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Original article

Assessment of carbapenem resistant *Klebsiella Pneumoniae* in intensive care unit of Zagazig University Hospitals

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ABSTRACT

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has become a real threat to hospitals worldwide with significant morbidity and mortality. Aim: to determine the rate of CRKP in ICUs of Zagazig University hospitals (ZUHs) by confirmatory phenotypic and molecular methods **Subjects and Methods:** This prospective cross-sectional study included 86 *K. pneumoniae* isolates from hospitalized patients in ICUs. Carbapenem resistance was identified by VITEK2 COMPACT system and Carbapenemase activities were identified by Modified Hodge Test, (MHT), modified Carbapenem Inactivation Method (mCIM) and KPC/MBL and OXA-48 Confirm Kit (KMOC test). Carbapenemase-encoding genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}) were detected by Multiplex PCR. **Results:** 66.3% (57/86) of *K. pneumoniae* isolates were CRKP. Hospital acquired infection (HAI) accounts for 84.2% among CRKP isolates. The rate of resistance by MHT, mCIM and KMOC test for carbapenems was 49.1%, 86% and 80.7% for CRKP isolates respectively. The bla_{NDM-1}, bla_{OXA-48} and bla_{KPC} genes were detected at a rate of 31.6%, 24.6%, and 12.3% by PCR respectively. Coexistence of carbapenemases was detected in 15.7% of isolates. Resistance pattern of Eravacycline, Cefiderocol, Ceftazidime/avibactam and Ceftolozane/tazobactam was 52%, 65%, 82.2% and 98% respectively. **Conclusion:** CRKP was responsible for a significant number of HAI cases in the ICUs of ZUHs. Antibiotic resistance was shown to be widespread in our study. The identification of carbapenemases classes will be useful for the improvement of patient's treatment and prognosis and for infections control measures in ICUs.

Introduction

Klebsiella pneumoniae is Gram negative pathogen of Enterobacterales that had numerous mechanisms to escape from different antimicrobials actions [1]. With the widespread and abuse of antibiotics, particularly carbapenems, the prevalence of Carbapenem resistant *klebsiella pneumonia* (CRKP) has increased. Now CRKP strains have become a worldwide problem [2]. Patients infected with CRKP in critical care units have limited therapeutic options that may cause considerable clinical problems including the risk of high mortality, prolonged hospital stay, and increases medical costs [3]. In 2017, the WHO cited carbapenem-resistant Enterobacterales among the highest critical category of the global priority list of pathogens, probably because carbapenems are considered as part of the last resort antimicrobials for treatment of life-threatening infections caused by multidrug-resistant Enterobacterales [4].

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CRKP can develop resistance against a varied class of antibiotics including carbapenem by the production of extended-spectrum β-lactamases (ESBLs) and/or carbapenemase enzymes [5]. The production of different classes of carbapenemases is the most common mechanism among the various mechanisms described for carbapenem-resistance [6]. The emergence of CRKP due to carbapenemases is of great concern as this is mediated by mobile genetic elements (transposons and plasmids) facilitating their transmission [7].

Carbapenemases belongs to the class A, B and D of Ambler classification for β-lactamases which were classified into four molecular classes according to their sequence homology [8]. Based on the structure of their active site, carbapenemases are divided into two groups: Serine- carbapenemases include class A and class D, that contain serine at their active site and The second group is metallo-β-lactamases (MBL) belonging to class B which contain zinc atom at the active site [9]. Among the class B MBL producing strains, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$ are the most frequently reported genes whereas, *bla*$_{KPC}$ and *bla*$_{OXA}$ are the most reported genes in class A and D respectively [10].

Commonly, CRKP have carbapenemase-encoding genes, such as *bla*$_{KPC}$, *bla*$_{OXA-48}$, and *bla*$_{NDM}$, along with a variety of genes conferring resistance to drugs other than β-lactams that leads to all available antibiotics ineffective [11]. Furthermore, coproduction of multiple types of carbapenemases by CRKP has also been documented, and they have become widespread worldwide. Such coexistence of carbapenem-hydrolyzing enzymes in bacteria is increasing and found to be associated with high-level resistance to carbapenems [12].

Treatment of serious CRKP infections remains extremely limited and associated with poor outcomes and high mortality rate. Optimizing therapeutic options available including polymyxins and the new combinations of β-lactams/β-lactamase inhibitors may be the most suitable treatment strategies in the present time. [13]. Molecular and Phenotypic techniques are available to assess either carbapenemases genes or activity, but each still have some limitations. Therefore, rapid and accurate detection of carbapenemase type is vital for patient care and infection control purposes [14,15] . The purpose of our study is to determine the rate of CRKP in ICUs of Zagazig University hospitals (ZUHs) by confirmatory phenotypic tests and molecular methods.

Subjects and Methods

This cross-sectional study was conducted in Clinical pathology department, ZUHs, Egypt, over the period from March 2019 to February 2022. The study protocol was approved by Zagazig medical research ethical committee. 86 *K. pneumoniae* isolates included in this study, recovered from sputum, Pus, CSF, Blood, Peritoneal fluid, CVP tips and Urine of ICUs patients. HAI was considered if the positive sample was obtained 48 hours after hospitalization, instrumentation, received IV or wound care in the last 30 day [16]. Informed consent was obtained and data were collected from electronic medical records.

Bacterial identification and susceptibility testing: All isolates were subjected to growth characterization on blood and MacConkey agar as a routine step in microbiology unit in Zagazig University hospital lab (ZUHL). Isolates have been identified as *K. pneumoniae* depending on gram staining, colonial morphology. The isolates were identified, and antimicrobial susceptibility testing performed on the VITEK 2 automated platform (bioMérieux, Marcy l'Etoile, France) using the GN-ID and 222- AST cards [17]. Minimum inhibitory concentration (MIC) data for each organism were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2013) [17] and the VITEK 2 Advanced Expert System (AES) [18]. Also carbapenem resistance was reassessed by MIC of Imipenem by E-test strip (bioMérieux, France)Lot No(10044022200).

Phenotypic Detection of carbapenemase activity:

1. The modified Hodge test (MHT): was performed based on CLSI 2020 recommendations. Briefly, standard suspension of E. coli 25922 was prepared and inoculated into Mueller Hinton agar (MHA) plate. A 10 µg Imipenem disk (Oxoid) was placed in the center of the plate. Suspected isolates were inoculated in straight line out from the edge of the disk. Finally, after an overnight incubation period, the presence of a "cloverleaf shaped" inhibition zone was considered positive [17].

2. Modified Carbapenem Inactivation Method (mCIM)

A suspension was prepared using one loop full of each isolate in 2 ml TSB (Oxoid). Then, a sensitivity testing disk containing 10 µg meropenem (Oxoid) was placed in the bacterial suspension and

then incubated for 4 hours at 37°C. Then the disk was removed from the bacterial suspension and placed on MHA plate that was inoculated with a susceptible E. coli indicator strain (ATCC 25922) followed by incubation at 37°C. If the bacterial isolate produced carbapenemase, then the meropenem was inactivated while the carbapenemase nonproducing isolate yielded a clear inhibition zone the test considered positive (carbapenemase positivity) with inhibition zone less than 15mm or with presence of colonies inside 16 – 18 mm zone , On the other hand the test considered negative with inhibition zone 19 mm or more [19].

3. Detection of carbapenemase activity by KPC/MBL and OXA-48 Confirm Kit (KMOC test):

1) The test was done using KPC/MBL and OXA-48 Confirm Kit (98015) Lot No 1601-1 (Rosco Diagnostica A/S Taastrup, Denmark) . Briefly; A suspension of the organism from fresh, pure culture to be tested was prepared equivalent to McFarland 0.5. Using a sterile swap the suspension spread uniformly over the entire area of a MHA plate. Tablets provided by the kit (Meropenem 10 µg (MRP10), Meropenem 10 µg + Phenylboronic Acid (MRPBO), Meropenem 10 µg + Cloxacillin (MRPCX), Meropenem 10 µg + Dipicolinic acid (MRPDP) and Temocillin 30 ug} were placed on the inoculated agar plate. Incubate at 35±1°C for 18±2 hours (overnight). The results were interpreted according to Kit manual by comparing the inhibition of the Meropenem 10 µg disc to the zones of inhibition of each of the Meropenem+ inhibitor discs. If the zones are within 3mm of each other, The organism neither expressing KPC nor MBL activity. when the zone around MRPBO disc was 5 mm or more and the zone around MRPCX within 3 mm in comparison to the single Meropenem disc, the organism demonstrates KPC activity (Class A). when the zone around MRPDP was 5 mm or more in comparison to the single Meropenem disc, the organism was positive for Metallo B lactamase activity (Class B). When there was no zone of inhibition for Temocillin 30µg disc the strain was presumptively OXA-48 positive (Class D).

Carbapenemase genes detection by PCR:

Extraction of DNA: DNA was extracted from overnight broth culture of *K. pneumoniae*, using HiPurA Bacterial Genomic DNA Purification Kit (MB505 Himedia, India) according to the manufacture's protocol.

PCR amplification: PCR amplification for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$ was performed for all CRKP isolates. A total volume of 50 µL was adequately prepared for the PCR reaction mixture, including 4 µL of template DNA, 12.5 µL of PCR master mix (QIAGEN Multiplex PCR Kit, Lot No.157013675). and 1 µL (10 pmol) of each primer. The volume was then completed with nuclease-free water up to 25 µL. The primers are namely, blaOXA-48 gene: OXA48_F: F- 5'-GCGTGGTTAAGGATGAACAC-3', OXA48_R: 5'-CATCAAGTTCAACCCAACCG-3' with Amplicon size 438bp. blaNDM-1 gene: NDM-1_F: F- 5'-GGTTTGGCGATCTGGTTTTC-3', NDM-1_R: R- 5'- CGGAATGGCTCATCACGATC-3' with Amplicon size 621 bp. blaKPC gene: KPC_F: F- 5'- CGTCTAGTTCTGCTGTCTTG-3', KPC_R: R- 5'- CTTGTCATCCTTGTTAGGCG-3' with Amplicon size 798 bp. blaIMP gene: IMP_F: F- 5'-GGAATAGAGTGGCTTAAYTCTC-3', IMP_R: R: R- 5'- GGTTTAAYAAAACAACCACC-3' with Amplicon size 232 bp. And blaVIM gene: VIM_F: F- 5'- GATGGTGTTTGGTCGCATA-3', VIM_R: R- 5'- CGAATGCGCAGCACCAG-3' with Amplicon size 390 bp. multiplex PCR was performed using thermal cycler (Applied Biosystem thermal cycler) with cycling conditions of initial denaturation at 94c for 30 sec. Annealing at 52c for 40 sec. . Extension at 72c for 5 min [20].

Novel antibiotic sensitivity by disc diffusion method:

The test was performed by inoculating pure cultures of clinical isolates onto the test medium and placing the AST disk on the surface of the medium. The antibiotic within the disk diffuses into the agar. After incubation, the zones of inhibition around the disks are measured and compared against recognized zone diameter ranges for the specific antimicrobial agent/organism combinations being tested.

Statistical analysis: All data were collected and analyzed by SPSS (version 25) for windows (SPSS Inc., Chicago, IL, USA). Data were expressed as number and percentage for qualitative variables.

Results

The distribution of CRKP clinical isolates in relation to the type of clinical specimens and the antimicrobial sensitivity pattern of the studied isolates are described in Table (1 and2) respectively. Agreement between imipenem E-test and VITEK2 compact 55 out of 57 isolates were

imipenem resistant by E-test with categorical agreement 96.4% Table. 3 . 57 CRKP isolates of this study were screened for detection of carbapenemases by MHT, mCIM, KMOC test and PCR. Carbapenemase-producers were 28 (49.1%) isolates by MHT and 49 (86%) by mCIM test , 46 (80.7%) isolates by KMOC test while 50 (87.7%) isolates were positive by PCR for carbapenemase genes as shown in Table. 4 and Fig.1. Among the 50 PCR positive isolates, the rate of isolates harboring *bla*$_{NDM}$ gene, *bla*$_{OXA-48}$ and *bla*$_{KPC}$ were 18 (31.6%), 14 (24.6 %) and 7 (12.3 %) respectively. one (1.8%) isolate was positive for each of *bla*$_{VIM}$ and *bla*$_{IMP}$ and 9 (15.8%) isolates were positive for more than one carbapenemase gene are described in Table. 5. The relation between the phenotypic tests (MHT, mCIM and KMOC test) and PCR were described in Tables 6. The performance of phenotypic tests in comparison to PCR is described in Table 7. The susceptibility pattern of the studied CRKP according to AST of the Novel antibiotic discs, 30 (52%) and 55 (98%) isolates were resistant to Eravacycline (ERV) and Ceftolozane / tazobactam as described in Table (8).

Table 1. Distribution of CRKP clinical isolates according to clinical samples.

Clinical samples	**(n=57)**	
	N	%
Sputum	35	61.4
Urine	14	24.6
Peritoneal fluid	3	5.3
CVP	2	3.5
Blood	2	3.5
CSF	1	1.8

Table 2. Pattern of antimicrobial susceptibility testing of CRKP isolates**.**

Antimicrobial drugs	**Sensitive**		**Intermediate**		**Resistant**	
	n	%	N	%	N	%
Piperacillin/ tazobactam, Piperacillin, Cefepime, Imipenem, Ceftazidime, Azithromycin, Ticarcillin	0	0.0	0	0.0	57	100.0
Ciprofloxacin	0	0.0	2	3.5	55	96.5
Amikacin	3	5.3	0	0.0	54	94.7
Tobramycin	8	14.0	0	0.0	49	86.0
Minocycline	7	12.3	5	8.8	45	78.9
Gentamicin	15	26.3	5	8.8	37	64.9
Sulfamethoxazole and trimethoprim	26	45.6	0	0.0	31	54.4
Colistin	49	86.0	0	0.0	8	14.0

Table 3. Results of Imipenem E-test and Agreement with vitek 2 compact.

VITEK2 Compact (Resistant=57)			**Categorical Agreement (CA)**	**Minor Error**
Imipenem E-test				
Susceptible 0 (0%)	**Intermediate 2 (3.5%)**	**Resistant 55 (96.4%)**	**96.4%**	**3.5%**

Table 4. Rate of carbapenemase detection by phenotypic and genotypic methods among CRKP clinical isolates.

Carbapenemase detection methods	CRKP (No= 57)			
	Positive		Negative	
	No	%	No	%
MHT	28	49.1	29	50.9
mCIM test	49	86.0	8	14.0
KMOC test	46	80.7	11	19.3
Multiplex PCR	50	87.7	7	12.3

Table 5. Frequency distribution of carbapenemases genes in CRKP isolates.

Carbapenemase-Encoding Genes					
Single Gene	**N**	**%**	**Coexistent Genes**	**N**	**%**
***bla*$_{NDM}$**	18	31.6	*bla*$_{NDM}$ + *bla*$_{OXA-48}$ + *bla*$_{KPC}$	1	1.8
***bla*$_{OXA-48}$**	14	24.6	*bla*$_{NDM}$ + *bla*$_{OXA-48}$	3	5.3
***bla*$_{KPC}$**	7	12.3	*bla*$_{VIM}$+ *bla*$_{KPC}$	2	3.5
***bla*$_{VIM}$**	1	1.8	*bla*$_{NDM}$ + *bla*$_{IMP}$	1	1.8
***bla*$_{IMP}$**	1	1.8	*bla*$_{NDM}$ + *bla*$_{VIM}$	1	1.8
Non	7	12.3	*bla*$_{NDM}$ + *bla*$_{KPC}$	1	1.8

Table 6. Agreement between PCR genes and (MHT, mCIM and KMOC test) for carbapenemases detection in CRKP isolates**.**

Phenotypic test			**PCR Genes**		**Kappa Co-efficient Factor**
			Negative	**Positive**	
MHT		Negative	5 (8.8%)	24 (42.1%)	0.1315±0.017
		Positive	2 (3.5%)	26 (45.6%)	
mCIM		Negative	6 (10.5%)	2 (3.5%)	0.7698±0.05
		Positive	1 (1.8%)	48 (84.2%)	
KMOC test	**Class A**	Negative	44 (77.2%)	6 (10.5%)	0.4638±0.01
		Positive	2 (3.5%)	5 (8.8%)	
	Class B	Negative	27 (47.3%)	5 (8.8%)	0.5547±0.00
		Positive	1 (1.8%)	24 (42.1%)	
	Class D	Negative	37 (64.9%)	6 (10.5%)	0.5945± 0.00
		Positive	2 (3.5%)	12 (21.1%)	

0.01-0.20 = slight agreement, 0.41-0.60 = moderate agreement, 0.61-0.80 = substantial agreement, 0.81-1.00 = almost perfect or perfect agreement.**: highly significant

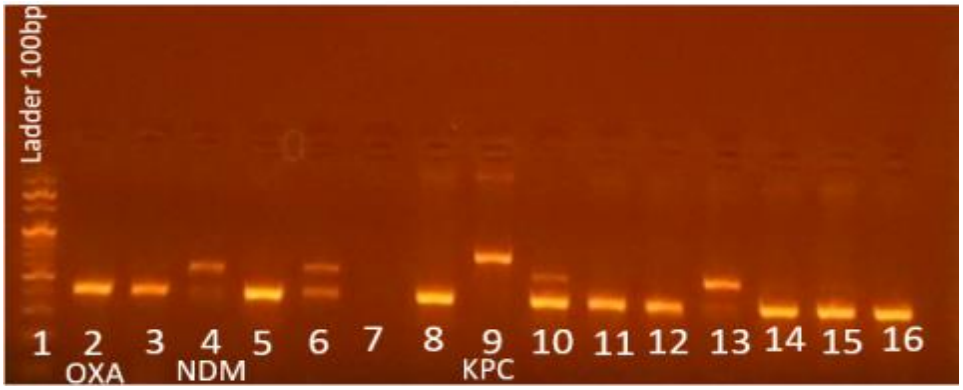
Table 7. The performance of phenotypic tests (MHT test, mCIM and KMOC test for detection of carbapenemases activity in comparison to PCR among *Klebsiella pneumonia*e isolates (N=57).

Parameter	TP	TN	FP	FN	Accuracy	Sensitivity	Specificity	PPV	NPV
KMOC test									
Class A	**5**	**44**	**2**	**6**	**85.96**	**45.5**	**95.65**	**71.4**	**88.0**
Class B	**24**	**27**	**1**	**5**	**89.47**	**82.8**	**96.43**	**96**	**84.4**
Class D	**12**	**37**	**2**	**6**	**85.96**	**66.7**	**94.9**	**85.7**	**86.0**
MHT	**26**	**7**	**2**	**24**	**55.9**	**52**	**77.78**	**92.9**	**22.6**
mCIM	**48**	**6**	**1**	**2**	**94.74**	**96**	**85.7**	**98**	**75.0**

Table 8. Antimicrobial susceptibility testing of the Novel antibiotic among the studied CRKP clinical isolates using disc diffusion method.

Novel antibiotic discs	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
Ceftolozane / tazobactam (C/T 40 µg)	0	0.0	2	3.5	55	98
Ceftazidime / Avibactam (CZA 30/20 µg)	7	12.3	2	3.5	48	82.2
Cefidricol (FDC 30 µg)	15	26.3	5	8.8	37	65
Eravacycline (ERV 20 µg)	26	45.6	1	1.8	30	52

Figure 1. PCR results for carbapenemase -encoding genes.



PCR results for carbapenemase -encoding genes; lane **(1):** DNA ladder 100bp, lanes **(2,3):** positive for bla_{OXA} (438bp), lane **(4):** positive for bla_{NDM} (621bp), lane **(5):** positive for $bla_{OXA,}$ lane **(6):** positive for bla_{NDM} & $bla_{OXA,}$ lane **(7):** negative, lane **(8):** lane positive for $bla_{OXA,}$ lane **(9):** positive for bla_{KPC} ((789bp), lane **(10):** positive for bla_{NDM} & $bla_{OXA,}$ lanes **(11,12):** positive for $bla_{OXA,}$ lane **(13):** positive for $bla_{NDM,}$ lanes **(14,15,16):** positive for $bla_{OXA.}$

Figure 2. positive test for Muller-Hinton agar showed cloverleaf like indentation (positive MHT).

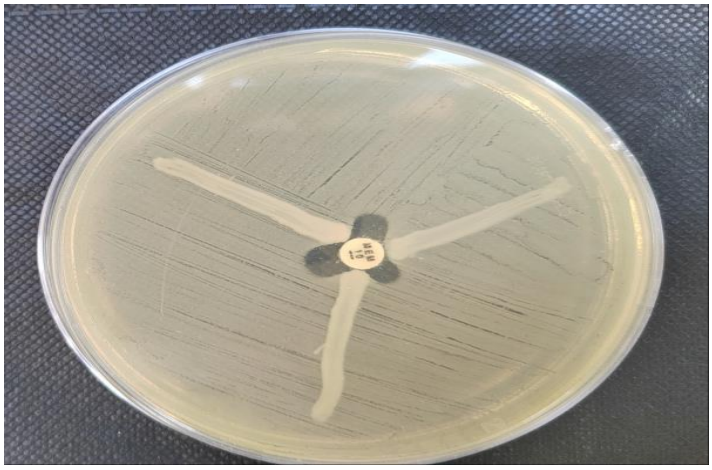


Figure 3. KMOC test we compare the inhibition zone of the Meropenem 10µg disc (A) to the inhibition zones of both; Meropenem + phenylboronic (MRPBO) (B) and Meropenem + cloxacillin (MRPCX) (C), the zones around (MRPBO) (B) and (MRPCX) (C) are < 3 mm so, isolate was negative for KPC. The inhibition zone around the disc of Meropenem + DPA (MRPDP) (D) comp[ared to (A) is < 3 mm, so it was negative for Metallo-β-lactamase activity. There was no zone of inhibition around the Temocillin 30µg (E), so it was positive for OXA-48. This isolate was negative for class A & B carbapenemases and positive class D (OXA-48) carbapenemases.

Discussion

Globally, *Klebsiella pneumoniae* is one of the most frequent pathogens that exhibit resistance to multiple antibiotics by producing Carbapenemase enzymes and by other mechanisms [1]. Carbapenems are considered as part of the last resort antimicrobials for treatment of life-threatening nosocomial infections caused by *K. pneumoniae* [4], The CRKP prevalence have been reported worldwide. In the present study, 66.3% of the isolates were carbapenem-resistant, consistent with other studies that reported high incidence of CRKP infections of about 66.9%, 66.1% and 64.7% respectively [21-23]**.** Although, others reported lower incidence of CRKP infections of about 29.7%, 43.1% and 46.2% [23-25]**.** The high incidence of CRKP in the current study could be related to the frequent use of carbapenems as an empiric therapy in ICUs at our institution.

In this study, 84.2% of CRKP isolates were HAI. Similarly, Gandor et al. found that most of the isolates (91.6%) were hospital-acquired infections. Moreover, the incidence rate of CRKP was elevated by prolonged hospitalization [22]. In agreement with El-Kholy et al. and Zhao et al. who showed that fatal nosocomial infections occurred during the period of hospitalization in the ICU and prior researches indicated that ICU admission was a substantial risk factor for developing CRKP [26,27,28].

In current study the majority of CRKP samples were from sputum (61.4%), followed by urine samples (24.6%). In an Egyptian study, the respiratory samples were the predominant source of CRKP(62%), followed by urine (14%)[29]. Also, Han et al. showed that 95.7% of the strains isolated from clinical specimens were from sputum [30]. In contrast to, Anani et al who reported that CRKP were more prevalent in urine (82%), followed by sputum specimens (14%) [31].

The results of the current study indicated that all 57 CRKP isolates were highly resistant to most antibiotics used for AST (table2) and the most sensitive antibiotics were colistin 86%, and Sulfamethoxazole/ trimethoprim 45.6%. These results were nearly similar to another study where the most sensitive antibiotics were colistin, 89.1%, and tigecycline, 55.5% [22]. Moreover, others studies reported that CRKP isolates showed higher rates of resistance to all antibiotic classes [32,33].

Rapid and accurate identification of carbapenemases is critical for targeted therapy and implementing of infection control strategies. To date, phenotypic methods are the tools available in the routine Microbiology laboratory. In this study, three phenotypic techniques for detection of carbapenemase in K. pneumoniae were used. The modified Hodge test (MHT) employs reagents readily available in most laboratories and does not require expensive devices, furthermore, it is

considered to have a low cost in relation to molecular tests [34].

In the current study, only 49.1% (28/57) of tested CRKP isolates were positive by MHT with (52%) sensitivity and (77.8%) specificity. This was in agreement with results obtained by Gandor et al who reported that MHT was positive for 48.7% among the CRKP isolates with a sensitivity of 56.8 % and same specificity [22]. Higher results were reported in other studies regarding to the sensitivity and specificity [35,36,37]. In this study, false-positive and false-negative results were recorded by MHT in two and 24 isolates respectively. Because, the MHT had low sensitivity for NDM-producers and poor specificity due to false-positives in strains with ESBL production or AmpC overexpression combined with outer membrane porin loss [36]. So, it has been removed from the recent 2018 guideline [39].

mCIM recommended by CLSI in 2018 as a simple and inexpensive method to perform , well established in many clinical microbiology laboratories based on its high sensitivity and specificity to detect carbapenemase-producing Enterobacteriaceae isolates [39] . The present study showed that mCIM detected 49 out of 57 (86%) of CRKP isolates, with a sensitivity (96%) and specificity (85.7%). Research in the United States found that (98.9%) carbapenemase-infected isolates tested positive for mCIM and added that the mCIM test is uncomplicated and easily to interpret, but the lengthy incubation period (from eight hours to overnight) and inability to identify the class of carbapenemases cannot be neglected [34]. Also, Better performance of mCIM was achieved in other studies, Zhong et al. [37] with sensitivity and specificity of 99%, Tsai et al and Aktaş et al with 100% sensitivity and specificity. [36,40]. In our study, one isolate was falsely positive upon testing with mCIM. In a study by Pierce et al stated that TEM-1 and TEM-52 enzymes may produce false positive results in mCIM tests [34].

The phenotypic identification of carbapenemases classes carried out using Rosco Diagnostica Neo-Sensitabs assay (KMOC test) was positive in 46 (80.7%) of CRKP isolates. Class B was the most common found in the investigated isolates 24 out of 29 PCR positive isolates, followed by the Class D, 12 out of 18 PCR positive isolates and then the Class A 5 out of 11 of PCR positive isolates table (5). In agreement with Van Dijk et al who reported that carbapenemase inhibition tests with Phenylboronic Acid and Dipicolinic Acid combined with a temocillin disc (KMOC) test provide a reliable phenotypic confirmation method for class A, B and OXA-48 carbapenemases in Enterobacteriaceae [41]. In this study the sensitivities and specificities were (45.5%, 82.8%, 66.7%) and (95.7%, 96.4%, 94.9%) for class A, B and D respectively. Pantel et al reported higher sensitivity (98.8%) results but same specificities (93.1%) [42]. While Doyle et al [43] reported that the Rosco Diagnostica Neo-Sensitabs had a sensitivity of 80% and specificity of 93% and can be used to confirm the presence of carbapenemases, if a clinical laboratory does not able to perform molecular tests

Molecular assays are considered the gold standard for carbapenemase detection, but it requires additional equipment, skilled staff and is not available in many laboratories. Additionally, only targeted genes can be detected, with new enzyme variants and mutations possibly being missed [44].

Regarding to PCR results, the detection of Carbapenemase-encoding genes among CRKP isolates was carried out for 57 CRKP isolates, where 50 (87.7%) isolates were positive (table 3), 3 (5.2%) isolates were negative by PCR but positive by other phenotypic tests (MHT, mCIM). table (5). Carbapenem - resistance shown in these isolates may be mediated by other carbapenemase genes.

In our study, the most prevalent gene among the 50 CP-CRKP isolates was $bla_{\text{NDM-1}}$ gene 18/57 (31.6%) followed by $bla_{\text{OXA-48}}$ gene 14(24.6%). Our results were in agreement with Badran et al who reported that the most prevalent gene detected was bla_{NDM} (84.4%) followed by the $bla_{\text{OXA-48}}$ (73.3%) [45]. This finding was in total agreement with several studies from Egypt [46-48]. In contrast to previous studies, where $bla_{\text{OXA-48}}$ (58%) were the most common in K. pneumoniae [49] also, Raheel et al. and El-Badawy et al. from Egypt reported $bla_{\text{OXA-48}}$ as the most commonly present gene followed by bla_{NDM} [50,51]. The predominance of bla_{NDM} might be explained by the fact that they are encoded on a range of highly mobile conjugative plasmids, which enable horizontal inter and intra-transfer rather than clonal spread between bacteria [52].

In our study, 7(12.3%) isolates were positive for bla_{KPC} gene. Lower frequency was

reported in a previous study in Egypt, where one (1.6%) isolate of *K. pneumoniae*, identified as blaKPC producer [29] While a much higher prevalence (89 %) was reported in another study [53]. 1(1.8%) were positive for each of bla_{VIM} gene and bla_{IMP} gene by PCR.

This study revealed that MHT, mCIM and KMOC test detected 26, 48 and 41 cases out of the 50 PCR CRKP positive isolates with slight , substantial and moderate agreement respectively table (5). In our study 9 isolates (15.8%) co-expressed more than one carbapenemases genes. In another study, nearer results were reported (12.5%) [30]. The coproduction of genes may lead to false negative results for phenotypic detection methods. Moreover, a previous study in Egypt stated that absence of phenotypic resistance to carbapenem could be due to a lack of gene expression and a pronounced inoculum effect on MIC determinations for IPM with some KPC-producing Klebsiella spp. [54].

With limited therapeutic options available for treatment of Carbapenem resistant Enterobacteriaceae (CRE)in Egypt,Ceftazidime-avibactam(CZA) was recently introduced into the Egyptian market. It has been reserved for patients showing resistance to the last line of traditional CRE treatment (e.g., Colistin and Tigecycline). In our institution, and in an unexpected speediness, resistance to CZA was reported about (82.2%), also about 14% of CRKP are resistant to colistin although it did not recommended by physicians due to its side effect as nephrotoxicity, moreover , it was not tested by one of the reference methods recommended by CLSI (broth or agar dilution or colistin disk broth elution) . Furtherly, data regarding novel agents are still limited and slowly emerging. Therefore, continuous surveillance and epidemiological investigation of carbapenemases are of great importance to control infections. As the Novel antibiotics demonstrated that the least resistant was Eravacycline (ERV) was 52% and the highest resistant was Ceftolozane/tazobactam (C/T) was 98% table (7). Our results were agreement with Badran et al who reported that 100%, 86.7%, 51.1% and 42.2% were resistant to Ceftolozane/tazobactam (C/T), CZA, ERV and Cefidricol (FDC) respectively [42]. Although, these drugs are unavailable in the Egyptian market, the resistance rate of ERV and FDC was unexpectedly high. Inconsistently with this finding other studies stated that retained activity of ERV and FDC had a good antibacterial effect on CRE [27,55,56].

Conclusion: CRKP was responsible for a significant number of HAI cases in the ICUs of ZUHs. Antibiotic resistance was shown to be widespread in our study. The identification of carbapenemases classes using KMOC test will be useful for the improvement of patient's treatment and prognosis and for infections control measures in ICUs.

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