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In Vitro Activity of Single and Combined Antibiotics against Carbapenem Resistant *Enterobacteriaceae* Clinical Isolates in Relation to their Resistance Genes

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Abstract

BACKGROUND: Mortality due to infection with carbapenem-resistant *Enterobacteriaceae* (CRE) is reported globally and carbapenemase production is the main mechanism of resistance in these isolates. The detection and treatment of carbapenemase-producing *Enterobacteriaceae* (CPE) are a major challenge in health care facilities.

AIM: The aim of the current study was to evaluate the *in vitro* effect of different single and combined antibiotic agents against CRE clinical isolates.

METHODS: Out of total 775 *Enterobacteriaceae* clinical isolates, fifty CRE isolates were detected using disk diffusion test as a screening test. Species identification and antibiotic susceptibility testing was done using Vitek 2 system. Carbapenemase enzyme production was confirmed by Carba NP test. Multiplex PCR was done to detect carbapenem resistance genes. Antibiotics were tested in the form of single agents (colistin and tigecycline) and combined (tigecycline/colistin, doripenem/colistin and dual carbapenem therapy (ertapenem and doripenem) against CRE isolates using E-test method.

RESULTS: Most of the CRE isolates were *Klebsiella pneumoniae*, 68%, followed by *Escherichia coli*, 22%, *Serratia marcescens*, 4%, *Enterobacter cloacae*, 4%, and *Citrobacter freundii*, 2%. CPE was confirmed in 46 isolates by multiplex PCR; $bla_{\text{NDM-like}}$ was the main carbapenem resistance gene in (84%) of the isolates, followed by $bla_{\text{OXA-48-like}}$ (6%) and $bla_{\text{KPC-like}}$ (2%). Carba NP test detected 90% of CPE isolates. Single use of colistin and tigecycline showed 100% sensitivity against all tested CRE isolates except in $bla_{\text{NDM-like}}$ (83%). Combination of colistin/tigecycline showed synergetic activity in 18% of CRE that was correlated to their carbapenemase R genes showing a significant increase in $bla_{\text{OXA-48-like}}$ and $bla_{\text{KPC-like}}$ positive isolates (100%) compared to $bla_{\text{NDM-like}}$ (7%). Other combinations showed indifferent effect whereas antagonism was not detected in any of the tested combinations.

CONCLUSIONS: $bla_{\text{NDM-like}}$ is the main carbapenemase-producing gene detected among our CPE isolates followed by $bla_{\text{OXA-48-like}}$. Colistin and tigecycline are still effective when used as single agents and may offer effective treatment options when used in combination for CRE infections. Characterization of carbapenemases is crucial in determining treatment options. There is urgent demand for the development of novel therapeutic agents against NDM-producing CPE isolates.

Introduction

Antibiotic resistance (AMR) is a global health threat especially to developing countries limiting their planned attainment on sustainable development goals [1], [49]. Carbapenems, with their broad spectrum antibacterial activity, have long been considered as one of the most reliable drugs for treating bacterial infections. Hence, the emergence of resistance to carbapenems represents a major health concern [2]. *Enterobacteriacea* are among the most common human pathogens. They cause wide array of hospital as well as community acquired infections including urinary tract, respiratory tract, bloodstream, intra-abdominal, skin, and soft-tissue infections [3], [46]. AMR is worsened with the emergence of carbapenem resistant *Enterobacteriaceae* (CRE) due to the excessive inadequate and uncontrolled use of carbapenems against multidrug-resistant (MDR) bacteria and the rapidly disseminating extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* [4], [5].

The most important mechanism of carbapenem resistance in CRE is the production of carbapenemases. Other mechanisms of carbapenem resistance among *Enterobacteriaceae* include excessive production of ESBL and/or AmpC enzymes in combination with loss of outer membrane protein or up-regulation of efflux pump [6], [45].

Carabapenemases identified in *Enterobacteriaceae* belong to either of the three Ambler classes of β-lactamases: A, B, and D [7]. Class A carbapenemases include the plasmid encoded *Klebsiella pneumoniae* carbapenemases (KPC)

as well as other uncommon enzymes; IMI-2, GES derivatives [8]. KPC enzymes, being the commonest class A carbapenemases in CRE, hydrolyze all β-lactams (including cephamycins at a low level) and their activity is only inhibited partially *in vitro* by clavulanic acid, tazobactam, and boronic acid [9]. However, this class of carbapenemases is not common in the Middle East except in Israel [10]. Class B includes the metallo-β-lactamases (MBLs) which include the New Delhi metallo-lactamase (NDM-1), the latest and the most alarming [8]. Death rates associated with carabapenemases producing *Enterobacteriaceae* (CPE) producers range from 18% to 67% with MBL [11]. The OXA-48–type producers (group D β-lactamases), with their weak hydrolytic activity, are likely the most difficult carbapenemases to be detected in the lab. Thus, their true prevalence could be underestimated. The attributed mortality rate from infections with OXA-48 producers is still unknown [8].

It is reported previously that human mobility is the main cause in the dissemination and the transmission of CPE in endemic areas such as KPC from the USA, Greece, and Israel, VIMs from Greece, OXA-48 from Turkey, and NDMs from the Indian subcontinent. Different types of carbapenemases were reported in the Middle East however OXA-48 and NDM are the most commonly reported enzymes [2], [10].

Regarding antibiotic treatment, there is an urgent need for new antibiotics for the treatment of these infections. It is not settled up till now whether combination or monotherapy antibiotic regimens are more effective against CRE [12]. Some studies show that a significant proportion of CPE are still susceptible to imipenem, meropenem or doripenem (and only rarely to ertapenem) [8]. Tigecycline, which was approved by the Food and Drug Administration in 2005, as well as the “old” antibiotics colistin and fosfomycin, which have been revived, and aminoglycosides are among the last resort treatment options for CRE infections [13].

Combination therapy including tigecycline with colistin, carbapenem in combination with colistin, and dual carbapenem therapy was introduced as possible treatment regimens subjected for more studies [12], [13].

In the present study, we aimed to evaluate the *in vitro* effect of different single and combined antibiotic agents against CRE clinical isolates and the possible relation to the carbapenemase-producing genes.

Methods

Bacterial isolates and collection

Various clinical samples were collected from outpatient clinic attendants and hospitalized patients of Theodor Bilharz Research Institute During the period from January 2016 to February 2017, 866 Gram-negative bacilli were isolated, out of which 775 isolates were identified as *Enterobacteriaceae.* Clinical samples were cultured on microbiological media and isolates recovered were identified microscopically and biochemically to species level.

Confirmation of species identification and antibiotic sensitivity for each isolate was done by Vitek 2 compact system (BioMérieux, France)

Minimum inhibitory concentrations (MICs) to different antibiotics were determined using Vitek 2 compact system. Antibiotics tested include ampicillins, cephalosporins (cefazolin, ceftriazone, and cefepime), aztreonam, cabapenems (imipenem, meropenem, and ertapenem), aminoglycosides (amikacin, gentamycin, and tobramycin), quionolones (ciprofloxacin and moxifloxacin), tigecyclin, nitrofurantoin, and combinations (trimethoprime/sulfamethoxazole and ampicillin/sulbactam). Results were interpreted according to the European Committee of antibiotic susceptibility testing (EUCAST) [15].

Determination of MIC of carbapenems by E-test

MICs of the four carbapenems (imipenem, meropenem, doripenem, and ertapenem) of CRE isolates were detected by E-test *(Bio-Mérieux, France)* and results were interpreted according to EUCAST breakpoints [15] (Table 1).

Table 1: MIC breakpoints of suspected carbapenemase producers (EUCAST, 2017)

Agent	Sensitive	Resistant
Doripenem	≤1	≥4
Ertapenem	≤0.5	≥1
Imipenem	≤2	≥8
Meropenem	≤2	≥8

Detection of carbapenemase activity using Rapidec Carba NP test (Bio-Mérieux, France)

Which is a colorimetric test that detects carbapenem hydrolysis through acidification of the medium resulting from imipenem hydrolysis, which results in the change in color of the pH indicator from red to yellow/orange [16].

Assessment of susceptibility to single and combined antibiotic options

Single antibiotic options

MIC of colistin and tigecycline were detected by E-test *(Bio-Mérieux, France)*: Resistance to each of these antibiotics was defined when MIC was ≥2 mg/L according to EUCAST recommendations [15].

Combined antibiotic options tested included

Tigecycline/Colistin., Doripenem/colistin and Doripenem/Ertapenem. Antibiotic interaction testing was performed using the E-test method in which two strips one containing antibiotic A and another containing antibiotic B were aligned at 90° at the respective MICs (mg/L) of the two antibiotics, MIC A and MIC B (Figure 1). To evaluate interactions between antibiotics, the fraction inhibitory concentration index (FICI) was calculated according to the formula: FICI = (MIC of antibiotic A in combination/MIC of antibiotic A alone) + (MIC of antibiotic B in combination/MIC of antibiotic B alone). Antibiotic combinations were evaluated based on the FICI as follows: <1 (synergistic); ≥1 but <4 (indifferent); and ≥4 (antagonistic) [17], [18].

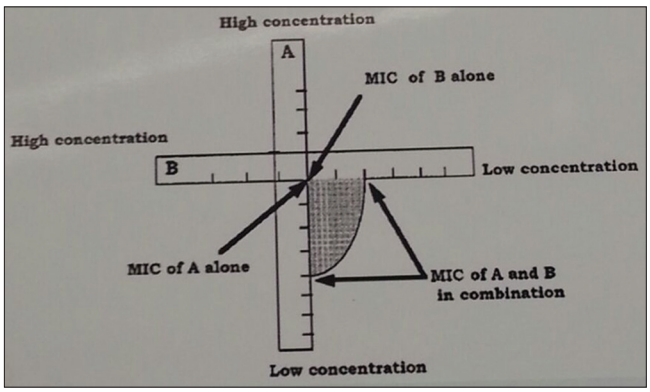

Figure 1: Evaluation of antibiotic interactions using E-test method [18]

Genotypic detection of carbapenamase-producing genes using multiplex PCR

DNA extraction was done by boiling method. Master mix was prepared using primers for carbapenemase-producing genes (Table 2). Master mix samples without DNA were used as negative controls. Amplification was done with the following thermal cycling conditions: 10 min at 94°C and 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C, with 5 min at 72°C for final extension. Amplified products were stored at −20°C until use [19]. Detection of PCR amplified products using gel electrophoresis for separating and analyzing mixtures of charged molecules. Images of the stained DNA bands were captured using a gel documentation system (Cleaver Scientific, UK).

Table 2: Primers for different carbapenem resistant genes and expected band sizes

Target gene	Nucleotide sequences (5'–3')	Amplicon size (bp*)
bla_{VIM}	F: GATGGTGTTTGGTCGCATA	390
	R: CGAATGCGCAGCACCAG	
bla_{IMP}	F: GGAATAGAGTGGCTTAAYTCTC	232
	R: GGTTTAAYAAAACAACCACC	
bla_{NDM}	F: GGTTTGGCGATCTGGTTTTC	621
	R: CGGAATGGCTCATCACGATC	
bla_{OXA-48}	F: GCGTGGTTAAGGATGAACAC	438
	R: CATCAAGTTCAACCCAACCG	
bla_{KPC}	F: CGTCTAGTTCTGCTGTGTTG	798
	R: CTTGTCATCCTTGTTAGGCG	

*bp: Base pair.

Statistical analysis

Data entry, processing, and statistical analysis were carried out using MedCalc ver. 15.8. Tests of significance (Chi-square, Kruskal-Wallis and Kappa statistical tests) were used. Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. $p < 0.05$ (5%) was considered to be statistically significant.

Results

1-phenotypic detection of carbapenem resistance

Out of 775 *Enterobacteriaceae* clinical isolates, fifty CRE isolates that showed resistance to either one or all of the tested carbapenems by disk diffusion were recovered from urine specimens (28/50; 56%), wound swabs (7/50; 14%), sputum (6/50; 12%), blood (6/50; 12%), and ascitic fluid (3/50; 6%).

Vitek 2 compact system confirmed identification of recovered isolates. *K. pneumoniae* was the most common (n = 34, 68%), followed by *Escherichia coli* (n = 11, 22%), *Enterobacter cloacae* (n = 2, 4%), and *Serratia marcescens* (n = 2, 4%), while *Citrobacter freundi* was only one isolate (2%). Most of the CRE were isolated from hospitalized patients (80%) particularly from urology department (28%), followed by ICU (26%) and from outpatient clinic (8%).

2-antibiotic susceptibility using Vitek 2 and E tests

Vitek 2 compact system showed that the tested isolates were 100% resistance to β-lactam antibiotics (ceftazidime, azteronam, and cefipime), 98% to quinolones (ciprofloxacin), and 82% to trimethoprim/sulfamethoxazole. Resistance to aminoglycosides ranged from 84% for gentamicin, to 94% for amikacin. Comparison between different antibiotic susceptibilities revealed significant increase in susceptibility to gentamcin 8/50 (16%) and trimethoprim/sulfamethoxazole 9/50 (18%) compared to the remaining antibiotics ($p < 0.0001$).

In vitro activity of different carbapenems tested by E test showed that: 9 of 34 (26.5%) of *K. pneumoniae* isolates were sensitive to doripenem, 2 of 34 (6%) were imepenem sensitive and none were sensitive to either meropenem or ertapenem. All other isolated species showed complete resistance to the four tested carbapenems.

3-PCR detection of carbapenem resistant genes

The most common detected gene was bla_{NDM} that was found in 42 isolates (42/50; 84%). Its distribution

among the species was: 31 in *K. pneumoniae*, 9 in *E. coli* and 2 in *E. cloacae* isolates. Three isolates were positive for bla_{OXA-48}; one *K. pneumoniae* and two *E. coli* isolates (3/50; 6%), bla_{KPC} was detected in only *Citrobacter freundii* isolate (1/50; 2%) while bla_{VIM} and bla_{IMP} were not detected in any of the tested isolates (Figure 2 and Table 3).

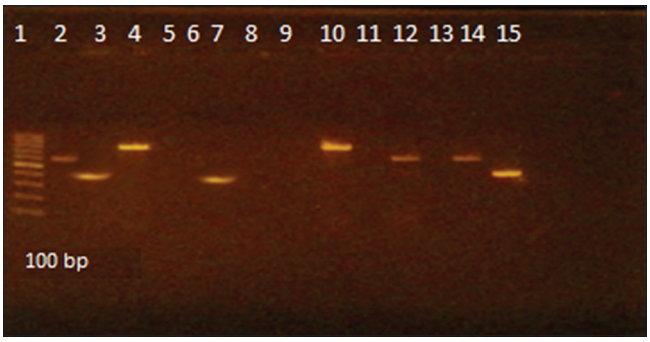

Figure 2: (2%) Agarose gel electrophoresis of multiplex PCR products
Lane 1: L; Molecular weight marker (ladder 100 bp)
Lane 2: P; Positive control for blaNDM
Lane 3: P; Positive control for blaOXA-48
Lane 4: P; Positive control for blaKPC
Lane 5: N; Negative control
Lane (7 and 15): Positive DNA samples blaOXA-48 PCR amplification product
Lane (10): Positive DNA samples blaKPC PCR amplification product
Lane (12 and 14): Positive DNA samples blaNDM PCR amplification product

4-carba NP results in relation to PCR results

45 CPE were detected by Carba NP (45/50; 90%). Complete agreement was detected between the results of carba NP test and multiplex PCR regarding bla_{NDM} and bla_{KPC} (Kappa = 1), whereas poor agreement between the 2 tests was found regarding bla_{OXA-48} (Kappa = 0).

5- In vitro activity of single and combined antibiotics against CRE isolates

All CRE isolates were completely susceptible to colistin (100%), whereas 42 isolates were sensitive to tigecycline (84%) by E-test. Antibiotic interactions testing using the E-test FICI method showed that synergy was only detected with colistin/tigecycline combination (9/50; 18%) ($p < 0.0001$), but was not detected in the other tested combinations. Indifference was observed in colistin/doripenem and ertapenem/doripenem combinations (50/50; 100%). Antagonism was not detected in any of the tested combinations (Table 4 and Figure 3).

Table 4: Results of antibiotic combinations against CRE isolates

Antibiotics in combination	Synergy n (%)	Indifference n (%)	Antagonism n (%)
Colistin with tigecycline	9 (18)**	41 (82)	0 (0)
Colistin with doripenem	0 (0)	50 (100)	0 (0)
Doripenem with ertapenem	0 (0)	50 (100)	0 (0)

$p < 0.0001$.

6-antibiotic interactions in CRE isolates in relation to resistance genes

Synergy between colistin and tigecycline was detected in 9/50 CRE isolates; in three OXA-48- producing isolates (100% of all OXA-48 positive isolates) and in one KPC isolate (100% of the totally detected KPC gene) ($p = 0.0001$). Whereas only three NDM-positive isolates (7% of all NDM positive) showed synergy as well as in two isolates with unidentified genetic basis (positive carba NP and negative with PCR to all tested genes). Indifference was detected in all of the other tested combinations (100%). Antagonism was not detected in any of the tested combinations (Table 5).

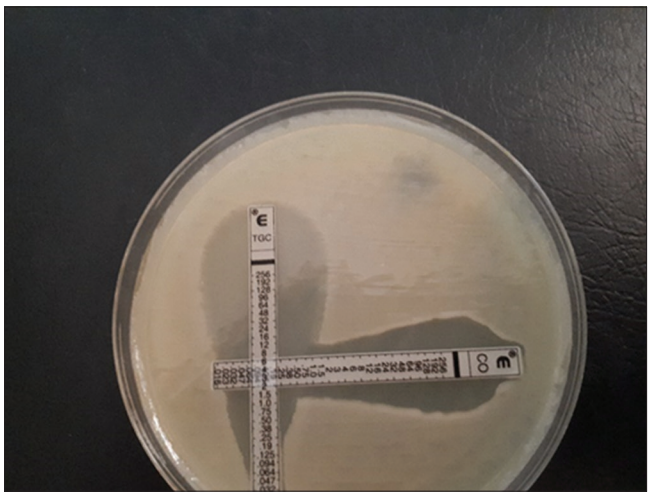

Figure 3: CRE isolate showing synergistic effect between tigecycline and colistin (FICI = 0.3) where MIC of colistin alone was 0.064 mg/L, and MIC of tigecycline alone was 1.5 mg/L

Comparative study between different resistance genes in relation to the tested combinations of antibiotics showed that: colistin/tigecycline combination had significantly higher synergy in OXA-48 and KPC positive genes compared to NDM gene ($p = 0.0001$). Whereas colistin/doripenem and ertapenem/doripenem

Table 3: Distribution of carbapenemase resistance genes among different species of CRE isolates

Carbapenemase resistance genes	*Klebsiella pneumoniae* n (%)	*Escherichia coli* n (%)	*Enterobacter cloacae* n (%)	*Serratia marcescens* n (%)	*Citrobacter freundii* n (%)	Total n (%)
	34 (68)	11 (22)	2 (4)	2 (4)	1 (2)	50 (100)
NDM	31 (91.2)	9 (81.9)	2 (100)	0 (0)	0 (0)	42 (84)
KPC	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (2)
OXA-48	1 (2.9)	2 (9.1)	0 (0)	0 (0)	0 (0)	3 (6)
VIM	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
IMP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

E. coli: *Escherichia coli*, *E. cloacae*: *Enterobacter cloacae*, *K. pneumonia*: *Klebsiella pneumonia*.

Table 5: Antibiotic interactions in CRE isolates in relation to resistance genes

Ertapenem/Doripenem		Colistin/Doripenem		Colistin/Tigecycline		Resistance genes
Indifference (%)	Synergy (%)	Indifference (%)	Synergy (%)	Indifference (%)	Synergy (%)	
42 (100)	0 (0)	42 (100)	0 (0)	39 (93)	3 (7)	NDM (42)
3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)**	OXA (3)
1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)**	KPC (1)

p = 0.0001.

combinations revealed indifference between all three genes; with no significant statistical difference (p = 1.0000, respectively).

Discussion

The emergence and dissemination of CRE are associated with worsening in the clinical outcomes, longer hospitalization periods, excess in mortality and increase in the burden and the costs of the health-care infrastructure [1], [46]. The Middle East is now an endemic region for CPE strains that could be transmitted to other parts of the world with lower prevalence by the extensive international exchange [2]. The shortage of novel antibiotic drugs in the past few years with substantial activity against CRE improvises the development of novel therapeutic options [44], [49].

The aim of the current study was to test the *in vitro* effect of single antibiotic agents: colistin and tigecycline as well as combined antibiotic agents: Tigecycline/Colistin, doripenem/colistin, and doripenem/ertapenem against CRE clinical isolates in our region in relation to their resistance genes.

In the present study, the frequency of carbapenem resistance among *K. pneumoniae* (68%) was higher than that of *E. coli* (22%) and *E. cloacae* (4%). Similar results were found in other studies from China, Taiwan, and Egypt where *K. pneumoniae* accounted for 47.8%, 53.6%, and 47%; respectively [21], [22], [23]. *K. pneumoniae* has been the most identified CRE species representing about 75% of all reported strains from Middle East countries [2]. This confirms the global special concern that has been given to carbapenem-resistant *K. pneumoniae* (CRKp) isolates and their worsening epidemiological situation in the Mediterranean countries in the last years [47].

Categorization of clinical CRE infection sources showed that urine specimens represented the majority (56%) of specimens harboring CRE. Our results were in agreement with those of other studies where CRE were mostly recovered from urine specimens representing 42% and 40%, respectively [21], [24]. This alarming finding raises the attention that CRE may be currently considered as a prominent cause of urinary tract infection [47].

In the present study, CRE were detected phenotypically using disk diffusion test and confirmed carbapenemase production was done by Carba NP test. Results were confirmed using multiplex PCR technique.

The overall detection rate by carba NP test was 90%. This was in line with two other studies in which the overall detection rate of carbapenem resistance by carba NP test reached 89.2% and 95%, respectively [26], [27]. In this study, comparing results of carba NP test to multiplex PCR showed false-negative results regarding OXA-48 producing isolates, this is in agreement with many other studies who found that carba NP test gives false-negative results OXA-48 and OXA-48-like enzymes [26], [27], [31], [32]. This might be explained by the known low hydrolytic activity of OXA-48 like enzymes [26], [48].

Carbapenemase resistance genes were detected in 92% of the CRE isolates where bla_{NDM} was the most prevalent (84%), followed by bla_{OXA-48} (6%) and only one isolate with bla_{KPC} (2%). This was in line with the results of a Mumbai study that found a high rate of carbapenemase production (more than 98%) with similar distribution of the studied genes; bla_{NDM} (76.5%); bla_{OXA-48} (4.5%); with no detection of $bla_{VIM,}$ bla_{IMP}, and bla_{KPC} [28]. The dissemination of OXA-48-like and NDM-like carbapenemases was reported in all Middle East countries. NDM was also the most commonly detected carbapenemase-producing gene in other studies from South Africa (59%) and from Kuwait (34%) [29], [30]. A recent review included 492 strains in 23 Egyptian published articles on carbapenemase-producing *Enterobacteriaceae* reported the production of NDM-like as the main enzyme produced in 70.5% (n = 130), followed by OXA-48-like in 20.7% (n = 102), then VIM (n = 32) then sporadic cases of IMP (n = 12), SIM (n = 1) and SME (n = 2_ and GIM (n = 1), whereas KPC was detected in combination with other carbapenemases as NDM, OXA-48, VIM, and IMP. The same review involved other studies from Iran, Turkey, Israel, Lebanon, Saudi Arabia, UAE, Oman, Jordan, Kuwait, Palestine, Yemen, and Qatar showed that OXA-48-like was most frequently identified (48.6%), followed by NDM-like (17.7%), KPC-like (13.4%), and VIM-like (5.1%) [2].

Antibiotic sensitivity testing to CRE isolates showed resistance to all groups of tested antibiotics, ranging from 84% to gentamycin, 82% against SXT to 100% against β-lactams. Similar results were reported from India where resistance ranged from 65.2% against SXT to 100% against β-lactams [21], Using the E-test for testing MIC of different carbapenems, 18% of the isolates showed sensitivity to doripenem and 30% were intermediately susceptible, with wider MIC range than that for other carbapenems. This may be due to the suggestion that doripenem has high stability against the hydrolyzing activity of KPC- producing *Enterobacteriaceae* [33], [34]. Other carbapenems showed minor or no activity against

the studied CRE isolates (imipenem showed 4% sensitivity whereas meropenem and ertapenem both showed 0%). This is in accordance with other study that also showed that doripenem had better *in vitro* activity than that of meropenem and imipenem against challenging Gram-negative pathogens, including resistant enteric bacilli [35].

All CRE isolates in the current study were completely susceptible to colistin 100%, and 84% were susceptible to tigecycline by E-test. This is in agreement with previous study from Egypt which reported that susceptibility to colistin and tigecycline were 98% and 81%, respectively [23]. Other studies also showed that CRE strains are often reported to be susceptible to these drugs [12], [16], [36]. Whereas, another study from Taiwan reported that only 58.3% and 50% of the tested isolates were susceptible to colistin and tigecycline, respectively [22].

Although CRE strains are often reported to be susceptible to polymyxins (polymyxin B and colistin) and tigecycline, there are concerns with the use of these drugs as monotheraputic agents, with debate over how to safely dose colistin and warnings over the efficacy of tigecycline in the treatment of bloodstream and other serious infections [37], [38]. Rapid emergence of resistance has also been documented with these two agents if either one is used alone in the treatment of MDR Gram-negative infections [39]. Thus, these antibiotics are often used clinically in combination to improve their antibiotic activity [13]. Colistin mechanism of action is mainly through disruption of the bacterial outer-membrane and thus facilitating tigecycline uptake allowing a potentiation of the tigecycline\colistin combination [13].

In this study, we assessed the activity of colistin in combination with tigecycline and in combination with doripenem against CRE isolates *in vitro* by using the E-test FICI method. Combination of colistin and tigecycline showed synergistic effect in 18%, in contrast to the previous studies where higher rates of synergy of 80% and 47% were reported respectively for colistin and tigecycline combination [36], [40]. On the other hand, combination of colistin with doripenem showed 100% indifference whereas better results were reported previously of 80%, 82%, and 90% synergy between colistin and doripenem among the tested isolates [33], [41], [42]. In this study, synergy between colistin and tigecycline was detected mostly in Non-NDM- producing CRE isolates only; 100% of all OXA-48 and KPC isolates whereas only 7% of NDM-producing CRE isolates as well as two isolates with uncharacterized carabapenemase production showed synergistic effect.

In contrast, no synergy was detected in any of the other tested combinations. This comes along with the results of two other studies who found that more than 90% of detected CRE isolates that showed synergistic activity in different combination therapy regimens belong to Class A carbapenemases (KPC), while the rest of the isolates harbored genes for Class B metallo-β-lactamases and Class D carbapenemases (OXA-48) [34], [42]. A recent study reported the successful combination of colistin and tigecycline as a therapeutic alternative for infection caused by CRE *E. coli* that harbored both bla_{NDM-5} and *mcr-1* [13].

The association of two synergistic carbapenems (ertapenem plus either meropenem, doripenem, or imipenem), alone or combined with other antibiotics, has been proposed as rescue treatment for CRE infections [34]. Such combination therapy relies on the use of ertapenem as a suicide antibiotic facing carbapenemase activity produced by CRE isolates. Hence, it should be given 1 h before the main carbapenem, thus allowing the active carbapenem to express stronger activity at the site of infection. However, the data are encouraging, there are limited *in vitro* studies [42]. In this study indifferent effect resulted from ertapenem combination with doripenem against all CRE isolates using the E-test method. Better results were reported by two other studies who found ertapenem and doripenem combination synergistic in 78% and 73% of the tested isolates, respectively [34], [42]. This difference in the success rates of combination therapies might be attributed to the difference in the types of carbapenemases produced by the isolates in different studies [43].

Conclusions

NDM is the main carbapenemase detected among CRE isolates in our hospital. Our study emphasizes that colistin and tigecycline continue to be active against most CRE, however with the reported emergence of resistance against both agents if used as monotheraputic options, combination of both antibiotics is a possible successful option for treatment of CRE infections. Characterization of carbapenemases is imperative for deciding treatment options in CRE isolates. Additional *in vivo* studies are mandatory to assess the performance of this combination against various types of carbapenemases produced by different CRE isolates. There is crucial demand for the development of novel therapeutic agents against NDM-producing CPE isolates.

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