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Carbapenemase typing and resistance profile of enteric bacteria isolate with reduced sensitivity to carbapenems in a Lebanese tertiary care center

Abstract

Objective: Nowadays-resistant bacteria represent worldwide a public health problem Leading in some cases to a stalemate without any possible treatment. Therefore, early detection and identification of carbapenemase-producing gram-negative bacteria (GNB) is of crucial importance. Consequently, we conducted a study in a tertiary care hospital to analyze the resistance phenotype of the carbapenem-resistant GNB (CRGNB).

Methods: This study investigated all the CRGNB from September 2014 till January 2016. A total of 40/126 isolates were randomly collected and tested by susceptibility test in addition to a real time multiplex PCR to detect the carbapenemase-coding genes (bla SPC, bla IMP1, bla VIM, bla NDM, bla KPC, et bla OXA-48). The examined isolates were: *Escherichia* coli (70%), Klebsiellapneumoniae (20%), Enterobacteraerogenes (2,5%), *Enterobacter cloacae* (2.5%) and *Klebsiellaoxytoca* (2.5%).

Results: All (100%) of the examined isolates were intermediate or resistant to ertapenem, 85% intermediate or resistant to imipenem and/or meropenem. Additionally, 33/40 of the isolates (82.5%) were bla OXA-48 positive, and one isolate (2.5%) is bla NDM positive. *E. coli* was the bacteria that expressed the most OXA-48 (26/40) (65%). Out of the 40 isolates in our study, 6 did not express carbapenemases although they have shown resistance to carbapenems.

Conclusion: This study reports the emergence of CPGNB in our medical center especially bla OXA-48 with a high resistance pattern leading to narrow therapeutic options. This study indicts the importance of

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rapid detection of such strains in order to initiate quickly the right preventive and therapeutic measures and to avoid hospital epidemics with disastrous consequences.

Keywords
Carbapenems, Carbapenemase, Bacterial Resistance, Resistance Mechanisms, Enterobacteriacae, Molecular Typing.

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Introduction

The discovery of penicillin in 1928 was one of the major events that changed the course of life on earth. Unfortunately, since the start of antibiotics use, bacterial resistance has rapidly developed and kept on increasing mainly due to the inappropriate use of these drugs.

Carbapenems are a group of βlactam antibiotics used to treat serious infections, especially in hospital settings [1]. Compared to cephalosporins, penicillins, and βlactams. βlactamase inhibitors such as carbapenems have a very broad antimicrobial spectrum covering both Gram-positive and negative bacteria [2]. In recent years, the emergence of carbapenemase secreting bacteria has been reported worldwide including in many hospitals and in the environment of the Middle East countries, especially OXA-48 in strains of *E coli* and OXA-48/NDM1 in *K pneumoniae* early as 2012 [3]. Both of these bacteria types produce different enzymes that degrade carbapenems, especially KPC, VIM, IMP, NDM, and OXA-48 [4].

This study analyses the different enzymes produced by *Enterobacteriaceae* isolates in order to find out which species are the most prevalent and their resistance profile in our hospital and region. Also, to consider the most appropriate treatment for the carbapenem-resistant *Enterobacteriaceae*.

Materials and methods

Study type

This study is an observational epidemiological cross-sectional study with two main objectives: Typing carbapenemases in *Enterobacteriaceae* with reduced susceptibility to carbapenem, and studying the resistance profile of these bacteria comparing it to regional data, and suggesting to determine the best therapeutic choice.

Target population

The population includes all patients with an infection or colonization of *Enterobacteriaceae* with a decreased susceptibility to carbapenems between September 2014 and January 2016.

Methods

All strains of *Enterobacteriaceae* isolates with reduced susceptibility to carbapenems were collected according to the antibiogram committee of the French Society of Microbiology – recommendations 2015 [5]. Ertapenem: MIC>0.5mg/L or an inhibition zone diameter <25mm for a disk load of 10ug/ml (CASFM2015),imipenem: MIC>2mg/L or an inhibition diameter <22mm for a disk load of 10ug/ml (CASFM2015), meropenemMIC>2mg/L or an inhibition diameter <22mm for a disk load of 10ug/ml

(CASFM2015). Ertapenem is the most susceptible carbapenem for the detection of carbapenemase-producing *Enterobacteriaceae* strains (CPE). The susceptibility profile included the following: ampicillin, amoxicillin + clavulanate, piperacillin, piperacillin + tazobactam, ticarcillin, cephalothin, cefoxitin, cefuroxim, cefotaxim, ceftazidim,cefepim, cefixim, aztreonam, imipenem, meropenem, ertapenem, gentamicin, amikacin, colistin, trimethoprim/sulfamethoxazole, ciprofloxacin, nitrofurantoins and fosfomycin.

After suspecting CPR isolates, a PCR confirmation test was performed using GeneXpertCarba-R kits (Cepheid USA).

Ethical Considerations

Confidentiality and anonymity of patient information were respected. This was determined by assigning a number for each medical record. Only the investigator and the laboratory technicians know this number. Outcomes 126 bacteria isolates with reduced susceptibility to carbapenems were isolated between September 2014 and January 2016, of these 40 isolates were randomly selected using the InfoWebMaster software. The average age of patients is 58 years, with an age limit of 1 month and 90 years. It should be noted that a study done in the same tertiary care center between 1/11/2014 and 31/12/2014, showed that 5.6% of *Enterobacteriaceae* strains (4% *Klebsiella*spp. and 1.6% *E. coli* have a reduced sensitivity to carbapenems) **(Table 1)**.

Table 1. Type and number of bacteria with reduced susceptibility tocarbapenems

Bacteria	No.
Enterobacter spp	13
Klebsiella spp	26
E. coli	42

Sources of isolates

Urine culture, catheter culture, wound culture, pressure ulcer culture, and rectal culture. The majority of isolates (30) were from urine *cultures* **(Table 2)**.

Table 2. Sources of specimens.

Source	No.	%
Urine	30	75
Rectal screening	4	10
Wound	3	7.5
Catheter	2	5
Pressure ulcer	1	2.5

Species of Bacterial isolates

There were *E. coli, Enterobacteraerogene*s, *Enterobacter cloacae*, *Klebsiella*pneumoniae, *Klebsiellaoxytoca* and *Morganellamorganii*, with the majority of *E. coli* (28) followed by *K. pneumoniae* (8).

Table 3. Type, number and percentage of bacteria isolates.

Bacteria	No.	%
E. coli	28	70.0
K. pneumoniae	8	20.0
E. aerogenes	1	2.5
E. cloacae	1	2.5
K. oxytoca	1	2.5
Morganellamorganii	1	2.5

Results

The result of susceptibility profile is summarized in **(Table 4)**.

Resistance to different carbapenems

All the investigated bacterial isolates were resistant to at least one of the three carbapenems: Ertapenem, imipenem and meropenem. The percentage of bacteria resistant to different carbapenems is shown in **(Figure 1)**.

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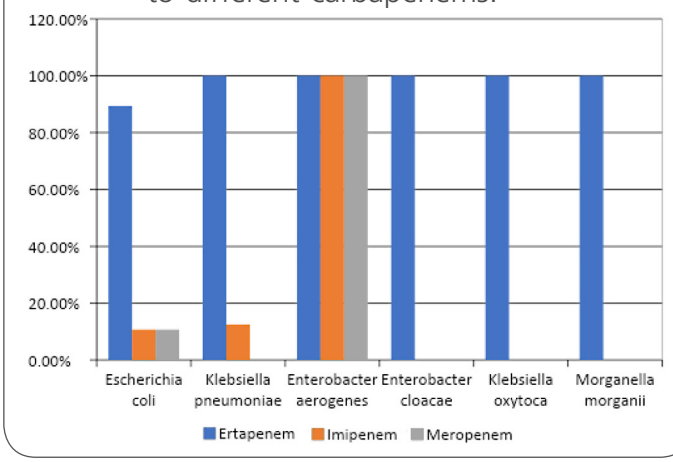
Table 4. Percentages of antibiotic resistance of bacteria isolates.

Antibiotic	Bacteria isolates					
% resistant	*E. coli*	*E. aerogenes*	*E. cloacae*	*K. oxytoca*	*K. pneumoniae*	*M. morganii*
Ampicillin	100	100	100	100.0	100	100.
Amoxicillin + Clavulanate	100	100	100	100.0	100	100
Piperacillin	100	100	100	100.0	87.5	100
Piperacillin + Tazobactam	96.4	100	100	100.0	100	0.0
Ticarcillin	100	100	100	100.0	100	100
Cephalothin	100	100	100	100.0	100	100
Cefoxitin	50	100	100	100	87.5	0.0
Cefuroxim	100	100	100	100	100	100
Cefotaxim	100	100	100	100	100	100
Ceftazidim	100	100	100	100	100	100
Cefepim	100	100	100	100	100	100
Cefixim	92.9	100	100	100	100	100
Aztreonam	100	100	100	100	100	100
Imipenem	10.7	100	0.0	0.0	12.5	0.0
Meropenem	10.7	100	0.0	0.0	0.0	0.0
Ertapenem	89.3	100	100	100	100	100
Gentamicin	28.6	100	0.0	0.0	62.5	100
Amikacin	0.0	0.0	0.0	0.0	12.5	0.0
Colistin	0.0	0.0	0.0	0.0	0.0	100
Sulfamethoxazole + Trimethoprim	67.9	100	0.0	0.0	75	100
Ciprofloxacin	57	100.	0.0	100	50	100
Nitrofurantoins	17.9	100	0.0	100	50	100
Fosfomycin	14.3	0.0	0.0	100	37.5	100

Figure 1: Percentage of bacteria isolates resistant to different carbapenems.



Results of expression of different carbapenemases

After selecting bacteria with reduced susceptibility to carbapenems, a PCR confirmation test was performed and showed that 34 of the 40 isolates expressed carbapenemases, and 33 were OXA-48 and only one strain expressed NDM **(Table 5)**. Difference in susceptibility depending on the expression of carbapenemases. Comparison of the resistance profile between bacteria not expressing any carbapenemases and bacteria expressing OXA-48 did not show a significant difference for any of the tested antibiotics. However, a statis-

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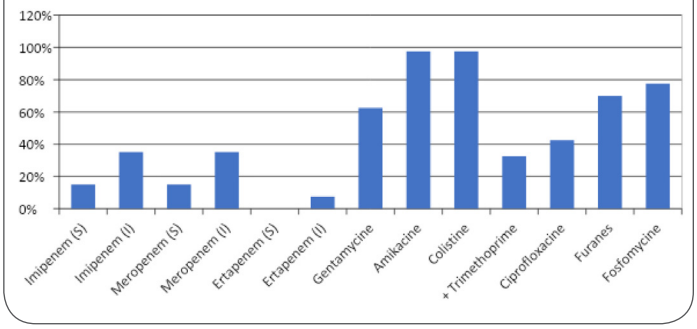
Table 4. Number of bacteria isolates expressing the different carbapenemases.

Detected enzymes	No. of Bacteria spp.					
	E. coli	*E. aerogenes*	*E. cloacae*	*K. oxytoca*	*K. pneumoniae*	*M. morganii*
SPC	0	0	0	0	0	0
IMP1	0	0	0	0	0	0
VIM	0	0	0	0	0	0
NDM	0	0	0	0	1	0
KPC	0	0	0	0	0	0
OXA48	26	1	0	1	5	0

tical analysis showed a more significant profile with *K. pneumonia* which expresses NDM, where one isolate was completely resistant to ertapenem, intermediatly susceptible to imipenem and meropenem, the was the only isolate resistant to amikacin, but was susceptible to both colistin and fosfomycin.

Out of all the bacteria studied producing different carbapenemases, 76.5% are *E. Coli* expressing OXA-48.

Figure 2: Percentage of susceptible bacteria isolates to antibiotics other than B-lactams (carbapenems excluded).



The susceptibility profile of isolates to antibiotics other than B-lactams excluding carbapenems is demonstrated in **(Figure 2)**.

In an attempt to find an alternative to the treatment of *Enterobacteriaceae* with reduced susceptibility to carbapenems, we add together in a table the percentage of bacteria susceptible to imipenem, meropenem, ertapenem, gentamicin, amikacin, colistin, sulfamethoxazole + trimethoprim, ciprofloxacin, furans, and fosfomycin. The results are presented in **(Figure 2)**.

Discussion

Emergence of OXA-48

The OXA-48 was first identified in Turkey in 2001 and was subsequently identified in several Middle Eastern countries, Europe and North Africa [6]. *E coli* is the most OXA-48-expressing bacteria species found in the Middle East except in a Jordanian study by Abdelfattah et al where *K pneumonia* accounted for 82% among carbapenemase-producing *Enterobacteriacae* [7]. The first carbapenem*ase*-secreting enteric bacteria isolated in Lebanon was OXA-48 in 2008, expressed by *E. coli* and *K. pneumonia* [8]. As early as 2012 OXA-48 and OXA-48/NDM-1 positive *E coli* and *K. pneumonia* were isolated from cultures of Iraqi patients. Our results are consistent with those of the other studies stating that 70,4% of blaOXA-48 found among *Enterobacteriacae* [9]. In fact, 85% of our bacteria isolates showed decreased susceptibility to carbapenems or were carbapenemase-producers (97% OXA-48-producers). Furthermore, of the 33 OXA-48 identified isolates in our study, 26 were *E. coli* (78%) and 5 (15%) were *K. pneumoniae*. The emergence of the OXA-48 in Lebanon can be explained by the exchange of tourists between Lebanon and Turkey. Thousands

of Lebanese travelling annually to Turkey and vice versa, in addition to the medical tourism of Iraqi and Syrian patients due to the geographic proximity, and the available good level of medical services in Lebanon.

Resistance to carbapenems may be attributed to three major mechanisms: porin-mediated resistance to reduce uptake of carbapenems, efflux pumps. Additionally, it has been reported that the OXA-48 may emergence very rapid due to the spread of a transferable plasmid especially in *E coli* [6]. The OXA-48 gene is located on the same plasmid in *E coli* and *K. pneumonia* highlighting the mobile genetic elements role in the dissemination [3].

NDM strain

In our study, only one strain of *K. pneumoniae*, expressed NDM. A recent study showed that NDM-producing bacteria are mainly found in Pakistan, Iraq, Afghanistan and the United Arab Emirates [6]. Enteric bacteria expressing NDM was first reported fromLebanon in two Iraqi patients in 2012 [10]. The only patient in our study who has been isolated with *K. pneumonia* expressing NDM is an Iraqi patient living in Lebanon. Therefore, we have performed screening for NDM producing bacteria in all Iraqi patients and for those who were residents for more than three months in Iraq. The isolated NDM-*K. pneumoniae* has more resistance patterns than other bacteria isolates, it showed intermediate susceptibility toimipenem and meropenem, and was susceptible only to colistin and fosfomycin. This isolate has exactly thesame resistance profile as found in the 2 patients from Iraq during 2012 [10].

Other resistance mechanisms

This study found that 6/40 (15%) of the isolates did not express carbapenemases, although they have reduced susceptibility to carbapenems. Two hypotheses arise, either these isolates secrete carbapenemases that have not been identified by our PCR protocol (SPC, IMP1, VIM, NDM, KPC, OXA-48) or express other resistance mechanisms; impermeability, efflux pump, modification of the binding protein. Our study has not investigated for other express carbapenemases, and has not searched for binding protein mutations and porin expression. However, the detection of carbapenemases remains the most important resistance feature used by enteric bacteria [11]. Patient records in our medical center were reviewed, showing that the majority of patients (82%) had taken an antibiotic within 6 months before the onset of their infection, of these 64% have taken at least one carbapenem antibiotic (ertapenem, Imipenem and/or meropenem). This makes the history of antibiotic use in general and carbapenems, in particular, a very important risk factor for the emergence of multiresistant isolates.

Therapeutic choices of carbapenems

All bacteria isolated in this study are of intermediate susceptibility or resistance to carbapenems, however, these drugs can still be used for the treatment of such bacteria butby altering the mode of administration. The Monte Carlo simulator study showed that high doses of meropenem (6000mg/d), with prolonged infusion over 4 hours, increasedits efficacy for a MIC between 8-16ug/mL [12]. A second study has also shown that the probability of reaching the therapeutic threshold, for a MIC=4ug/mL, is 93% over a 3-hour infusion of 1000mg of meropenem, 3 times per day as compared to 69% using thetraditional model [13]. In this study, 50% of the isolates were resistant to imipenem and meropenem, and 35% of these were intermediate and 15% completely susceptible. This fact has allowed us to use carbapenems with prolonged infusions.

Polymyxins

All 39/40 examined isolates were susceptible to colistin, except one isolate of *Morganellamorganii* that exhibits natural resistance. Colistin (polymyxin E) and polymyxin B are normally active in vitro against carbapenem-resistant enteric bacteria. The two

drugs differ by a single amino acid. The advantage of polymyxin B is that it does not require a change of dose regarding renal clearance [14]. The therapeutic dose is not well defined, especially with colistin that requires dose adjustment depending on renal clearance, and not to administrate underdoes in severe infections. A retrospective study of 258 patients showed lower mortality in the group treated with a maximum dose of colistin (9 million IU/d), compared with higher mortality in the lower dose groups (6 million and 3 million IU/d). Kidney clearance with colistin should be estimated closely and a neurological examination for paresthesia and ataxia should be done regularly [15].

Tigecycline

Our study did not test the susceptibility of the isolates to tigecycline, but several articles describe the utility of tigecycline as a possible treatment for carbapenem-resistant enteric bacteria, but in combination with other antibiotics and at high doses [16].

Fosfomycin

A total of 77.5% of the isolates were susceptible to fosfomycin. Few studies have been done to show the efficacy of fosfomycin in treating this kind of infection. Fosfomycin should never be given alone in such infections because there will be increased risk of emergence of resistant mutants [17].

Aminoglycosides

Gentamicin is the most active aminoglycoside in vitro against *K*pneumoniae, but amikacin is the most active against other enteric bacteria [18, 19]. In our study, 62.5% of the bacteria were suceptible to gentamicin, and 97.5% were sensitive to amikacin. Aminoglycoside could be used in combination, especially for severe urinary tract infections. Many studies have shown that aminoglycoside and carbapenems have the lowest mortality rate [20]. Like other countries in the Middle East and around the world, Lebanon is facing a very dangerous infectious crisis, with the emergence of carbapenemase-producing enteric bacteria, especially with OXA-48. Multi-resistant microorganisms such as *Pseudomonas*, *Acinetobacter,* and *Stenotrophomonas*, which are generally responsible for health-associated infections, are not the only bacteria type's difficult-to-treat infections. *Enterobacteriaceae* which are much more common causing infections in hospitals has become the major cause of concern [18]. Therapeutic choices are becoming very limited with the majority of carbapenems becoming more ineffective, but infusion over a long period can be a good choice in certain circumstances. Fosfomycin and colistin can be reconsidered as therapeutic options despite the renal and nervous toxicity of colistin. Recently, antimicrobial resistance worsened with the Covid-19 pandemic with the overuse of antimicrobials especially in critically ill patients but also in outpatients settings [21].

Conclusion

This study shows the widespread of carbapenemase-producing *Enterobacteriaceae* in our medical center. The only way to counter this global emergence of antibiotic resistance is through prevention, discontinuing all unnecessary antibiotic prescriptions, establishing antimicrobial stewardship programs, and applying strict isolation and hygiene methods in all medical centers.

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References

1. Norrby SR. Carbapenems. Med Clin North Am 1995; 79(4):745-59.
2. Queenan AM, Bush K. Carbapenemases: The Versatile β-Lactamases. Clin Microbiol Rev 2007; 20(3):440-58.
3. Dandachilman, Chaddad Amer, Hanna Jason, Matta Jessika, Daoud Ziad. Understanding the Epidemiology of Multi-Drug Resistant Gram-Negative Bacilli in the Middle East Using a One Health Approach. FrontMicrobiol 2019; 23.
4. Meletis G, Oustas E, Bagkeri M. Carbapenemase reports from the Balkans: a systematic review. Infez Med Riv Period Eziologia Epidemiol Diagn Clin E Ter Delle Patol Infett. 2014; 22(2):85-106.
5. Société Française de Microbiologie [Internet]. [cited 2016 Feb 23]. Available from: http://www.sfm-microbiologie.org
6. Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, Asgharzadeh M, Aghazadeh M. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol 2015; 7(5):226-46.
7. Aqel AA, Findlay J, Al-Maayteh M, Al-Kaabneh A, Hopkins KL, Alzoubi H, Masalha I, Turton J, Woodford N, Ellington MJ. Characterization of Carbapenemase-Producing Entero bacteriaceae from Patients in Amman, Jordan. Microb Drug Resist 2018; 24(8):1121-1127. doi: 10.1089/mdr.2017.0238.
8. El-Herte RI, Kanj SS, Matar GM, Araj GF. The threat of carbapenem-resistant Enterobacteriaceae in Lebanon: an update on the regional and local epidemiology. J Infect Public Health 2012; 5(3):233-43.
9. Hammoudi Halat D, Moubareck CA, Sarkis DK. Heterogeneity of Carbapenem Resistance Mechanisms among Gram-Negative Pathogens in Lebanon: Results of the First Cross-Sectional Countrywide Study. Microb Drug Resist 2017; 23(6):733-743. doi: 10.1089/mdr.2016.0077. Epub 2017 Jan 12. PMID: 28080212.
10. El-Herte RI, Araj GF, Matar GM, Baroud M, Kanafani ZA, Kanj SS. Detection of carbapenem-resistant Escherichia coli and Klebsiellapneumoniae producing NDM-1 in Lebanon. J Infect DevCtries. 2012; 6(5):457-61.
11. Girlich D, Poirel L, Nordmann P. CTX-M expression and selection of ertapenem resistance in Klebsiellapneumoniae and Escherichia coli. Antimicrob Agents Chemother. 2009; 53(2):832-4.
12. Roberts JA, Kirkpatrick CMJ, Roberts MS, Robertson TA, Dalley AJ, Lipman J. Meropenem dosing in critically ill patients with sepsis and without renal dysfunction: intermittent bolus versus continuous administration? Monte Carlo dosing simulations and subcutaneous tissue distribution. J Antimicrob Chemother 2009; 64(1):142-50.
13. Kuti JL, Dandekar PK, Nightingale CH, Nicolau DP. Use of Monte Carlo simulation to design an optimized pharmacodynamic dosing strategy for meropenem. J Clin Pharmacol 2003 Oct; 43(10):1116-23.
14. Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin Bagainst a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). J Antimicrob Chemother 2011; 66(9):2070-4.
15. Akajagbor DS, Wilson SL, Shere-Wolfe KD, Dakum P, Charurat ME, Gilliam BL. Higher incidence of acute kidney injury with intravenous colistimethate sodium compared with polymyxin B in critically ill patients at a tertiary care medical center. Clin Infect Dis Off Publ Infect Dis Soc Am. 2013; 57(9):1300-3.
16. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment Options for Carbapenem- Resistant Enterobacteriaceae Infections. Open Forum Infect Dis [Internet]. 2015 May 5 [cited 2016 Feb 25]; 2(2). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4462593/
17. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing Enterobacteriaceae infections: a systematic review. Lancet Infect Dis 2010; 10(1):43-50.
18. Souli M, Galani I, Antoniadou A, Papadomichelakis E, Poulakou G, Panagea T, et al. An outbreak of infection due to beta-Lactamase Klebsiellapneumoniae Carbapenemase 2-producing K. pneumoniae in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis off Publ Infect Dis Soc Am 2010; 50(3):364-73.
19. Livermore DM, Mushtaq S, Warner M, Zhang J-C, Maharjan S, Doumith M, et al. Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. J Antimicrob Chemother2011; 66(1):48-53.
20. Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. Treating infections caused by carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect Off Publ EurSoc Clin Microbiol Infect Dis 2014; 20(9):862-72
21. Rizk NA, Moghnieh R, Haddad N, Rebeiz MC, Zeenny RM, Hindy JR et al. Challenges to Antimicrobial Stewardship in the Countries of the Arab League: Concerns of Worsening Resistance during the COVID-19 Pandemic and Proposed Solutions. Antibiotics (Basel). 2021; 10(11):1320. doi: 10.3390/antibiotics10111320. PMID: 34827257; PMCID: PMC8614706.