

# Carbapenem-Resistant *Klebsiella pneumoniae* Among Patients with Ventilator-Associated Pneumonia: Evaluation of Antibiotic Combinations and Susceptibility to New Antibiotics

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**Background:** Carbapenemase-producing Gram-negative bacteria, particularly *Klebsiella pneumoniae* (*K. pneumoniae*), are at the forefront of the list of causative agents of ventilator-associated pneumonia (VAP). The treatment options for such infections are limited, and various antimicrobial combinations have been suggested as alternatives in clinical practice. New antibiotics, such as ceftazidime/avibactam, ceftolozane/tazobactam and cefiderocol, have shown advantages in both in vitro and clinical studies.

**Purpose:** To evaluate the in vitro effect of meropenem–ciprofloxacin and meropenem–colistin combinations on carbapenem-resistant (CR) *K. pneumoniae* VAP isolates and to determine their susceptibility to new antibiotics.

**Methods:** Seventy-three *K. pneumoniae* isolates from 176 endotracheal samples from VAP cases were studied. Antibiotic susceptibility testing and phenotypic detection of extended-spectrum  $\beta$  lactamase (ESBL) and carbapenemase production were done. CR *K. pneumoniae* isolates were tested for the five predominant carbapenemase genes (*bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>IMP</sub>). In vitro evaluation of meropenem–ciprofloxacin and meropenem–colistin combinations was done by MIC test strips. Susceptibility to new antibiotics was tested by disk diffusion method.

**Results:** Sixty-three (86.3%) of the isolates were ESBL producers and 52 (71.2%) were carbapenem resistant. *bla*<sub>NDM</sub> was the most prevalent carbapenemase gene (50%), followed by *bla*<sub>OXA-48</sub>, (36.5%) then *bla*<sub>KPC</sub> in (11.5%). *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> were not detected. Meropenem–ciprofloxacin combination showed indifferent effect on all isolates, while meropenem–colistin combination showed 25% synergism, 15.4% addition and 59.6% indifference. All (100%) CR *K. pneumoniae* isolates were resistant to ceftolozane/tazobactam and 79% were resistant to ceftazidime/avibactam, while 96% were sensitive to cefiderocol.

**Conclusion:** A high rate of carbapenem resistance exists among VAP *K. pneumoniae* isolates. Meropenem–colistin combination and cefiderocol appear to be potential treatment options for infections caused by CR *K. pneumoniae*. Resistance to the tested new  $\beta$ -lactam/ $\beta$ -lactamase inhibitors was high, signifying a major threat.

**Keywords:** ventilator-associated pneumonia, *Klebsiella pneumoniae*, carbapenem resistant, carbapenemases, combination, new antibiotics

## Introduction

Ventilator-associated pneumonia (VAP) is the most serious intensive care unit (ICU)-acquired infection of the lung parenchyma that develops after 48 hours of endotracheal intubation and mechanical ventilation.<sup>1</sup>

Multidrug-resistant (MDR) bacteria, both Gram-positive and Gram-negative, have been associated with VAP but in low- and middle-income countries, it was found that the MDR Gram-negative bacilli like *K. pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter* species predominate.<sup>2</sup>

Carbapenems have been the last therapeutic option in the treatment of infections induced by MDR Enterobacterales.<sup>3</sup> Nevertheless, the development of different resistance mechanisms to carbapenems, as a result of their misuse, has reduced their effectiveness.<sup>4</sup>

The use of antibiotic combinations has emerged as an alternative option for treatment of such infections seeking the synergism in suppressing bacterial resistance and curtailing the toxicity of the used antibiotics.<sup>5</sup>

The use of broad-spectrum  $\beta$ -lactam and fluoroquinolone in combination has been recommended for treatment of VAP patients with risk factors for MDR Gram-negative bacilli or at high risk of death.<sup>6</sup>

Colistin has been increasingly prescribed as the last treatment option for infections caused by CR bacteria, but limited efficacy of colistin monotherapy, its high toxicity and colistin hetero-resistance, have led to its use in combinations with other antibiotics.<sup>7</sup>

In 2017, WHO defined CR *Enterobacterales* as one of the highest priority pathogens for the development of new antibiotics and some new antibiotics have been marketed or have approached late stages of development, new  $\beta$ -lactam/ $\beta$ -lactamase inhibitors such as ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam have brought additional choices for clinicians treating these MDR organisms. With a broader spectrum of activity, the clinicians' repertoire furtherly welcomed newer agents including cefiderocol, a catechol-substituted siderophore cephalosporin, eravacycline, a fluorocycline of the tetracycline class, and the synthetic aminoglycoside, plazomicin.<sup>8</sup>

This study aimed to evaluate the in vitro effect of the frequently used combination in our ICU, meropenem–ciprofloxacin, in comparison to meropenem–colistin combination on CR *K. pneumoniae* isolates and to determine the susceptibility of these isolates to three new antibiotics with previously reported favorable outcomes in nosocomial pneumonia.

## Materials and Methods

### Study Design and Setting

This cross-sectional study was carried out during the period from January 2020 to November 2021 in the Medical Microbiology and Immunology Department and the Emergency ICU of Zagazig University Hospitals, a set of tertiary referral hospitals that serves five governorates in eastern Egypt. The Emergency ICU is a 24-bed unit that provides care for the most challenging and life-threatening acute, severely injured and critical cases.

The study was approved by the Institutional Review Board of Faculty of Medicine, Zagazig University (approval #5347-31-3-2019) and carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from patient first-degree relatives.

### Patients

Mechanically ventilated patients who met the clinical and laboratory criteria of infection-related ventilator-associated complication as described by CDC's National Health Safety Network (NHSN) in 2013 were subjected to microbiological evaluation for possible VAP.<sup>9</sup> Immunocompromised patients or those with evidence of chest infection prior to intubation were excluded.

Sample size was estimated using Epi Info 6, to be 176 VAP patients assuming that the frequency of *K. pneumoniae* among VAP patients is 43%<sup>10</sup> and the total number of possible VAP patients was 328 with 80% statistical power and 95% confidence interval.

## Microbiological Evaluation

### Specimen Collection, Culture and Bacterial Identification

Endotracheal aspirates (ETAs) were obtained under complete aseptic conditions. Microscopic examination of Gram-stained smears showing more than 25 neutrophils and less than 10 squamous epithelial cells/LPF was indicative of purulent lower respiratory secretions and possible infection.

Quantitative cultures were performed as follows: ETAs were liquefied and homogenized by vortexing for 1 minute and centrifuged at 3000 rpm for 10 minutes. They were serially diluted in sterile normal saline to 1/10, 1/100, and 1/1000, then 0.01 mL of 1/1000 dilution was inoculated on blood agar and MacConkey agar (Oxoid, UK) and incubated

aerobically at 37 °C for 48 hours. Colony count was done by multiplying the number of colonies by the dilution and inoculation factor and expressed as CFU/mL. A colony count  $\geq 10^5$  CFU/mL was considered a diagnostic growth.<sup>9,11</sup>

Isolates from 176 non-duplicate VAP samples were identified by standard bacteriological methods: colonial morphology, Gram-stained films, and biochemical tests,<sup>12</sup> and *K. pneumoniae* species was then confirmed by API-20E (BioMérieux, USA).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of *K. pneumoniae* isolates was done using the modified Kirby–Bauer disk diffusion method, and colistin minimum inhibitory concentration (MIC) value was detected by the colistin broth disk elution (CBDE) method according to the CLSI guidelines.<sup>13</sup> *K. pneumoniae* ATCC 2146 and *K. pneumoniae* ATCC 1705 were used as the quality control strains (Global Bioresource Center of American Type Culture Collection).

MDR strains were those showing non-susceptibility to one or more drugs in at least three antibiotic classes.<sup>14</sup>

### Phenotypic Determination of ESBL-Producing Isolates

*K. pneumoniae* isolates were first screened phenotypically for ESBL production according to the previous standard disk diffusion procedure by the four antimicrobial disks: Ceftazidime 30 µg, cefotaxime 30 µg, ceftriaxone 30 µg, and aztreonam 30 µg. ESBL production was considered if the inhibition zones were less than 22, 27, 25, and 27 mm respectively and was further confirmed by double disk synergy test (DDST).<sup>13</sup>

### Phenotypic Detection of Carbapenem-Resistant and Carbapenamase-Producing Strains

*K. pneumoniae* isolates that showed non-susceptibility to at least one of the four tested carbapenem antimicrobials (imipenem 10 µg, meropenem 10 µg, ertapenem 10 µg, and doripenem 10 µg) were considered carbapenem resistant and were phenotypically screened for carbapenemases production by modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) as illustrated by CLSI 2021.<sup>13</sup> Briefly, one µL loopful of *K. pneumoniae* isolates from an overnight blood agar plate was resuspended in a 2 mL tube of tryptic soy broth (TSB) and in another 2-mL tube of TSB supplemented with EDTA at a final concentration of 5mM (20 µL of 0.5M EDTA was added to 2 mL of TSB). A meropenem disk was placed in each tube, and the tubes were incubated at 35°C for 4 h. Meropenem disks were then removed and applied to Mueller Hinton agar plates freshly plated with a 0.5 McFarland suspension of a carbapenem-susceptible *E. coli* ATCC25922 strain. The plates were incubated at 35°C for 16–20 h. The diameter of the inhibition zone around each meropenem disk was then measured. As for mCIM test interpretation, a tested isolate is considered carbapenemase producer if the inhibition zone diameter is 6–15 mm or presence of pinpoint colonies within a 16- to 18-mm zone while a clear zone of diameter 19 mm or more indicates a carbapenemase-negative isolate. eCIM results were interpreted only if mCIM was positive; an isolate is positive for MBL production when the eCIM zone size increases by  $\geq 5$  mm compared to the zone size observed for the mCIM, and is negative or MBL if the increase in zone size is  $< 4$  mm.

### Molecular Detection of Carbapenemase Genes in CR *K. pneumoniae* Isolates

DNA extraction was done using GeneJET™ Genomic DNA Purification Kit (ThermoFisher Scientific, Germany). Five carbapenemase genes were sought by two multiplex PCRs; the first reaction was for genes encoding class A (*bla*<sub>KPC</sub>) and those encoding class D carbapenemases (*bla*<sub>OXA48</sub>). The other was for genes encoding class B “metallo-beta-lactamases” (MBL) (*bla*<sub>NDM</sub>, *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub>). Amplification was performed using DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Germany). Primers and conditions were as described by Poirel et al<sup>15</sup> *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-2146 were used as the positive controls for *bla*<sub>KPC</sub> and *bla*<sub>NDM</sub>, respectively.

### Antimicrobial Combination Testing

The in vitro effect of two combinations (ie, meropenem/ciprofloxacin and meropenem/colistin) was evaluated for CR *K. pneumoniae* isolates by “Perpendicular MIC test strip” method.<sup>16</sup>

MIC values of meropenem, ciprofloxacin, and colistin were determined by MIC test strip (Liofilchem, Italy) separately and in combinations and the fractional inhibitory concentration index ( $\Sigma$  FIC) was calculated for each combination using the formula:  $\Sigma$  FIC = FIC [A]+ FIC [B].

FIC [A]= MIC of drug A in combination with drug B/ MIC of drug A alone.

FIC [B]= MIC of drug B in combination with drug A/ MIC of drug B alone.

The  $\Sigma$  FIC values determine the effect of the tested combination as follows:  $\leq 0.5$ , synergy;  $>0.5$  to  $\leq 1.0$ , additively;  $>1.0$  to  $<4.0$ , indifference; and  $\geq 4$ , antagonism.<sup>17</sup>

### New Antibiotics Susceptibility Testing

The new agents obtained from (Liofilchem, Italy): ceftazidime/avibactam (CZA) (30/20  $\mu\text{g}$ ), ceftolozane/tazobactam (C/T) (30/10  $\mu\text{g}$ ) and cefiderocol (FDC) (30 $\mu\text{g}$ ) were tested against CR *K. pneumoniae* isolates using the disk diffusion method. The diameters of the inhibition zones were interpreted according to CLSI 2021 guidelines.<sup>13</sup>

### Statistical Analysis

The data were statistically analyzed using (Statistical Package for Social Science) SPSS software version 20.0. Terms of numbers and percentages were used for representation of categorical variables. The distribution differences in categorical variables were calculated using the Chi square and Fisher exact test. A p value  $<0.05$  was considered statistically significant and  $p > 0.05$  was considered non-significant.

### Results

Seventy-three (41.5%) *K. pneumoniae* isolates were recovered from 176 non duplicate endotracheal samples.

Most of the isolates were resistant to most classes of antibiotics with the highest resistance to penicillin (97.3%), cephalosporins (85%), monobactams (Aztreonam 83.6%), aminoglycoside (gentamycin 85%, and amikacin 80.8%), quinolones (ciprofloxacin and levofloxacin 85%) and carbapenems (imipenem 68.5% and meropenem 71.2%). While the least resistance was observed with doxycycline (11%) and chloramphenicol (20.5%). For colistin, 89% of the isolates were of intermediate susceptibility (MIC  $\leq 2$   $\mu\text{g}$ ) while 11% were resistant with (MIC  $\geq 4$   $\mu\text{g}$ ). Most of the isolates ( $n = 66$ , 90.4%) showed MDR phenotype. (Table 1).

A high prevalence of ESBL production was detected among *K. pneumoniae* isolates reaching 86.3%.

Recent antibiotic therapy (within 1 month prior to mechanical ventilation) and previous hospitalization of 5 days or more were significant risk factors for CR *K. pneumoniae* in VAP cases (Table 2).

Out of 52 CR *K. pneumoniae* isolates, carbapenemase production was detected in (38/52, 73.1%) using mCIM test (Table 3); of which 22 (58%) showed MBL production by eCIM test (Figure 1).

Regarding carbapenemase genes, PCR results showed that 42/52 (80.8%) of CR isolates harbored one or more of the tested carbapenemase genes. The most prevalent gene was *bla*<sub>NDM</sub>, it was found in 50% of the isolates (32.7% *bla*<sub>NDM</sub> only and 17.3% both *bla*<sub>NDM</sub> + *bla*<sub>OXA-48</sub>) Figure 2A, followed by *bla*<sub>OXA-48</sub> in 36.5% of the isolates (19.2% *bla*<sub>OXA-48</sub> only and 17.3% both *bla*<sub>NDM</sub> + *bla*<sub>OXA-48</sub>), then *bla*<sub>KPC</sub> in 11.5% of the isolates Figure 2B, while *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> were not detected. None of the studied genes were detected in (10/52, 19.2%) of the isolates. Co-existence of *bla*<sub>NDM</sub> and *bla*<sub>OXA-48</sub> carbapenemase genes were observed in (9, 17.3%) of the isolates (Table 3).

Correlating the results of the phenotypic mCIM/eCIM for carbapenemases production and PCR results for carbapenemase genes showed that, only 38 out of 42 carbapenemase gene-positive isolates showed positive results by mCIM/eCIM. In addition, four of the isolates that co-harbor *bla*<sub>OXA-48</sub> and *bla*<sub>NDM</sub> showed negative results for eCIM test (Table 3).

Meropenem MICs for the 52 CR *K. pneumoniae* isolates by E-test ranged from 8 to more than 32  $\mu\text{g}/\text{mL}$ . All these isolates were ciprofloxacin resistant, where ciprofloxacin MIC values ranged from 8 to 64  $\mu\text{g}/\text{mL}$ . Forty-four isolates (84.6%) were colistin-intermediate (MIC  $\leq 2$   $\mu\text{g}/\text{mL}$ ), and 8 isolates (15.4%) were resistant (MIC  $\geq 4$   $\mu\text{g}/\text{mL}$ ) where colistin MICs ranged from 0.125 to 16  $\mu\text{g}/\text{mL}$ .

Meropenem–ciprofloxacin combination test showed indifferent effect against all CR *K. pneumoniae* isolates. On the other hand, meropenem–colistin combination showed 25% synergism (Figure 3), 15.4% addition and 59.6% indifference. No antagonism was detected for either combination (Table 4).

**Table 1** Antibiotic Susceptibility Pattern of *K. pneumoniae* Isolates by Disk Diffusion Method (n=73)

Antibiotics	<i>K. pneumoniae</i> (n=73)		
	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
<b>Amoxicillin/clavulanate (AMC)</b>	2 (2.7)	0 (0.0)	71 (97.3)
<b>Ampicillin/sulbactam (SAM)</b>	2 (2.7)	0 (0.0)	71 (97.3)
<b>Piperacillin/tazobactam (TPZ)</b>	2 (2.7)	0 (0.0)	71 (97.3)
<b>Cefepime (FEP)</b>	11 (15)	–	62 (85)
<b>Cefotaxime (CTX)</b>	11 (15)	0 (0.0)	62 (85)
<b>Cefoxitin (FOX)</b>	11 (15)	0 (0.0)	62 (85)
<b>Ceftazidime (CAZ)</b>	11 (15)	0 (0.0)	62 (85)
<b>Aztreonam (ATM)</b>	12 (16.4)	0 (0.0)	61 (83.6)
<b>Imipenem (IMP)</b>	23 (31.5)	0 (0.0)	50 (68.5)
<b>Meropenem (MEM)</b>	21 (28.7)	0 (0.0)	52 (71.2)
<b>Ertapenem (ET)</b>	33 (45.2)	8 (11)	32 (43.8)
<b>Doripenem (DOR)</b>	37 (50.7)	6 (8.2)	30 (41.1)
<b>Gentamycin (CN)</b>	9 (12.3)	2 (2.7)	62 (85)
<b>Amikacin (AK)</b>	11 (15.1)	3 (4.1)	59 (80.8)
<b>Ciprofloxacin (CIP)</b>	11 (15.1)	0 (0.0)	62 (85)
<b>Levofloxacin (LEV)</b>	11 (15.1)	0 (0.0)	62 (85)
<b>Doxycycline (DO)</b>	60 (82.2)	5 (6.8)	8 (11)
<b>Chloramphenicol (C)</b>	54 (74)	4 (5.5)	15 (20.5)
<b>Colistin*</b>	–	65 (89)	8 (11)

Note: \*Colistin MIC value was detected by the colistin broth disk elution (CBDE) method.

**Table 2** Risk Factors Associated with VAP Caused by CR *K. pneumoniae* Isolates

Risk Factors	<i>K. pneumoniae</i> Isolates (73)				$\chi^2$	P
	CR <i>K. pneumoniae</i> (n = 52)		Non-CR <i>K. pneumoniae</i> (n = 21)			
	N	%	N	%		
<b>Age</b>						
<40 (n=51)	35	67.3	16	76.2	0.56	0.45
≥40 (n=22)	17	32.7	5	23.8		
<b>Recent antibiotics intake</b>						
Yes (n=40)	34	65.4	6	28.6	8.18	0.004*
No (n=33)	18	34.6	15	71.4		
<b>Previous hospitalization of 5 days or more</b>						
Yes (n=60)	51	98.1	9	42.9	Fisher	0.000*
No (n=13)	1	1.9	12	57.1		
<b>Duration of mechanical ventilation</b>						
<5 days (early onset VAP) (n=5)	3	5.8	2	9.5	Fisher	0.89
≥5 days (late onset VAP) (n=68)	49	94.2	19	90.5		
<b>Associated comorbidities</b>						
Yes (n=63)	44	84.6	19	90.5	Fisher	0.80
No (n=10)	8	15.4	2	9.5		

Note: \*Statistically significant at  $p \leq 0.05$ .

Regarding susceptibility to new antibiotics (52/52) 100% of CR *K. pneumoniae* isolates were resistant to ceftolozane/tazobactam and (41/52) 79% were resistant to ceftazidime/avibactam; however, (50/52) 96% were sensitive to cefiderocol. All the MBL producing isolates were resistant to CZA and C/T (100% resistance), while resistance rates in serine producing strains were 62.5% and 100%, respectively (Table 5).

**Table 3** Carbapenemase Genes Detected in CR *K. pneumoniae* Isolates and the Corresponding mCIM and eCIM Results (n=52)

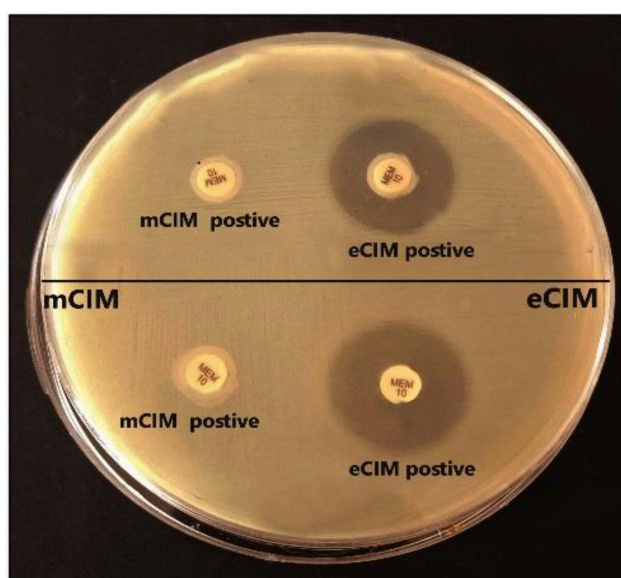
PCR results	CR <i>K. pneumoniae</i>		mCIM/eCIM Results			
	N	%	mCIM		eCIM	
			Positive	Negative	Positive	Negative
<b>Carbapenemase genes positive</b>	<b>42</b>	<b>80.8</b>				
– Class A ( <i>bla<sub>KPC</sub></i> )	6	11.5	6	0	0	6
– Class B MBL						
• <i>bla<sub>NDM</sub></i>	17	32.7	17	0	17	0
• <i>bla<sub>VIM</sub></i>	0	0.0	–	–	–	–
• <i>bla<sub>IMP</sub></i>	0	0.0	–	–	–	–
– Class D ( <i>bla<sub>OXA-48</sub></i> )	10	19.2	6	4	0	10
– Both <i>bla<sub>OXA-48</sub></i> + <i>bla<sub>NDM</sub></i>	9	17.3	9	0	5	4
<b>Carbapenemase genes negative</b>	<b>10</b>	<b>19.2</b>	<b>0</b>	<b>10</b>	<b>0</b>	<b>10</b>
<b>Total</b>	<b>52</b>		<b>38</b>	<b>14</b>	<b>22</b>	<b>30</b>

**Abbreviations:** mCIM and eCIM, modified carbapenem inactivation method and EDTA-modified carbapenem inactivation method; MBL, metallo beta lactamases.

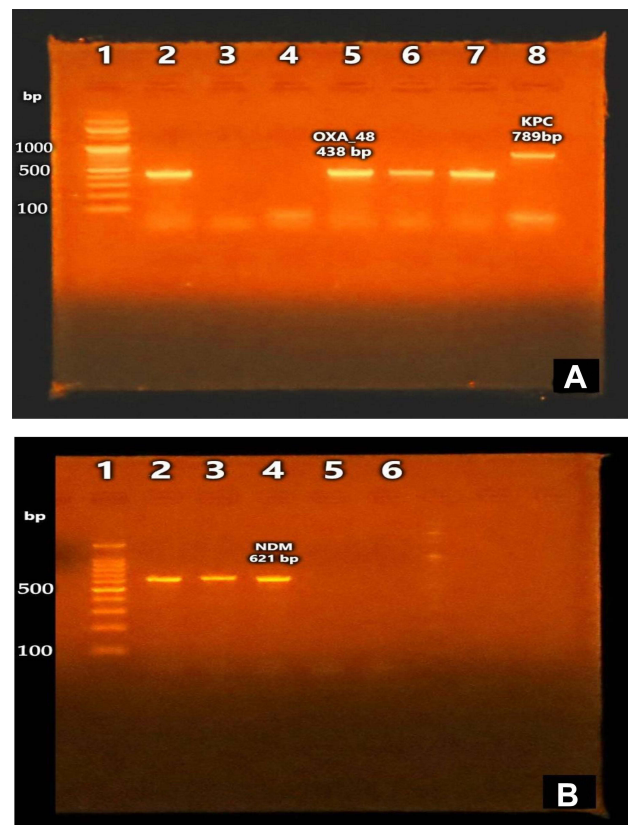
### Discussion

VAP accounts for one-fourth of the infections occurring in critically ill patients and is the reason for half of antibiotic prescriptions in mechanically ventilated patients.<sup>1</sup> CR *K. pneumoniae* is associated with high morbidity and mortality and is considered a significant public health challenge worldwide.

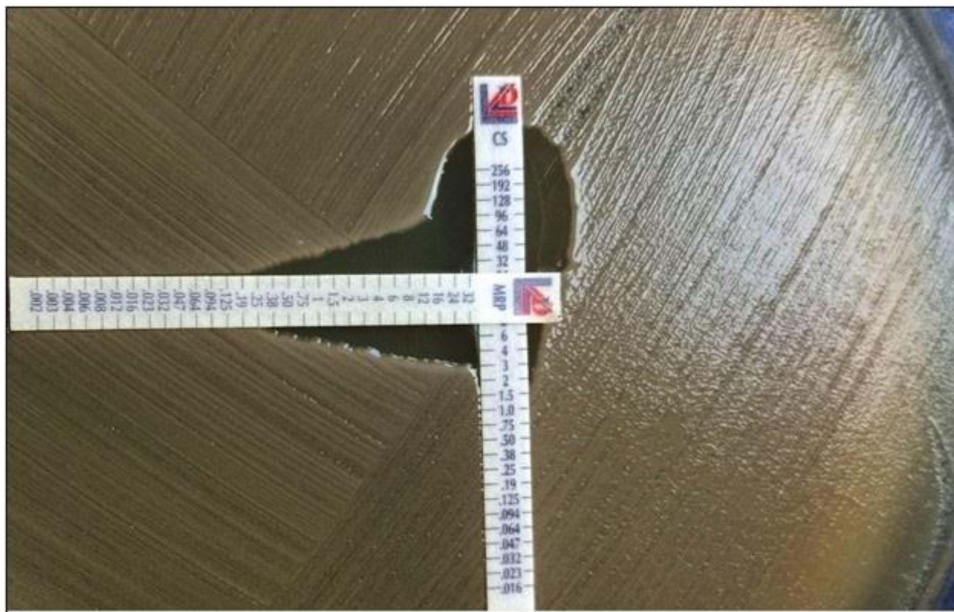
In the present study, *K. pneumoniae* was the most frequent VAP isolate with a rate of 41.5% (73/176). Similar results were obtained by a previous study on VAP in Emergency ICU in Zagazig University Hospitals where *K. pneumoniae* represented (43%) of VAP agents.<sup>10</sup> Lower results (29%) were reported by El-Kholy et al<sup>18</sup> The outstanding ability of *K. pneumoniae* to colonize skin, respiratory and gastrointestinal tracts of ICU patients as well as devices and instruments, its rapid spread between patients and its resistance to commonly used empirical antibiotics, account for the high



**Figure 1** Results of mCIM/eCIM test of 2 CR *K. pneumoniae* isolates. The two isolates show positive mCIM/eCIM test results indicating MBL production.



**Figure 2** PCR results for carbapenemase genes. **(A)** Agarose gel electrophoresis of *bla*<sub>KPC</sub> and *bla*<sub>OXA-48</sub> amplicons. Lane (1): DNA ladder 100 bp, lanes (2, 5, 6, 7): positive for *bla*<sub>OXA-48</sub> gene (438bp), lane (8): positive for *bla*<sub>KPC</sub> gene (789bp). **(B)** Agarose gel electrophoresis of *bla*<sub>NDM</sub> amplicons. Lane (1): DNA ladder 100 bp, lanes (2–4): positive for *bla*<sub>NDM</sub> gene (621bp).



**Figure 3** Meropenem–colistin combination testing. This was a synergistic effect. Meropenem MIC was >32 µg/mL when tested alone but was 0.25 µg/mL in combination. Colistin MIC was 8 µg/mL when tested alone but was 1.5 µg/mL in combination.

**Table 4** The Effect of Meropenem–Ciprofloxacin and Meropenem–Colistin Combinations on CR *K. pneumoniae* Isolates (n = 52)

Combination	Synergistic	Additive	Indifferent	Antagonistic
Meropenem–ciprofloxacin	0 (0%)	0 (0%)	52 (100%)	0 (0%)
Meropenem–colistin	13 (25%)	8 (15.4)	31 (59.6%)	0 (0%)

**Table 5** Antibiotics Susceptibility Pattern of Carbapenem-Resistant *K. pneumoniae* Isolates to New Antibiotics According to Their Carbapenemase Genes (n=52)

Antibiotic Carbapenemase Gene	CZA			C/T			FDC		
	S N (%)	I N (%)	R N (%)	S N (%)	I N (%)	R N (%)	S N (%)	I N (%)	R N (%)
<b>CR <i>Klebsiella</i> (52):</b>	11	-	41	0	0	52	50	2	0
- Carbapenemase genes negative (n=10)	21	-	79	0.0	0.0	100	96	4	0.0
- Carbapenemase genes positive (n=42)	5	-	5	0	0	10	10	0	0
• Class B MBL ( <i>bla<sub>NDM</sub></i> ) positive (n=17)	50	-	50	0.0	0.0	100	100	0.0	0.0
• Class A ( <i>bla<sub>KPC</sub></i> ) positive (n=6)	6	-	36	0	0	42	40	2	0
• Class D ( <i>bla<sub>OXA-48</sub></i> ) positive (n=10)	14.3	-	85.7	0.0	0.0	100	95.2	4.8	0.0
• Both <i>bla<sub>NDM</sub></i> + <i>OXA-48</i> positive (n=9)	0	-	17	0	0	17	17	0	0
	0.0	-	100	0.0	0.0	100	100	0.0	0.0
	2	-	4	0	0	6	6	0	0
	33.3	-	66.7	0.0	0.0	100	100	0.0	0.0
	4	-	6	0	0	10	10	0	0
	40	-	60	0.0	0.0	100	100	0.0	0.0
	0	-	9	0	0	9	7	2	0
	0.0	-	100	0.0	0.0	100	77.7	22.2	0.0

**Abbreviations:** CZA, ceftazidime–avibactam; C/T, ceftolozane–tazobactam; FDC, cefiderocol.

prevalence of *K. pneumoniae* particularly in ICU patients with multiple risk factors including invasive mechanical ventilation.<sup>19</sup>

Information about the prevalent VAP agents and their susceptibility to antimicrobials is regularly required to guide initial empirical therapy. In the present study, antibiotic resistance was high among *K. pneumoniae* isolates even to typically recommended antibiotics for the treatment of VAP. High resistance was detected with penicillins (97.3%) and cephalosporins (85%). Similarly, high rates of resistance were noticed for quinolones; 85% for each of ciprofloxacin and levofloxacin, and for aminoglycosides; 85% for gentamycin and 80.8% for amikacin. More or less similar high rates were reported by other studies.<sup>10,20–22</sup> A high rate of carbapenem resistance was noticed where 71.2% of *K. pneumoniae* were carbapenem resistant, which is near to the results of a previous study in different ICUs in our facility.<sup>20</sup> The high rates of resistance are due to rapid horizontal transmission of resistance determinants between different species that is augmented by non-adherence to infection control standards.

Multidrug resistance among Gram-negative bacilli is a growing problem in hospital settings that led to the resurrection of polymyxin antibiotics as last options in life-threatening infections by these superbugs. In our study, 90.4% of *K. pneumoniae* isolates were MDR; this high rate was comparable to Azzab et al, who reported a similar rate of MDR *K. pneumoniae* causing VAP.<sup>10</sup> Colistin resistance, however, was detected in 11% of our isolates (MIC ≥ 4 µg) which goes in line with a recent study conducted on hospitalized patients at Egyptian National Cancer Institute where 8.8% of MDR *K. pneumoniae* isolates were colistin-resistant.<sup>23</sup>



Evaluating the risk factors of VAP caused by CR *K. pneumoniae* revealed that recent antibiotic use (1 month prior to mechanical ventilation) and previous hospitalization of 5 or more days were significant risk factors. Other studies reported similar findings as Patro et al.<sup>24</sup> Both prolonged hospitalization and the use of antibiotics enhances patients' upper airway and endotracheal tube colonization with MDR bacteria that finally gain access to the lower airways causing pneumonia. In this perspective, routine surveillance endotracheal cultures, that detect colonizers, are proposed as predictors of invasive infection and a guide for empirical therapy in case VAP develops.

In the present study, the prevalence of ESBL-producing *K. pneumoniae* was extremely high among our isolates representing 86.3%. Similarly, a high rate (80%) was reported by another Egyptian study by Muhammed et al.<sup>25</sup> However, these are much higher than ESBL rates reported in many European countries that range from 2.4% to 5.1%.<sup>26</sup> The less controlled use of antibiotics in Egypt, where many drugs are still available over the counter, and the over reliance on third-generation cephalosporins, being broad spectrum and safe drugs, led to this high rate of ESBL production.

In fact, the hospital authorities and staff did not stand idly by on the issue of antibiotic resistance and since 2016, a comprehensive infection control program was started comprising bundle care to reduce hospital-acquired and device-associated infections, and thus reduce antibiotics use.<sup>27</sup> Moreover, an antibiogram was developed in 2019 to rationalize antibiotic prescription,<sup>20</sup> but unfortunately, these endeavors were hampered by the emergence of COVID-19 global pandemic and shortage of medical staff. In addition, being a tertiary hospital, the majority of the patients most probably have received medical care at some primary centers before admission where they get colonized with resistant pathogens and consequently these pathogens are continuously introduced into the hospital environment.

Carbapenem resistance is due to the production of carbapenemases or a combination of porin deficiency and other  $\beta$ -lactamases. As the susceptibility results of new antibiotics differ according to the type of carbapenemase, whether serine (KPC or OXA-48) or MBL, CR *K. pneumoniae* isolates were screened for carbapenemases production by mCIM and eCIM tests and were tested for detection of carbapenemase genes by PCR. In this study, 38 (73.1%) of the CR isolates were carbapenemase producers, of which 22 (58%) were MBL producers. Other studies reported carbapenemases as the main carbapenem-resistance mechanism;<sup>28</sup> Mohamed et al, in a previous Egyptian study, detected carbapenemase activity in 61.1% of the *Klebsiella* isolates; 66.2% of them were MBL producers.<sup>29</sup>

PCR results for the commonly reported carbapenemase genes in Enterobacterales revealed that 80.8% of the isolates harbored carbapenemase genes, which is in concordance with another Egyptian study where carbapenemase genes were detected in 74% of the isolates.<sup>30</sup> The most prevalent gene was *bla*<sub>NDM</sub> (50%) followed by the *bla*<sub>OXA-48</sub> (36.5%). This finding agrees with another study on Enterobacterales from Egyptian cancer patients,<sup>31</sup> but Raheel et al reported *bla*<sub>OXA-48</sub> as the most common gene followed by *bla*<sub>NDM</sub>.<sup>28</sup> Predominance of *bla*<sub>NDM</sub> could be related to the fact that they are encoded on a variety of readily transmissible conjugative plasmids that are capable of horizontal inter- and intra-species transfer.<sup>32</sup> A low incidence rate of *bla*<sub>KPC</sub> (11.5%) was reported in our study, which is lower than Azzab et al, who reported a rate of 23.1%.<sup>10</sup> Absence of *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> genes in our study was also reported by other studies<sup>30,32</sup> suggesting that both genes are not prevalent in our geographical region. In addition, *bla*<sub>VIM</sub> genes are uncommon among Enterobacterales.<sup>33</sup> Co-existence of carbapenemase genes was observed where *bla*<sub>NDM</sub> and *bla*<sub>OXA48</sub> were both detected in 17.3% of the isolates, a similar finding was reported by Raheel et al and Ahmed El-Domany et al.<sup>28,30</sup> On the other hand, 10 (19.2%) of the CR isolates were negative for carbapenemase genes; these isolates may possess other mechanisms of carbapenem resistance such as ESBL production coupled with disruption in porin expression.<sup>34</sup>

Four of the isolates that harbored carbapenemase genes showed negative results by mCIM. In addition, eCIM failed to detect MBL production in four isolates that co-harbor *bla*<sub>NDM</sub> and *bla*<sub>OXA-48</sub>; the co-production of a serine carbapenemase had probably masked the inhibitory effect of EDTA on the accompanying MBL. Moreover, carbapenemase genes may be not expressed or may be truncated producing nonfunctioning enzymes.<sup>35</sup> Therefore, phenotypic detection of carbapenemases production is better coupled to molecular detection of carbapenemase genes, particularly if targeted antibiotic therapy with one of the new drugs is to be considered as discussed later.

Antibiotic combination therapy and novel antibiotics are the current available strategies for treatment of infections by CR GNB. Combination therapy has long been considered as the standard for treating severe infections by CR GNB.<sup>6</sup>  $\beta$ -

lactams and fluoroquinolones have been frequently used in our ICU for empiric treatment of VAP and in the present study, the in vitro effect of meropenem–ciprofloxacin combination was compared to meropenem–colistin combination against CR *K. pneumoniae* isolates. Meropenem–ciprofloxacin combination showed indifferent effect against all tested isolates. This is similar to Karki et al, who reported 100% indifferent effect of this combination when tested on XDR *K. pneumoniae* isolates.<sup>36</sup> On the other hand, Sueke et al reported 10% synergy, 80% addition and 10% indifference for the same combination.<sup>16</sup>

Synergy testing of meropenem–colistin combination, showed 25% synergism, 15.4% addition and 59.6% indifference. No antagonism was observed for this combination. Better results with this combination were reported by a recent Indian study where the effect of meropenem–colistin combination tested by the checkerboard technique showed 56% synergism, 36% addition and indifference rate of 8%.<sup>37</sup>

The favorable outcomes of the colistin-based combinations result from its ability to disrupt the outer membrane of Gram-negative bacteria by targeting lipopolysaccharides which increases the outer membrane permeability to the other drug. On the other hand, discrepant effects of antimicrobial combinations in different studies could be due to different methods of synergy testing with different interpretation methods and synergy definitions. Moreover, different genetic constitution of the tested isolates and the different antibiotic selective pressures possibly influence their response to drug combinations.

Regarding susceptibility to new antibiotics, resistance rates to ceftazidime/avibactam and ceftolozane/tazobactam were 79% and 100%, respectively. These are extremely high rates of resistance in comparison to the results of the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2017, which reported susceptibility rates of ceftazidime/avibactam and ceftolozane/tazobactam for *K. pneumoniae* isolates of 100% and 80%, respectively.<sup>38</sup> All the MBL-producing isolates were resistant to CZA and C/T (100% resistance), while resistance rates in serine-producing strains were 62.5% and 100%, respectively. These findings partially agree with previous studies, which stated that most MBL-positive strains were resistant to CZA and C/T, and the resistance rates ranged from 90.8% to 100%.<sup>39</sup>

Avibactam is not effective against class B (MBL) but was found effective against serine carbapenemases by reversibly acylating them<sup>40</sup> and its reported resistance rates in serine carbapenemase-producing strains ranged from (16.7–21%) which contradicts our high rates of resistance.<sup>39</sup> Chemical modification of the target, presence of multiple (or even novel) beta-lactamases, changes in cell permeability and expression of efflux pumps are suggested causes for resistance exerted by non-MBL isolates. It is worth mentioning that avibactam, by its potent activity against class A  $\beta$ -lactamases (as ESBL) and AmpC-type determinants, restores the activity of aztreonam against MBL-producing Gram-negative bacteria that co-produce these  $\beta$ -lactamases that hydrolyze aztreonam.<sup>41</sup> Consequently, CZA plus aztreonam combination was recommended for treatment of MBL-producing Enterobacterales.<sup>42</sup>

Cefiderocol had a good activity against our isolates as 96% of the isolates were sensitive, This was similar to a previous study that reported 100% sensitivity to cefiderocol.<sup>43</sup> Resistance to cefiderocol was also reported by previous studies where resistant mutants as well as cross resistance with ceftazidime/avibactam have been described.<sup>44</sup> Also, Yamano suggested that cefiderocol resistance could be attributed to simultaneous production of *bla*<sub>NDM</sub> and some serine- $\beta$ -lactamases.<sup>45</sup>

The previous findings point out to an alarming situation that might be encountered in the future, particularly with the continuous worsening of the issue of carbapenem and multidrug resistance in Egypt.

## Conclusion

In this study, high resistance rates were detected among *K. pneumoniae* isolates to carbapenems and the tested new  $\beta$ -lactam/ $\beta$ -lactamase inhibitors. Meropenem–colistin combination appears to be a potential treatment option for infections caused by CR *K. pneumoniae* isolates but cefiderocol is superior for its greater in vitro activity, low resistance rates, stability to most carbapenemase classes and low toxicity, particularly if carbapenem-sparing strategies are to be implemented.

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