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Original Article

Antimicrobial agents active against carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in Lebanon

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Abstract

Introduction**:** It is not yet clear which antimicrobial agents should be used to treat the ominously increasing infections with carbapenem-resistant (CR) bacteria. We therefore investigated the activity of different antimicrobial agents against CR *Escherichia coli* and *Klebsiella pneumoniae* in Lebanon.

Methodology: This retrospective study assessed the minimum inhibitory concentrations (MICs) of three carbapenems (by Etest), as well as the *in vitro* activity of eight other antimicrobials (by disk diffusion) against CR *E. coli* (n = 300) and *K. pneumoniae* (n = 232) isolates recovered at a major University Medical Center in Lebanon.

Results: Higher percentages of isolates showing carbapenem MICs of ≤ 8 µg/mL were noted among the CR *E. coli* compared to the CR *K. pneumoniae* for ertapenem (48% vs 27%), imipenem (74 % vs 58%) and meropenem (82% vs 63%). Among the eight other antimicrobials, activity was generally higher when the MICs for the three carbapenems were ≤ 8 µg/mL. Regardless of the MIC level of the three carbapenems, very low susceptibility rates (≤ 33%) were noted for ciprofloxacin, trimethoprim-sulfamethoxazole and aztreonam against both *E. coli* and *K. pneumoniae* isolates. With Amikacin, higher susceptibility rates were seen against *E. coli* isolates (81%-97%) than against *K. pneumoniae* isolates (55%-86%), also reflecting higher activity than gentamicin (44%-54%). The best activity (66%-100%) was observed for tigecycline, colistin and fosfomycin against both CR species.

Conclusions: Based on the in vitro findings in this study, the combination of a carbapenem showing an MIC of ≤ 8 µg/mL together with an active colistin, tigecycline, or fosfomycin, would offer a promising treatment option for patients infected with CR *E. coli* or *K. pneumoniae*.

Key words: Carbapenem-resistance; *E. coli*; *K. pneumoniae*; *in vitro* testing; antimicrobial resistance; Lebanon.

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Introduction

Globally, infection with carbapenem resistant (CR) *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (Kp) as well as other CR Enterobacteriaceae (CRE) had been on the rise, often accompanied by high rates of resistance to a wide range of antimicrobials. These pathogens, together with the dwindling antimicrobial armament, constitute a most concerning contemporary threat to health in many countries [1-3]. The Center for Disease Control and Prevention in the USA noted an increase in the percentages of CRE from 1.2% in 2001 to 4.2% in 2011, where *Klebsiella* spp. accounted for the highest increase in proportion, a rise from 1.6% to 10.4% [4]. In Lebanon, a notable increase was observed at our medical center in the percentages of CR, from 2010 to 2016 for Ec (0.1% to 5%) and Kp (0.7% to 8%), as well as in other hospitals [5,6].

The ominous spread of these pathogens together with the scarcity of treatment options results in a serious health impact, seen in high rates of morbidity and mortality (up to 75% of infected patients) and increasing health costs. This situation is a major challenge for the treating clinicians and infection control professionals [1-3,7,8]. In Lebanon, similar problems and challenges have been encountered since CRE pathogens were introduced in this country several years ago [9,10]. As a result, several studies have been conducted to describe the phenotypic and molecular features of CR *E. coli* and *K. pneumoniae* in this country [5,11-15].

Because of the very limited available options to treat CR isolates, this study was undertaken to identify the types and percentages of antimicrobial agents active against CR *E. coli* and *K. pneumoniae* resistant to ertapenem, imipenem, and/or meropenem.

Methodology

Bacterial Isolates

Consecutive non-duplicate isolates of carbapenem resistant *E. coli* (300 isolates) and *K. pneumoniae* (232 isolates) at the Clinical Microbiology Laboratory, Department of Pathology and Laboratory Medicine,

American University of Beirut Medical Center (AUBMC) during the period March 2008 to June 2016 were investigated. Identification of the isolates was based on standard biochemical methods [5].

The source distribution of these *E. coli* and *K. pneumoniae* isolates was: urine (39% and 32%), body screen (19% and 23%), wound/pus/abscess (17% and 18%), blood (10% and 9%), respiratory (9% and 5%), and other fluids (6% and 13%).

Antimicrobial Susceptibility Testing

The minimal inhibitory concentrations (MICs) of ertapenem, imipenem and meropenem were determined using the Etest methodology (AB BIODISK, Solna, Sweden) according to the manufacturer's guidelines. The span of MIC levels on these strips ranges between ≤ 0.025 and ≥_32 µg/mL. The 2016 Clinical and Laboratory Standards Institute (CLSI) MICs' breakpoints (µg/mL) for Enterobacteriaceae were used to interpret the susceptibility category as susceptible, intermediate and resistant, respectively; for ertapenem: 0.5, 1, 2; for imipenem: 1, 2, 4; for meropenem: 1, 2, 4 [16].

The disk diffusion (DD) antimicrobial susceptibility testing (except for colistin) was done and interpreted according to the CLSI standards [16]. Commercial disks (BBL, Becton Dickinson, USA) were used, with antimicrobial concentrations of: amikacin 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg, trimethoprim/sulfamethoxazole 1.25/23.75 µg, tigecyline 15 µg, colistin 10 µg, fosfomycin/trometamal (200 µg), and aztreonam 30 µg. Colistin disk diffusion was used and interpreted according to the study of Gelani *et al*. [17], where the susceptible, intermediate and resistant zone of inhibition were ≤ 11 mm, 12-13 mm, and ≥ 14 mm, respectively.

For screening of carbapenemase-producers, ertapenem disks (10 µg) were used. Those isolates showing < 25 mm zone of inhibition were then tested by Etest to confirm the MICs of ertapenem, imipenem and meropenem.

Quality Control

The American Type Culture Collection (ATCC) quality control strains of *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used to ensure correct performance of the Etest and disk diffusion methods. When received in February 2016, the quality control reference strains for colistin [CDC *E. coli* reference strains MCR-1 (AR-Bank # 0349, specified colistin MIC of 2-4 µg/mL) and MCR-1 (AR-Bank # 0346, specified colistin MIC of 4 µg/mL)] were also used to ensure the quality of testing.

Statistical Analysis

Our data was analyzed using Stata v. 13 software Package (Stata Corp LP, College Station, Texas, USA) for determining the p value, considering less than ≤ 0.05 as significant.

Results

The $MICs_{50}$ (µg/mL) for ertapenem, imipenem and meropenem against the CR *E. coli* (n = 300) and *K. pneumoniae* (n = 232) isolates were 8 and 24, 2 and 4 and 1 and 4, respectively. The $MICs_{90}$ (µg/mL) for these carbapenems were ≥ 32 ug/mL against both *E. coli* and *K. pneumoniae* isolates.

The distribution of the MICs (µg/mL) for ertapenem, imipenem and meropenem among these CR *E. coli* and *K. pneumoniae* isolates are shown in Figures 1 and 2. The MIC (µg/mL) level for the three carbapenems ranged between ≤ 0.25 and ≥ 32. The

Figure 1. Distribution of carbapenem MICs among *E. coli* isolates (n = 300).

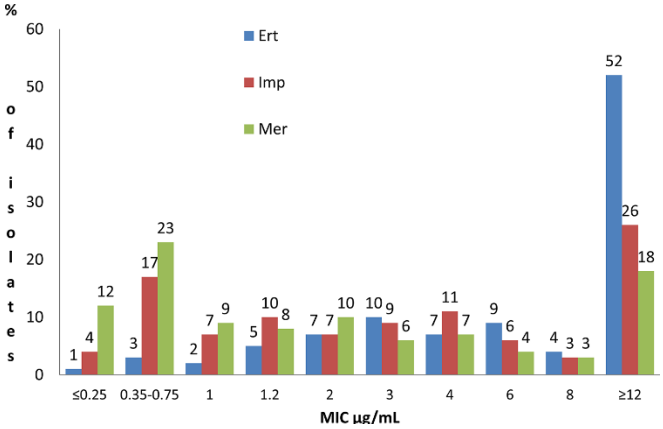
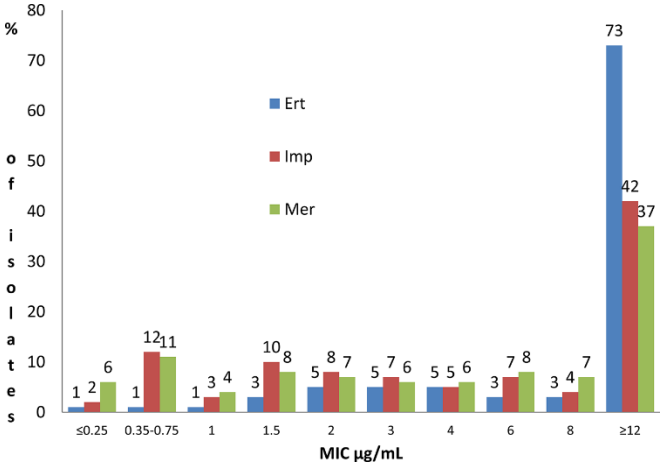


Figure 2. Distribution of carbapenem MICs among *K. pneumoniae* isolates (n = 232).



figures reflect the distribution of the MICs up to ≥ 12 µg/mL and not up to ≥ 32 µg/mL due to space limitation.

The distribution of the CR *E. coli* and *K. pneumoniae* isolates according to their overall carbapenems MICs, as well as carbapenems MICs of ≤ 8 or ≥ 8 µg/mL in relation to their susceptible rates to other antimicrobial agents, namely: amikacin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, tigecycline, colistin, fosfomycin and aztreonam are presented in Table1.

Three of the antimicrobial agents tested, ciprofloxacin, trimethoprim-sulfamethoxazole and aztreonam, showed very low susceptibility rates (≤ 33%) regardless of the MICs level against both *E. coli* and *K. pneumoniae* isolates, and with little difference among the three carbapenems. Among aminoglycosides, the susceptibility rates of gentamicin were low, ranging between 44% and 54%, for both *E. coli* and *K. pneumoniae* isolates, and with little difference among the 3 carbapenems. On the other hand, the *E. coli* isolates showed higher susceptibility rates to amikacin (range: 81% to 97%), compared to the *K. pneumoniae* isolates (range: 55% and 86%), as noted for the three carbapenems and regardless of the MICs category. Tigecycline, colistin and fosfomycin showed high activity (66%-100%) against both CR *E. coli* and *K. pneumoniae,* regardless of the MICs levels of ertapenem, imipenem and meropenem (Table 1).

Discussion

The clinical microbiology laboratory (CML) has a key role in promptly detecting and reporting multi-drug resistant (MDR) pathogens to guide targeted antimicrobial therapy, which will influence outcome. In this context, the CML at AUBMC reported the first alert about CRE in Lebanon, when the first imipenem resistant *K. pneumoniae* that harbors the *bla*-OXA-48 gene was recovered from the urine of an 8-year- old girl in 2008 [9].

Two years later (July 2010), three Iraqi patients presented at our medical center seeking treatment. CR *E. coli* and *K. pneumoniae* were isolated from different specimens of these patients, and the isolates harbored not only the *bla*-OXA-48 gene but also the very threatening *bla*-NDM-1 gene, producing the novel New Delhi Metallo ß- lactamase (MBL) gene [10]. Subsequently, the incidence of CR Enterobacteriaceae continued to rise [5]. Such critically resistant pathogens warranted publishing an overview to inform the medical and paramedical community on the local and regional epidemiology of carbapenem resistance, the mechanisms involved, screening and detection methods, as well as their treatment and control [11].

Table 1. Distribution of CR *E. coli* (n = 300) and *K. pneumoniae* (232) isolates according to carbapenem MICs in relation to susceptibility rates (%) of eight other antimicrobial agents.

Carbapenems & MICs (µg/mL)	CRE	Percent (%) of susceptible isolates to antimicrobial agents							
		AMK	GENT	CIP	SXT	TIGE[H]	COLI[H]	FOSFO[H]	AZT
Ertapenem									
≤ 8	*E. coli*	97*	53	28	16	100	82	89	20*
> 8	*E. coli*	88	47	11	16	99	86	93	10
All	*E. coli*	89	51	19	16	99	84	89	15
≤ 8	*K. pneumoniae*	84*	40	33	27	87	94*	86*	30*
> 8	*K. pneumoniae*	68	49	24	21	92	79	67	23
All	*K. pneumoniae*	73	47	15	23	90	82	73	25
Imipenem									
≤ 8	*E. coli*	94*	48	19	14	100	89*	94*	15
> 8	*E. coli*	85	54	16	23	98	74	87	16
All	*E. coli*	92	53	19	17	99	84	89	15
≤ 8	*K. pneumoniae*	86*	44	29	24	89	94*	83*	25
> 8	*K. pneumoniae*	57	49	21	21	94	73	61	24
All	*K. pneumoniae*	77	48	26	23	93	83	68	25
Meropenem									
≤ 8	*E. coli*	94*	49	21*	17	100	85	92*	15
> 8	*E. coli*	81	52	8	12	98	82	86	13
All	*E. coli*	92	51	19	15	99	83	91	14
≤ 8	*K. pneumoniae*	86*	45	31	25	89	88*	76*	30
> 8	*K. pneumoniae*	55	51	16	19	94	76	61	23
All	*K. pneumoniae*	73	47	26	24	92	84	66	25

CR: Carbapenem resistant; AMK: amikacin; GEN: gentamicin; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; TIGe: tigecycline; COLI: colistin; FOSFO: fosfomycin; AZT: aztreonam; *p value significance ≤ 0.05 determined between MICs ≤ 8µg/mL and > 8µg/mL; [H]The number of *E. coli* and K. *pneumoniae* isolates tested, respectively, were: tigecycline (198 and 160 isolates), colistin (156 and 125 isolates), fosfomycin (124 and 68 isolates).

Generally, physicians rely on the *in vitro* antimicrobial susceptibility results of pathogens to provide optimum therapy to their patients. Empirical combination therapy for CRE infected patients is usually based on the local resistance epidemiology. Definitive therapy, however, should be guided by determination of the *in vitro* antimicrobial susceptibility profiles especially when considering combined treatment for CRE pathogens. This is very important since only very few drugs remain as last-resort agents (e.g. colistin, fosfomycin, tigecycline, and aminoglycosides) [18-22].

In our study, the *in vitro* activity of these last-resort agents against the CR *E. coli* and *K. pneumoniae* isolates detected at our institution are the core of the following discussion. These results will be considered especially in relation to updates reported in the recent scientific literature [18,19].

Colistin has significant activity against various carbapenemase-producing isolates and is often used in combination therapy (e.g., with aminoglycosides, aztreonam, carbapenems, rifampin, tigecycline, or fosfomycin) with a high (82.1%) clinical cure rate [18,23,24]. Caution in its use is advised, to avoid development of colistin-resistant Ec & Kp. In our study, the overall high susceptibility rates (82%-84 %) of both Ec and Kp to colistin makes it an important first option to use in the treatment of such CR infections (Table 1).

Fosfomycin has also been considered as part of a combination regimen (including at least one more active agent, e.g. with tigecycline and colistin) in the treatment of invasive infections with CRE pathogens [25]. Its use in combinations, however, resulted in varied synergistic activity against CRE depending on the type of pathogen and the antibiotics used [26]. For example, Samonis *et al*. [27] reported that the synergistic effect of fosfomycin was 55% to 79% with carbapenems, 7.1% to 36% with colistin, 21% to 30% with tigecycline, and 25% to 43% with gentamicin. In this study, fosfomycin showed high activity (89-91%) against the CR Ec, while the susceptibility of CR Kp was lower (66-73%). We conclude that fosfomycin can remain a viable option for the treatment of CR pathogens especially Ec (Table 1). These levels are close to those we reported in 2012 on the fosfomycin susceptibility of ESBL-producing pathogens, where its activity was higher against ESBL-producing *E. coli isolates* (86%) than against *K. pneumoniae* isolates (62%) [28].

Aminoglycosides have been used in combination with other classes of antibiotics with different results [18,27]. For example, some *K. pneumoniae* carbapenemase (KPC) and OXA-48 -producers in *K. pneumoniae* isolates remain susceptible to gentamicin, while this is rare for New Delhi metallo beta-lactamase (NDM) - producers. An *in vitro* synergistic effect was observed when netilmicin and fosfomycin were tested against a wide range of resistant pathogens. Also, a combination of carbapenems and aminoglycosides showed *in vitro* and *in vivo* (in animals) synergistic effects against some KPC-producing *K. pneumoniae* isolates, but studies with carbapenemases other than KPC are lacking [18,27]. A lack of effect was noted when a combination of gentamicin and fosfomycin was tested against KPC-2-producing *K. pneumoniae*. In our current study, the overall higher activity of amikacin compared to that of gentamicin against both CR Ec and Kp may be attributed to the type of carbapenemase gene involved. For example, in the clinical setting, a study from Greece reported a favorable outcome in five patients infected with KPC-2-producing *K. pneumoniae* who were treated with aminoglycoside plus colistin (plus tigecycline in 2 patients) [29].

Tigecycline has also been recommended as part of the initial combination regimen in the treatment of patients with CRE infections in different body sites, other than UTI, especially when tigecycline MIC is ≤ 1 mg/L (the typical MIC_{s90} was 0.5 mg/L for *E. coli* and 1–2 mg/L for *K. pneumoniae*). The overall crude mortality when treating with tigecycline was higher with monotherapy than with combined treatment (40–80% vs 0–33%) [18]. In our study, tigecycline showed very high *in vitro* activity against both CR Ec and Kp, making it an excellent agent in combined treatment options for such CR pathogens. Low *in vitro* resistance rates against tigecycline were also reported in a 2008 study from our institution, in MDR and ESBL Ec (0%) and Kp (3%) [29]. The very low rates noted in the previous and current studies indicate a sustainable activity of tigecycline against resistant isolates at our center.

Aztreonam testing was included in this study because MBLs can hydrolyze carbapenems and all the available beta-lactams with the exception of aztreonam. Whether aztreonam remains an option for treating infections due to MBL producers that test susceptible to this agent remains to be determined; currently there is no clinical experience with aztreonam for the treatment of invasive infections due to CRE [30]. In our study, however, a very low aztreonam susceptibility (14-25%) was shown by both CR Ec and CR Kp isolates, indicating that the majority of these isolates were not MBL producers. An earlier study on the CR genes involved in *K. pneumoniae* and *E. coli* isolates from our

center indicated the prevalence of *bla*-OXA-1, *bla*-CTXM-15, *bla*-TEM-1, *bla*-CMY-2, *bla*-OXA-48, and porin channel genes, and few had the NDM-1 gene [12].

The antimicrobial agents discussed above have been considered for possible clinical relevance to treat patients infected with CRE. The question of the superior effectiveness of mono- or combined antimicrobial treatment of patients infected with CRE pathogens was excellently reviewed by Rodríguez-Baño and colleagues in Spain and Karaiskos and Giamarellou in Greece, looking at microbiological diagnosis and treatment of infections with carbapenemase-producing Enterobacteriaceae [18,19]. These comprehensive studies suggest a more favorable outcome (e.g. reduced mortality) using a combined antimicrobial treatment for patients infected with CRE pathogens. Besides, combined therapy may maximize bacterial killing (synergistic effect) and minimize bacterial resistance. For example, Tumbarello *et al*. 2012 [31] reported a higher mortality rate among patients treated with monotherapy compared to combined therapy (54.3% vs 34.1%); the combination included tigecycline, colistin, and meropenem.

In addition, the inclusion of a carbapenem in the combined treatment showed promising results. For example, Qureshi *et al*., reported lower mortality when carbapenem was used in combination with colistin or tigecycline, compared to a combination that lacked a carbapenem (12.5% vs 66.7%) [32]. Moreover, the effect of carbapenems in the combination was noticeable if the carbapenem MIC was ≤ 8 mg/L, and it was added to one or two *in vitro* fully active drugs (including colistin, tigecycline, an aminoglycoside or fosfomycin) for such infections. Carbapenems, despite being hydrolyzed by carbapenemases, may retain some activity against carbapenemase-producing isolates [18,19,33,34]. Such findings were vividly reflected in the study by Daikos *et al*. [20] who reported a lower mortality rate with treatment by a carbapenem-containing combination with another active drug, such as an aminoglycoside, colistin or tigecycline, than with a combination without a carbapenem (19.3% vs 30.6%). It was important that the carbapenem had a MIC ≤ 8 mg/L [20]. Akova *et al*. found similar results [35].

Interestingly, the efficacy of using dual carbapenem (i.e., meropenem plus ertapenem) therapy has been shown in animal model of infection (i.e., mouse pneumonia) and in humans infected with KPC producers. The suggested explanation was that ertapenem most likely acts as a "suicide" molecule for carbapenemase activity, whereas the more active drug, meropenem, retains its efficacy [36].

In our study the carbapenem rates of MIC ≤ 8 mg/L for ertapenem, imipenem and meropenem were higher when tested against *E. coli*: 48%, 74% and 82%, respectively, than against *K. pneumoniae*: 27%, 58% and 63%, respectively. Such rates, especially for imipenem and meropenem, in combination with the other *in vitro* active agents, can provide an adequate option in treating patients, particularly those with CR *E. coli* infection.

Studies at our medical center since 2008 have been addressing molecular characterizations, resistance mechanisms, genes involved, and other aspects of CR Ec and Kp isolates [9,10,12-14]. The first CRE detected in Lebanon was a *K. pneumoniae* recovered from a 7-year-old female child, which was characterized by PCR experiments using primers for multiple β-lactamase and carbapenemase genes: TEM, SHV, CTX-M, GES, KPC, IMI, OXA-1/2/OXA-10/18, OXA-23/58, IMP, VIM, SPM, GIM and SIM. The only positive result was for a *bla*OXA-48-like gene [9]. Interestingly, this was only the fourth report about this gene worldwide. Subsequently, increasing numbers of CRE have been noticed; some harbored the very threatening *bla* NDM-1 gene, detected for the first time in Lebanon, in *E. coli* and *K. pneumoniae* isolates recovered from Iraqi patients coming for medical care at AUBMC [10].

The underlying mechanisms and genes responsible for the carbapenem-resistance in *K. pneumoniae* and *E.* coli revealed the presence of different Beta-lactamase gene profiles including: *bla*-OXA-1, *bla*-CTXM-15, *bla*-TEM-1, *bla*-CMY-2, *bla*-OXA-48 and NDM-1 genes in both genera; in addition, the *K. pneumoniae* isolates were found to lack outer membrane porin (OmpC and OmpF) encoding genes, while *E. coli* harbored these porin genes [12]. In addition, the prevalence of carbapenem resistance encoding genes and their correlation with corresponding MIC_{90} against ertapenem, imipenem and meropenem were studied among CR *E. coli* (n=76) and CR *K. pneumoniae* (n=54) isolates. The prevalence of *bla*OXA-48, *bla*NDM-1, *bla*TEM-1 and *bla*CTX-M-15 among the *E. coli* isolates were 36%, 12%, 20% and 80%, respectively, while among *K. pneumoniae* isolates they were 37%, 28%, 28% and 72%, respectively. The presence of more than one carbapenem resistance encoding gene and/ or ESBL encoding gene did not have an effect on the MIC_{90} value in *K. pneumoniae* isolates, while in *E. coli* they resulted in higher MIC_{90} values [14].

We also assessed the effects of antimicrobial combination therapy against bacteria with different CR genes using BALB/c mice [13]. The mice were injected

with carbapenem-resistant Enterobacteriaceae strains, harboring either $bla_{\text{CTXM-15}}$, $bla_{\text{CTXM-15}}$ and $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, or $bla_{\text{KPC-2}}$ genes. The qRT-PCR revealed a significant decrease of transcript levels in all isolates upon using rifampicin or tigecycline, singly or in combination with colistin. However, variable levels were obtained using colistin singly or in combination with meropenem or fosfomycin. *In vivo* assessment showed that all combinations used were effective against isolates harboring $bla_{\text{CTXM-15}}$, $bla_{\text{OXA-48}}$, and $bla_{\text{NDM-1}}$. Conversely, the most significant combination against the isolate harboring the $bla_{\text{KPC-2}}$ gene was colistin with either carbapenem, fosfomycin, or kanamycin. Essentially, the findings indicated that combination therapy selected based on the type of carbapenemase produced, appeared to be non-toxic and effective in BALB/c mice [13]. Therefore, an approach based on gene detection may be useful to extrapolate for human use, as a step towards optimizing combination therapy and antimicrobial stewardship when in treating patients with CRE infections. The testing and logistics involved would, however, have to be feasible for the prompt and real time therapeutic practice.

The search for new and effective antimicrobial agents for optimal treatment of these highly threatening pathogens is continuing, especially for one that can cope with covering all genes involved in CR status. For example, one of the most recently introduced agents is avibactam with ceftazidime. Although this new agent showed promise for treating infections due to *K. pneumoniae* KPC producers, it lacks effectiveness against NDM producers [23].

Conclusions

The in vitro susceptibility testing of CRE and other pathogens is of utmost importance to provide local antimicrobial resistance epidemiologic data for selection of possible treatments. Our *in vitro* study highlighted the available options of antimicrobial agents to treat infections with CR *K. pneumoniae* and *E. coli* isolates in our institution. A larger nationwide study would be needed to reflect the overall situation in Lebanon. However, the control of such pathogens necessitates not only this technical information but also active and adequate antimicrobial stewardship program together with appropriate infection control measures following expert guidelines [30].

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References

1. Marston HD, Dixon DM, Knisely JM, Palmore TN, Fauci AS (2016) Antimicrobial Resistance. JAMA 316: 1193-1204.
2. Antimicrobial resistance: global report on surveillance (2014) World Health Organization, Geneva, Switzerland. Available:http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf. Accessed: 12 December 2016.
3. Theuretzbacher U (2017) Global antimicrobial resistance in Gram-negative pathogens and clinical need. Curr Opin Microbiol 39: 106–112.
4. Centers for Disease Control and Prevention (2013) Carbapenem-resistant Enterobacteriaceae. Morb Mortal Wkly Rep 62: 165–170.
5. Araj GF, Avedissian AZ, Ayyash NS, Bey HA, El Asmar RG, Hammoud RZ, Itani LY, Mallak MR, Sabai SA (2012) A reflection on bacterial resistance to antimicrobial agents at a major tertiary care center in Lebanon over a decade. Leb Med J 60: 125-135.
6. Chamoun K, Farah M, Araj G, Daoud Z, Salameh P, Saadeh D, Mokhbat J, Abboud E, Hamze M, Abboud E, Jisr T, Haddad A, Feghali R, Azar N, El-Zaatari M, Chedid M, Haddad C, Zouein_Dib Nehme M, Angelique Barakat M and Husni R (2016) Surveillance of antimicrobial resistance in Lebanon: A nationwide compiled data. Int J Infect Dis 46: 64-70.
7. Chang HJ, Hsu PC, Yang CC, Kuo AJ, Chia JH, Wu TL, Lee MH (2011) Risk factors and outcomes of carbapenem-nonsusceptible *Escherichia coli* bacteremia: a matched case-control study. J Microbiol Immunol Infect 44: 125–130
8. Ben-David D, Kordevani R, Keller N, Tal I, Marzel A, Gal-Mor O, Maor Y, Rahav G (2012) Outcome of carbapenem resistant *Klebsiella pneumoniae* bloodstream infections. Clin Microbiol Infect 18: 54–60.
9. Matar GM, Cuzon G, Araj GF, Naas T, Corkill J, Kattar MM, Nordmann P (2008) Oxacillinase-mediated resistance to carbapenems in *Klebsiella pneumoniae* from Lebanon. Clin Microbiol Infect 14: 887-888.
10. El-Herte RI, Araj GF, Matar GM, Baroud M, Kanafani ZA, Kanj SS (2012) Detection of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* producing NDM-1 in Lebanon. J Infect Dev Ctries 6: 457-461. https://doi.org/10.3855/jidc.2340
11. El-Herte RI, Kanj SS, Matar GM, Araj GF (2012) The threat of carbapenem-resistant Enterobacteriaceae in Lebanon: An update on the regional and local epidemiology. J Infect Public Health *5*: 233–243.
12. Baroud M, Dandache I, Araj GF, Wakim R, Kanj S, Kanafani Z, Khairallah M, Sabra A, Shehab M, Dbaibo G, Matar GM (2013) Underlying mechanisms of carbapenem resistance in extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates at a tertiary care center in Lebanon: Role of OXA-48 and NDM-1 carbapenemases. Int J Antimicrob Agents 41: 75-79.
13. Salloum N, Kohar K, Cheaito K, Araj GF, Wakim R, Kanj S, Kanafani Z, Dbaibo G, Matar GM (2015) Assessment of combination therapy in BALB/c mice injected with carbapenem-resistant Enterobacteriaceae strains harboring various carbapenemase encoding genes. Front Microbiol 6: 999.
14. Kissoyan KAB, Araj GF, Matar GM (2016) Prevalence of carbapenem resistance encoding genes and corresponding

MIC_{90} in Enterobacteriaceae at a tertiary medical care center in Lebanon. Int Arab J Antimicrob Agents 6: 5.
15. Tokajian S, Eisen JA, Jospin G, Matar G, Araj GF, Coil DA (2016). Draft genome sequence of *Klebsiella pneumoniae* KGM-IMP216 harboring *bla*CTX-M-15, *bla*DHA-1, *bla*TEM-1B, *bla*NDM-1, *bla*SHV-28, and *bla*OXA-1, isolated from a patient in Lebanon. Genome Announc 4:e01632-15.
16. Clinical and Laboratory Standards Institute (2016) Performance standards for antimicrobial susceptibility testing, 26th information supplement. CLSI document M100-S26 (ISBN 1-56238-924-6).
17. Galani I, Kontopidou F, Souli M, Rekatsina P-D, Koratzanis E, Deliolanis J, Giamarellou H (2008) Colistin susceptibility testing by Etest and disk diffusion methods. Intl J Antimic Agents 31: 434–439.
18. Rodríguez-Baño J, Cisneros JM, Cobos-Trigueros N, Fresco G, Navarro-San Francisco C, Gudiol C, Horcajada JP, López-Cerero L,Martinez JA, Molina J, Montero M, Pano-Pardo JR, Pascual A, Pena C, Pintado V, Retamar P, Tomas M, Borges-Sa M, Garnacho-Montero J, Bou G, Study Group of Nosocomial Infections (GEIH) of the Spanish Society of Infectious Diseases, Infectious Diseases (SEIMC) (2015). Diagnosis and antimicrobial treatment of invasive infections due to multidrug-resistant Enterobacteriaceae. Guidelines of the Spanish Society of Infectious Diseases and Clinical Microbiology. Enferm Infecc Microbiol Clin 33:337. e1–337.e21.
19. Karaiskos I, Giamarellou H (2014) Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: Current and emerging therapeutic approaches. Expert Opin Pharmacother 15: 1351–1370.
20. Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, Stefanou I, Sypsa V, Miriagou V, Nepka M, Georgiadou S, Markogiannakis A, Goukos D, Skoutelis A (2014) Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: Lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 58: 2322–2328.
21. Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends Mol Med 18: 263–272.
22. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS (2014) Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: Systematic evaluation of the available evidence. Antimicrob Agents Chemother 58: 654–663.
23. Temkin E, Adler A, Lerner A, Carmeli Y (2014) Carbapenem-resistant Enterobacteriaceae: Biology, epidemiology, and management. Ann N Y Acad Sci 1323: 22–42.
24. Michalopoulos AS, Tsiodras S, Rellos K, Mentzelopoulos S, Falagas ME (2005) Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: The renaissance of an old antibiotic. Clin Microbiol Infect 11: 115–121.
25. Pontikis K, Karaiskos I, Bastani S, Dimopoulos G, Kalogirou M, Katsiari M, Oikonomou A, Poulakou G, Roilides E, Giamarellou H (2014) Outcomes of critically ill intensive care unit patients treated with fosfomycin for infections due to pandrug-resistant and extensively drug resistant carbapenemase-producing Gram-negative bacteria. Int J Antimicrob Agents 43: 52–59.
26. Falagas ME, Kastoris AC, Kapaskelis AM, Karagoergopoulos DE (2010) Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing Enterobacteriaceae infections: A systematic review. Lancet Infect Dis.10: 43–50.
27. Samonis G, Maraki S, Karageorgopoulos DE, Vouloumanou EK, Falagas ME (2012) Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* clinical isolates. Eur J Clin Microbiol Infect Dis.31: 695–701.
28. Araj GF, Jaber FA (2012) In vitro activity of fosfomycin and other antimicrobials against uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care center in Lebanon. Leb Med J 60: 142-147
29. Araj GF, Ibrahim GY (2008) Tigecycline in vitro activity against commonly encountered multidrug- resistant Gram – negative pathogens in a Middle Eastern country. Diag Microbiol Infect Dis 62: 411-415.
30. Munoz-Price LS, Quinn JP (2013) Deconstructing the infection control bundles for the containment of carbapenem-resistant Enterobacteriaceae. Curr Opin Infect Dis 26: 378–387.
31. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M (2012) Predictors of mortality in bloodstream infections caused by carbapenemase-producing *Klebsiella pneumoniae*: Importance of combination therapy. Clin infect Dis 55: 943-950.
32. Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky P, Adams-Haduch JM, Doi Y (2012) Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: Superiority of combination antimicrobial regimens. Antimicrob Agents Chemother 56: 2108–2113.
33. Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL(2014)Treating infections caused by carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect 20: 862–872.
34. Doi Y, Paterson DL (2015) Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med 36: 74–84.
35. Akova M, Daikos GL, Tzouvelekis L, Carmeli Y (2012) Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clin Microbiol Infect 18: 439–48.
36. Giamarellou H, Galani L, Baziaka F, Karaiskos I (2013) Effectiveness of double-carbapenem regimen for infections in humans due to carbapenemase-producing pandrug-resistant *Klebsiella pneumoniae*. Antimicrob Agents Chemother 57: 2388–2390.

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