18.3%)**	**93 (68.9%)**	**8.51**	**<0.001**
Total	**113 (83.7%)**	**22 (16.3%)**	**135**	**10.95**	**<0.001**

*BMD: broth microdilution test.

Our study demonstrated that fosfomycin was the most effective antibiotic against CPKp (93.5%) followed by tigecycline (92.5%), gentamicin (90.3%), trimethoprim-sulfamethoxazole (81.7%), colistin (81.7%) and then β-lactam/β-lactamase inhibitors (76.3%). The CPKp isolates that were showed colistin resistance had greater susceptibility to tigecycline and fosfomycin (64.7%), gentamicin (58.8%), amikacin (41.2%) and then trimethoprim-sulfamethoxazole (29.4%) while showed high resistance to cephalosporin (100%) followed by fluoroquinolones and β-lactam/β-lactamase inhibitors (94.1%) as shown in ***Table 5***.

Table (5): Antibiotic susceptibility of CPKp in relation to their colistin susceptibility.

CPKp (n=93)	Colistin susceptible (n=76) (81.7%)	Colistin resistant MIC ≥ 4µg/mL (n=17) (18.3%)	Test (P value) X^2
			8.51 (<0.001)
Fluoroquinolones			
S= 70 (75.3%)	69 (90.8%)	1(5.9%)	53.80
R= 23 (24.7%)	7 (9.2%)	16 (94.1%)	<0.001
β-lactam/β-lactamase inhibitors			
S=71 (76.3%)	70 (92.1%)	1 (5.9%)	57.19
R=22 (23.7%)	6 (7.9%)	16 (94.1%)	<0.001
Cephalosporin			
S= 65 (69.9%)	65 (85.5%)	0 (0.0%)	48.29
R=28 (30.1%)	11 (14.5%)	17(100%)	<0.001
Gentamicin			
S= 84 (90.3%)	74 (97.4%)	10(58.8%)	23.61
R=9 (9.7%)	2 (2.6%)	7 (41.2%)	<0.001
Amikacin			
S= 80 (86.0%)	73 (96.1%)	7 (41.2)	34.79
R=13 (14.0%)	3 (3.9%)	10 (58.8%)	<0.001
Trimethoprim-sulfamethoxazole			
S=76 (81.7%)	71 (93.4%)	5(29.4%)	38.10
R=17 (18.3%)	5 (5.6%)	12(70.6%)	<0.001
Tigecycline			
S= 86 (92.5%)	75 (98.7%)	11 (64.7%)	23.04
R= 7 (7.5%)	1 (1.3%)	6 (35.3%)	<0.001
Fosfomycin			
S=87 (93.5%)	76 (100%)	11(64.7%)	28.67
R=6 (6.5%)	0 (0.0)	6(35.3%)	<0.001

X^2 = Chi-square test.

There was good agreement (K= 0.87) of CBDE in relation to the golden standard test, BMD method for detection of colistin susceptibility, where the sensitivity, specificity, PPV, NPV and accuracy were as follow, 97.3%, 90.9%, 98.2%, 87% and 96.3% respectively. as represented in ***Table 6***.

Table (6): CBDE method in relation to BMD method for colistin susceptibility determination among CRKp.

CBDE	BMD		Total
	Sensitive (n= 113)	Resistant (n= 22)	
Sensitive	110(97.3%)	2 (9.1%)	112
Resistant	3 (2.7%)	20 (90.9%)	23
Total	113	22	135
*K: 0.87, P value <0.001, Sensitivity: 97.3%, Specificity: 90.9%, PPV: 98.2%, NPV: 87.0%, Accuracy: 96.3%			

*Kappa between 0.81 and 1.00: almost perfect agreement, NPV: negative predictive value. PPV: positive predictive value. CBDE: Colistin broth disk elution test.

Our results showed highly significant difference between carbapenemases genes detected in colistin sensitive (P <0.001) and colistin resistant (P <0.0005) CRKp. Where the *bla*$_{KPC}$ *and bla*$_{OXA-48}$ were more predominant than *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{VIM.}$ as shown in ***Table 7***.

Table (7): Distribution of carbapenemases genes among colistin sensitive and colistin resistant CRKp clinical isolates.

Colistin susceptibility BMD	Carbapenemase genes (n=85)						Z test (P value)
	blaKPC (n=35)	blaOXA-48 (n=25)	blaKPC/ blaOXA-48 (n=2)	blaNDM-1 (n=7)	blaIMP (n=8)	blaVIM (n=8)	
Colistin sensitive (n= 113)	25 (22.1%)	19 (16.8%)	1 (0.9%)	6 (5.3%)	7 (6.2%)	5 (4.4%)	4.79 (<0.001)
Colistin resistant (n= 22)	10 (45.5%)	6 (27.3%)	1 (4.5%)	1 (4.5%)	1 (4.5%)	3 (13.6%)	2.79 (0.005)

Regarding colistin resistant CRKp clinical isolates (n=22), only *mcr-1* was detected in 2 isolates (9.1%) with negative *mcr2*, *mcr3*, *mcr4*, and *mcr5*.

DISCUSSION

In our study, *Klebsiella pneumoniae* isolated from 33.9% of patients (334/ 984) admitted to different wards of Menoufia University Hospitals (MUH) during the period of February 2021 to July 2022. Out of them, 135 (40.4%) were carbapenem resistant by disc diffusion method that is nearly lesser than study by **Tsai *et al.*[3]** who detected 310 CRKp out of 419 CRE. And nearly similar to results of **Ajlan *et al.*[19]** who found that, 36.1% of *Klebsiella* spp. isolated showed carbapenem resistance, and more than detected by **Ngbede *et al.*[20]** where 57 *Klebsiella* spp. out of 583 tested enterobacteria showed carbapenem resistance.

In this study, 93 of CRKp (68.9%) showed positive carbapenemases production with 31 isolate out of them (33.3%) showed positive Metallo-β-lactamases by combined mCIM/ eCIM. That varies from previous study[19], where 83.2% of tested CRGNB showed positive carbapenemases with 65.9% showed positive MBLs by combined mCIM/ eCIM testing. Our results nearly matched with the **Tsai *et al*.[3]** study, that detected 105 out of 193 (54.4%) ertapenem resistant enterobacteria showed positive carbapenemases with 26 out of them (24.7%) were MBLs positive.

In our study, 85 (62.9%) of CRKp (135) had carbapenemase genes, where 35 (25.9%), 25 (18.5%), 2 (1.5%), 7 (5.2%), 8 (5.9%) and 8 (5.9%) had *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{KPC, OXA-48,}$ *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ respectively. The most frequent genes in our MBLs positive CRKp, were *bla*$_{IMP}$ and *bla*$_{VIM}$ by 8/31 (25.8%) for each of them followed by *bla*$_{NDM-1}$ by 7 (22.6%), with 8 (25.8%) were negative for genes. While in serine carbapenemases positive CRKp, the most frequent gene was *bla*$_{KPC}$ 35 (56.5%), followed by*bla*$_{OXA-48}$ 25(40.3%) and 2 (3.2%) had *bla*$_{KPC, OXA-48}$. That is greatly matched with previous study[3] that detected 73 (61.8%) of 118 tested CRKp showed positive carbapenemases genes with 29 (24.6%), 23 (19.5%), 2 (1.7%), 3 (2.5%), 5 (4.2%) and 2 (1.7%), had *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{KPC, OXA-48,}$ *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ respectively. While other studies[21,22] showed higher incidence of carbapenemases genes.

Our results showed that sensitivity, specificity, PPV, NPV and accuracy of combined mCIM/ eCIM in relation to carbapenemases genotyping for detection of carbapenemases were as follow respectively 100%, 84.0%, 91.4%, 100% and 94.1% with total agreement in serine carbapenemases and fair agreement with total carbapenemases and MBLs detection. That is strongly matched with study by **Tsai *et al.*[3]** where both sensitivity and specificity were 100 for mCIM in relation to carbapenemases genes, the sensitivity and specificity of the eCIM and were 89.3% and 98.7% respectively as compared to genotyping. Higher sensitivity and specificity (100 % for both) of eCIM in study by **Sfeir *et al.*[23]**

In our study, 16.3% of CRKp isolates were resistant to colistin by BMD. That is nearly similar to recent study in Egypt[19]**.** However, higher than detected by **El-Sokkary *et al.*[24]** study where 2.79% CRE was

colistin resistant. However, our results are much lesser than that regarding **Anan *et al.*[25]** study, who detect 75% of *K. pneumoniae* isolates were colistin resistant by BMD.

Our results demonstrated that, fosfomycin was the most effective antibiotic (93.5%) against CPKp followed by tigecycline (92.5%), gentamicin (90.3%), amikacin (86.0%), trimethoprim-sulfamethoxazole (81.7%), colistin (81.7%) and then β-lactam/β-lactamase inhibitors (76.3%). That is higher than the susceptibility in **Kar *et al.*[26]** study who found that**,** netilmicin, amikacin, gentamicin, trimethoprim-sulfamethoxazole, piperacillin-tazobactam and fosfomycin were the most effective against CRE by only 23%, 21%, 20.5%, 15.5%, 8.5% and 5.5% respectively. And around the susceptibility pattern of **Ajlan *et al.*[19]** study, who detected that the most effective drug against CRGNB isolates was colistin (72.3%), followed by ceftazidime- avibactam (67.7%), then tigecycline (63.2%).

In our study, colistin resistant CPKp isolates showed resistance to cephalosporins (100%) followed by fluoroquinolones and β-lactam/β-lactamase inhibitors (94.1%) and their lesser resistance was to tigecycline and fosfomycin (35,3%), gentamicin (41.2%), amikacin (58.8%) and then trimethoprim-sulfamethoxazole (70.6%). That runs in accordance with other studies[24,27,28]**.**

Colistin broth disk elusion method (CBDE) is an alternative simple, easy, non-expensive reliable option for assessing colistin sensitivity. Our results detected good agreement (K= 0.87) of CBDE in relation to the golden standard test, BMD method for detection of colistin susceptibility, where sensitivity, specificity, PPV, NPV and accuracy were as follow, 97.3%, 90.9%, 98.2%, 87% and 96.3% respectively. That goes in line with other invistigators[19,29].

Our results showed that the *bla*$_{KPC}$ *and bla*$_{OXA-48}$ were more predominant than *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ among colistin sensitive and colistin resistant (Col R) CRKp. This goes around with **Gharaibeh *et al.*[30]** who found that *bla*$_{KPC}$ was the most frequently found in either colistin sensitive (6.6%) or colistin resistant Kp (6.7%). followed by *bla*$_{NDM}$ among colistin sensitive Kp (3.3%) and *bla*$_{IMP}$ among Col R Kp, also matched with other studies[31,32]**.**

Regarding colistin resistant CRKp clinical isolates (n=22), only *mcr-1* was detected in 2 isolates (9.1%) with negative *mcr2*, *mcr3*, *mcr4*, and *mcr5*. This runs in accordance with **Ajlan *et al.*[19],** who found only 3 out of 43 colistin-resistant CRGNB isolates had *mcr1*, with negative *mcr2*, *mcr3*, *mcr4*, and *mcr5*. Also, studies on CRE in Hong Kong[33] and Oman[34] revealed presence of *mcr1* in only 6.5% and 4.5% respectively. This can be explained by that, the most common cause of colistin resistance in *Klebsiella pneumoniae* isolates is chromosomal mutation that modifies LPS resulting in decreasing their negative charges and so decrease their affinity to colistin[30].

CONCLUSIONS

Our results revealed a major problem regarding *k. pneumoniae* resistance to colistin that is considered the essential substitute for treatment of MDR *k. pneumoniae.* The study findings demonstrated that *bla*$_{KPC}$ and *bla*$_{OXA-48}$ were the most frequent carbapenemases genes among *k. pneumoniae*. We are in a critical need to strictly apply the prevention and control procedures to decrease the spreading of antibiotic resistance in hospital and community environments.

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