Prevalence and Characterization of Carbapenem-Resistant *Klebsiella pneumoniae* isolated from the intensive care units of Zagazig University Hospitals

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ABSTRACT

Background: All b-lactamase enzymes, or carbapenemases, are able to hydrolyzing the beta-lactam antibiotics. The ability of *Klebsiella pneumoniae* to produce carbapenemases is the primary cause of carbapenem resistance in this organism.

Objective: To define the prevalence as well as characterization of carbapenem resistance with genes coding for the carbapenemase in carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates that was taken from patients in the intensive care units (ICUs) of Zagazig University Hospitals.

Patients and Methods: In a cross-sectional study we included patients who were diagnosed with *K. pneumoniae* infections from different ICUs of Zagazig University Hospitals, which are referral hospitals serving many patients in eastern Egypt.

Results: In this study 120 out of the 190 participants who had a confirmed *K. pneumoniae* infection were found to have CRKP infections. The XpertCarba-R assay was used to test the isolates for the presence of *blaNDM, blaIMP, blaVIM, blaKPC, and blaOXA-48.* The *blaNDM* (60%), *blaOXA-48* (47.6%), and *blaKPC* (8.4%) genes were the most common. None of the isolates had either *blaVIM* or *blaIMP* present.

Conclusion: These current data indicate spread of CRKP isolates in our institution poses a serious health threat. Limiting transmission depends on early detection of isolates that are resistant to carbapenem. In order to prevent the spread of the CRKP, it is necessary to pay more attention to rationalizing the use of antibiotics and strengthening the application of infection control precautions, such as hand hygiene, patient isolation, environmental cleaning and antibiotics stewardship programs.

Keywords: CRKP, *blaNDM, blaKPC, blaOXA-48,* Egypt.

INTRODUCTION

Klebsiella pneumoniae, an opportunistic pathogen that is facultative anaerobic and non-motile, is strongly related with higher morbidity and mortality among patients, mainly in immune compromised ones [1]. *K. pneumoniae* is also one of common sources of antibiotic resistance [2]. Transposons and plasmids aid in the spread of antibiotic-resistant bacteria [3].

A class of enzymes known as b-lactamases, which hydrolyze the beta -lactam ring and render the antibiotic ineffective against bacterial transpeptidases, are produced by bacteria when they are able to acquire these genes. Majority of beta-lactamases are extended-spectrum beta-lactamases (ESBLs)[4]. Because ESBLs can hydrolyse cephalosporins, monobactams, and extended-spectrum penicillins, carbapenems are the only obtainable therapeutic options [5]. Infectious illnesses caused by carbapenem-resistant bacteria are therefore typically treated with these medications as a last option [6]. However, due to selection pressure from their erroneous or excessive use, carbapenem-resistant *Enterobacteriaceae* (CRE) have appeared. The most dominant isolate of CRE is CRKP [7].

There are phenotypic techniques for detecting carbapenemase activity while the genes that encode for this enzyme can be found using molecular tests [8]. According to the CDC's review of hospital surveillance data, 8% of *Klebsiella* isolates are carbapenem-resistant [8]. While in other research [9], 5-24% of *Klebsiella* isolates were from hospitalised patients. Concerningly,

the prevalence of carbapenem resistance is increasing in Egypt. According to one study, 44.3% of *K. pneumoniae* isolates from hospitals along the Suez Canal exhibited this resistance [10].

Correct antibiotic prescribing and infection control measures are needed and are based on carbapenem resistance diagnosis to prevent the spread of resistant strains in various healthcare settings. As a result, we conducted this study to define the prevalence as well as characterization of carbapenem resistance and genes coding for the carbapenemase in CRKP isolates that were taken from patients at Zagazig University Hospitals (ZUHs) intensive care units (ICUs), considering that carbapenems are routinely used as empirical therapy in the intensive care units (ICUs) of Zagazig University Hospitals (ZUHs).

PATIENTS AND METHODS

Between October 2022 and March 2023, we conducted this cross-sectional study, All patients admitted to any ZUH Intensive Care Unit who had *K. pneumoniae* infection confirmed from any clinical sample were included; The East Delta, Sinai, and Suez Canal governorates are all served by Zagazig University Hospitals, which are tertiary care teaching hospitals.

1) Case definition:

In a patient with symptoms and evidence of infection, *K. pneumoniae* was isolated from various sterile sites such as blood, cerebral spinal fluid, peritoneal fluid, or pleural fluid. When a patient's symptoms included

coughing, dyspnea, and fever, a chest X-ray revealed an infiltrate, and culture findings revealed more than 10^4 CFU per millilitre of tracheal purulent secretions or lavage fluid of broncho-alveoli, pneumonia was diagnosed [11].

2) Isolation and identification of K. pneumonia:

Bacteria were identified to the species level using various methods, including Gram staining, colony morphology, the indole test, the oxidase test, the motility test, the methyl red test, the Voges-proskauer test, the citrate test, and the urease test., and results were confirmed using The VITEK® 2 compact system Microbial Detection System (bioMérieux, USA) in accordance with the manufacturer's instructions. Duplicate strains of *K. pneumonia* from the same patient were removed, and only the first tested sample of the patient was reserved.

3) Antibiotic Susceptibility Testing:

Antimicrobial susceptibility was determined with the help of the VITEK® 2 compact system and the AST-GN 222 card (BioMérieux, Marcy L'Etoile, France). Susceptibility to tigecycline was evaluated using the disc diffusion technique (15 µg). The results were interpreted in light of the CLSI 2021 standards [12]. Although there are currently no CLSI-approved cutoffs for TGC, the FDA has provided cutoffs for *Enterobacteriaceae* as (19 mm for sensitive, 15-18 mm for intermediate, and 14 mm for resistant)[12].

4) Phenotypic detection of carbapenemase:

The following assays were used to determine sample screening of carbapenemase:

A) Modified Hodge Test (MHT):

Streaks of a 1:10 dilution of a 0.5 McFarland standard *E. coli* strain ATCC 25922 suspension were made on Muller-Hinton agar plates after adding 0.5-4.5 mL of saline (45%). After that, meropenem (10 g) was added to the mixture and it was centred on the plate. The material was evenly distributed from the disc to the outer edge of the plate. The plates were kept at 37 degrees or higher all night. Positive and negative findings were interpreted according to CLSI standards[13].

B) Modified Carbapenem Inactivation Method (mCIM):

A suspension of organisms, measuring 1 µL in a measured loop, was vortexed in 400 µL of water for 15 seconds. A disc containing 10 g of meropenem was added to the suspension sterilely. The disc was added to the suspension and then incubated for four hours at 35-37 degrees Celsius. The 10 µL inoculating loop was then used to extract the meropenem disc from the suspension; before placing the loop on a Muller- Hinton agar plate inoculated with a 0.5 McFarland suspension of the carbapenem-resistant *E. coli* control strain ATCC 25922, we slid the Eppendorf down the edge to drain

any extra liquid. The size of the inhibitory zone around the disc was also determined after incubating the plates overnight at 35-37 degrees Celsius. Colonies inside an inhibitory zone of 6-15 mm or within a zone of 16-18 mm were considered carbapenemase positive. However, a larger than 19-millimeter carbapenem inhibition zone indicated a negative result[14].

5) Carbapenemase gene identification at the molecular level:

Molecular testing was performed using the XpertCarba-R Assay (Cepheid, Sunnyvale).

Bacterial isolates that cultured on either blood or MacConkey agar can be tested for the presence of the carbapenemase resistance genes bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM} by using a qualitative in vitro real-time polymerase chain reactiontechnique developed in California, United States[15].To be more specific, a sample was taken from the transport tube and vortexed vigorously for 10 seconds in the elution reagent tube .The manufacturer recommended run time for the XpertCarba-R cartridge was 47 minutes, after which the contents were transferred into the specimen chamber. The GeneXpert System was used to decipher the results.

Ethical approval:

The study was approved by the Medical Research Ethics Committee of Faculty of Medicine, Zagazig University (no. ZU-IRB # 9971-11-10-2022). All adult participants or caregivers of children participants provided written consent. The study was conducted out in line with the Helsinki Declaration.

Statistical analysis

Statistical Package for the Social Sciences (SPSS), version 20, was utilized to do the computational analysis of the collected data. Mean, standard deviation, and range were displayed with the numerical data. Qualitative data like frequency and percentage were used to illustrate the data, which were compared by Pearson Chi-Square. Significant results were considered to exist when the p-value was less than 0. 05.

RESULTS

1) Identification of patients:

The study enrolled 190 cases who had proved *K. pneumoniae* infections; 120 were found to have CRKP infections; females (54.2%) were the majority of studied patients, and the mean age was 44.58 ± 21.89 years (Table 1).

Table (1): Demographics of CRKP patients

	No	%
Gender		
Female	65	54.2
Male	55	45.8%

Age (year)	
Mean ± SD	44.58 ± 21.89
Range	(0 - 92)

2) *Characteristics of bacterial* isolates:

Sputum samples (45.8%) were the predominant source of CRKP, followed by urine (23.3%), blood (15%), pus (8.3%), CVP (3.3%), CSF (2.5%) and peritoneal fluid (1.7%) samples (Table 2).

Table (2): Distribution Characteristics of CRKP Specimens (n=120)

Specimen type	No	%	X^2	P-Value
Respiratory samples	55	45.8%		
Urine	28	23.3%		
Blood	18	15%	103.983	0.0001*
Pus	10	8.3%		
CVP	4	3.3%		
CSF	3	2.5%		
Peritoneal fluid	2	1.7%		

* Significant difference (P < 0.05), X^2: Chi-square test.

3) *Antibiotics susceptibility of CRKP*

CRKP isolates were 100% resistant to extended-spectrum penicillin, ceftazidime, cefepime, azithromycin, imipenem, and meropenem. Also, they were highly resistant to aminoglycosides, quinolones, minocycline and sulfamethoxazole/trimethoprim. Also 35.8% and 10.8% of these isolates were resistant to tigecycline and colistin respectively (Table 3).

Table (3): Susceptibility patternof CRKP to various antibacterial drugs

Antibiotic	Susce-ptible N (%)	Inter-mediate N (%)	Resis-tant N (%)
Piperacillin/Tazobactam	0(0.0)	0(0.0)	120(100)
Piperacillin	0(0.0)	0(0.0)	120 (100)
Tobramycin	7(5.8)	0(0.0)	113 (4.2)
Cefepime	0(0.0)	0(0.0)	120(100)
Imipenem	0(0.0)	0(0.0)	120(100)
Gentamicin	15(12.5)	6(5)	99(82.5)
Ceftazidime	0(0.0)	0(0.0)	120(100)
Meropenem	0(0.0)	0(0.0)	120(100)
Amikacin	0(0.0)	0(0.0)	105 (87.5)
Sulfamethoxazole-Trimethoprim	15(12.5)	0(0.0)	93(77.2)
Azithromycin	26(21.6)	0(0.0)	120(100)
Minocycline		5(4.2)	107 (89.2)
Ticarcillin		0(0.0)	120 (100)
Pefloxacin	0(0.0)	7(5.8)	113 (94.2)
Ciprofloxacin	8(6.7)	3(2.5)	117 (97.5)
Tigecycline	0(0.0)	11 (9.2)	43(35.8)
Colistin	0(0.0)	0(0.0)	13(10.8)
	0(0.0)		
	66(55)		
	107(89)		

4) *Genotypic detection of carbapenemases producing genes:*

The isolates were examined by the XpertCarba-R Assay to detect the most prevalent carbapenemases genes: *blaNDM, blaIMP, blaVIM, blaKPC, and blaOXA-48*. 105/120 of the CRKP isolates were positive for one or more carbapenemase, *blaNDM* (60%) was found to be most prevalent one followed by *blaOXA-48* (47.6%) and *blaKPC* (8.4%). Neither *blaVIM* nor *blaIMP* was detected in any of the isolates (Table 4).

Table (4): Carbapenmase encoding gene frequency among CRKP isolates

CRKP isolates	N=120	
	N	%
Carbapenmase Gene Presence	105	87.5%
No Gene Presence	15	12.5%

Around 105 of CRKP isolates were Carbapenemase-encoding genes where fifteen out of 105were found to be co-harbored and around two out of 105 (1.9%) existing all three genes, while thirteen out of 105 of the isolates (12.4%) existing both *blaNDM* and *blaOXA-48*genes)(Table 5).

Table (5): The prevalence of carbapenemase-encoding genes among CRKP strains

Genes encoding carbapenemase	N=105 n(%)
blaNDM	48(45.7%)
blaOXA-48	35 (33.3%)
blaKPC	7 (6.7%)
blaNDM + blaOXA-48+ blaKPC	2(1.9%)
blaNDM + blaOXA-48	13(12.4%)

5) *The correlation of phenotypic and genotypic methods of carbapenemase detection:*

From 64 MHT-positive isolates, 21 MHT-positive isolates were tested positive for *blaKPC*, 20 were tested positive for *blaOXA-48*, and 17 were tested positive for two or more carbapenemase-encoding genes at the same time. Only two of the *blaNDM* isolates were MHT positive, but none of the other four MHT positive isolates had any of these genes.

Eighteen of the 102 mCIM-positive isolates were *blaKPC* positive, thirty-five were *blaNDM* positive, twenty-four were *blaOXA-48* positive, twenty-one were tested positive for two or more carbapenemase-encoding genes, and four were negative for all of them(Table 6).

Table (6): Correlation between phenotypic and genotypic methods in carbapenemase detection

Genes encoding carbapenemase		

	Negative (susceptible) n = 15 (%)	Positive (Resistant) n = 105 (%)	Kappa Agreement	p-Value
MHT Susceptible (n =56)	6 (40)	50 (47.6)	0.093	0.10500
Resistant (n = 64)	9 (60)	55 (52.4)		
mCIM Susceptible (n = 18)	10 (66.7)	8 (7.6)	0.328	<0.00001
Resistant (n = 102)	5 (33.3)	97 (92.3)		
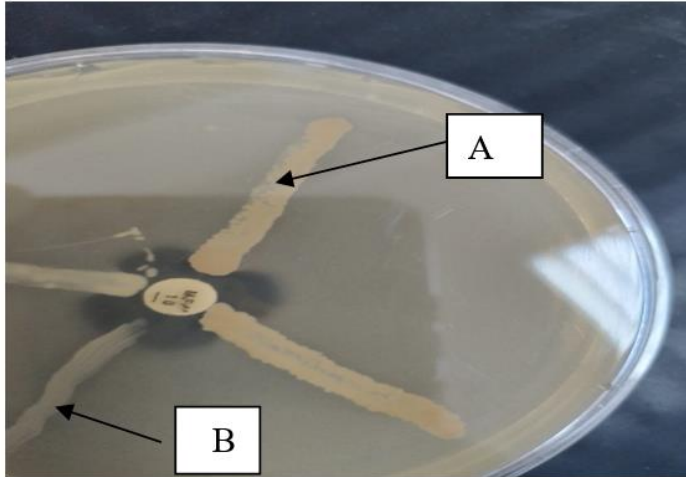
Figure (1): Modified Hodge test (MHT) A: clinical KP isolate with positive result, B: clinical KP isolate with negative result.

6) Susceptibility of CRKP to ceftazidime/avibactam (CZA):

Regarding susceptibility to ceftazidime/avibactam (CZA), 32/120 (26.7%) of CRKP isolates were susceptible. 95.8% of isolates harbouring *blaNDM* were resistant, while the serine-producing isolates have resistance rateof 57.1% (Table 7).

Table (7): Susceptibility of CRKP isolates to ceftazidime/avibactam

CRKP isolates	CZA	
	Susceptible n(%)	Resistant n(%)
CRKP (120)	32(26.7%)	88(73.3%)
Carbapenemase-encoding genes negative (15)	7(46.7%)	8(53.3%)
Carbapenemase-encoding genes positive (105)	25(23.8%)	80(76.1%)
blaNDM (48)	2(4.2%)	46(95.8%)
blaOXA-48(35)	16 (45.7%)	19 (54.3%)
blaKPC(7)	2(28.6%)	5(71.4%)
blaNDM + blaOXA-48 (13)	5(38.5%)	8(61.5)
blaNDM + blaOXA-48+ blaKPC(2)	0(0.0%)	2(100%)

DISCUSSION

Carbapenemase activity and genes can be detected using phenotypic and molecular methods, respectively.

Eight percent of *Klebsiella* isolates are carbapenem-resistant, according to CDC hospital surveillance data. In other studies, researchers discovered that anywhere from 5% to 24.0% of *Klebsiella* isolates were from hospitalised patients. Forty-three percent of *K. pneumoniae* isolates were carbapenem-resistant in university hospitals along the Suez Canal [16].

This study involved 190 patients who had confirmed *K. pneumoniae* infections; 120 were found to have CRKP infections; females (54.2%) made up the majority of the patients studied; and the mean age was 44.5 ± 21.89 years. The majority of the isolates (90.4%) were hospital-acquired infections with average of 16 days as intensive care unit stay.

In accordance with **Moemen et al.**[11] carbapenems are routinely utilized as an empiric therapy in the ICUs, we aim to assess our frequency of carbapenem resistance with genotypic detection of carbapenemase producing genes among *K. pneumonia* clinical isolates collected from ICUs patients. With same conditions and using primers as described by **Karuniawati et al.**[17], 125 patients with confirmed *K. pneumoniae* infections were included in the study; 42 of these patients had CRKP infections. Twenty-three men and nineteen women were sampled for CRKP isolates. The median age of patients was 60 years old (range, 43-77 years old). Most CRKP was collected from respiratory fluids (62%), then from urine (14%), wound (9%) and blood (9%) samples, and finally from a catheter tip (5%).

With ongoing rise of CRKP in **Moemen et al.**[11] study could be linked to the lack of an antimicrobial stewardship programme at their institution with misuse and abuse of carbapenems in intensive care units . They conducted their research on patients hospitalized to an ICU since it had previously been shown by **Kandeel et al.**[18] that being in the ICU was a significant risk factor for contracting CRKP.

In our study, about 55 (45.8%), 28(23.3%), and 18 (15%) of the 120 CRKP isolates were from respiratory samples, urine, or blood, respectively. 10 (8.3%) isolates were from pus. 4 isolates (3.3%) from central venous catheter,3 isolates (2.5%) from cerebrospinal fluid and 2 from peritoneal fluid. **Moemen et al.**[11] the majority of CRKP was found in respiratory samples (62%), then in urine (14%), then in wounds (9.5%), then in blood (9.5%), and finally in the catheter tip (5%).

In our study the predominant sources of CRKP were sputum samples as observed by a study of **Karuniawati et al.** [17] which was done in Indonesia and reported the highest incidence of carbapenemase-encoding genes was from sputum.

Possible explanations include transmission of multidrug-resistant clones across hosts and prolonged antibiotic exposure that accumulates resistance determinants in the microbiota of the respiratory system. In the future, these resistant microbes could cause infections in the respiratory system [19].

In our study, which is more serious piperacillin/tazobactam, piperacillin, ceftazidime,

cefepime, azithromycin, imipenem, and meropenem were all completely ineffective against CRKP isolates. Also, they showed a significant level of resistance to quinolones (pefloxacin 94.2% and ciprofloxacin 98.3%), aminoglycosides (amikacin 87.5%, gentamicin 82.5%, and tobramycin 94.2%), tetracyclines (minocycline 89.2%), and sulfamethoxazole/trimethoprim 77.2%), 35.8 % of patients were resistant to tigecycline, and 10.8 %were resistant to polymixins (colistin).

Based on the CLSI-recommended cutoffs (M100-S24) for carbapenem interpretation, **Moemen et al.** [11] showed that 33.6% (42/125) of *K. pneumoniae* isolates were non susceptible (intermediate and resistant) to ertapenem. Twenty-five point six percent were resistant to both meropenem and imipenem. Two isolates showed complete resistance to every antibiotic tested (panresistant); however, colistin and tigecycline showed good clinical activity against these isolates. Among the 42 CRKP isolates, *blaKPC* was the most common gene at 43.5%, contrasting with a previous study in Egypt that found *blaOXA-48* like types to be the most common at 28.6% and *blaKPC* to account for just 19%[20].

For CRE isolates, **Eltahlawi et al.**[21] found that colistin (98.9%) and tigecycline (88.9%) were the most effective antibiotics, followed by amikacin (52.2%), gentamicin (33.3%), cotrimoxazole (15.6%), and ciprofloxacin (8.9%).

Being the gold standard, molecular detection of carbapenemases was performed using the XpertCarba-R assay to detect *blaNDM, blaIMP, blaVIM, blaKPC*, and *blaOXA-48*. 105/120 of the CRKP isolates were positive for one or more carbapenemase, the most prevalent gene was *blaNDM* (60%) followed by *blaOXA-48*(47.6%) and *blaKPC* (8.4%). Neither *blaVIM* nor *blaIMP* was detected in any of the isolates.

One in ten of our isolates contained the *OXA-48* gene. Since its discovery in Turkey, both **Poirel et al.**[22] and **Memish et al.**[23] have documented the spread of this gene to new locations in the Middle East and North Africa, including India, Senegal, and Saudi Arabia.

Around 21 out of 64 MHT-positive isolates were tested positive for *blaKPC*, 20 isolates were positive for *blaOXA*-48, and 17 isolates were positive for two or more carbapenemase-encoding genes at the same time. Only two of the *blaNDM* positive isolates were MHT positive, but none of the other four MHT positive isolates had any of these genes. Among the 102 mCIM-positive isolates, 18 isolates were positive for *blaKPC,* 35 were isolates positive for *blaNDM* while 24 were tested positive for *blaOXA-48*, 21 were tested positive for two or more carbapenemase-encoding genes, and 4 were tested isolates negative for none.

Moemen et al.[11] found that 26/42 (61.9%) have carbapenamase activity when using the MHT method; 22/42 (52.4%) when using the Boronic acid screen; and 5/42 (11.9%) when using the EDTA test. Only 18 of the isolates tested negative using the MHT method but positive using the Boronic acid approach. Out of the 42

CRKP tested, 35 (83.3%) were found to produce carbapenemases.

On contrary to our results **Eltahlawi et al.**[21] reported that *blaOXA-48* was the most common carbapenemase gene (44.4%), followed by *blaNDM* (32.2%) while *blaKPC* gene was not found.

In our study, regarding susceptibility to ceftazidime/avibactam (CZA), 32/120 (26.7%) of CRKP isolates were susceptible. 95.8% of isolates harbouring *blaNDM* were resistant, while the serine-producing isolates had resistance rate of 57.1%

In a study by **Gandor et al.**[24]**,** only 23.5% of CRKP isolates were positive for susceptibility to the novel treatment drug, ceftazidime/avibactam (CZA). One hundred percent resistance was seen in isolates that had the *blaNDM* gene, but only 56.5% resistance was seen in serine-producing isolates.

Resistance to the novel therapeutic drug (CZA) was calculated to be 76.5%. The 2017 data from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) showed that all CRKP isolates tested were susceptible to CZA, making this resistance rate extremely high[16]**.**

One hundred percent of the isolates showed CZA resistance after harbouring *blaNDM* alone or in combination with additional carbapenemase-encoding genes, while only 56.5% of the isolates that produced serine were resistant. Our findings are in partial agreement with those of prior investigations, which found that between 90.8% and 100% of metallo-beta-lactamases positive isolates were resistant to CZA[25]**.**

CONCLUSION

Based on our results, the expansion of CRKP isolates in our institution poses a significant risk. Early recognizing carbapenem-resistant isolates is crucial for controlling transmission. This necessitates greater focus on rationalizing antibiotic use and strengthening the application of infection control measures such as hand hygiene, patient isolation, environmental cleaning, antibiotics stewardship programs. More research on new antimicrobial medicines against CRKP is needed, and they must be adopted soon to prevent the spread of these superbugs.

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