Molecular Detection of the Genes bla OXA, bla KPC and bla NDM Among Carbapenem-Resistant Klebsiella Pneumoniae Isolated From Different Hospitals in Duhok City, Iraq

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Citation

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Abstract

Background: Spreading of Carbapenem-resistant Klebsiella pneumoniae among hospitalized patients are beyond expected with potential infections and little is known in Iraq. The aims were to find out the prevalence of carbapenem genes among carbapenem-resistant K. pneumoniae isolates in major hospitals in Duhok city, Iraq.

Methods: For the 281 isolates recovered from different clinical specimens in 2017, species identification was done first by classical method and confirmed by Vitek 2 and antimicrobial susceptibilities were determined by both Kirby-Bauer method and Vitek 2. Isolates with reduced susceptibility to ertapenem were tested for production of carbapenemase by using the Modified Hodge Test and double disk synergy test (DDST). Positive isolates for carbapenemase were further characterized and subjected to PCR for carbapenemase genes including blaOXA-48, blaNDM-1 and blaKPC.

Results: Out of 281 K. pneumoniae isolates, 123 (43%) were carbapenem-resistant and showed high resistant to ampicillin, ceftazidime, ceftriaxone and pipracellin 100%, 76.9%, 74.0%, and 71.5 % respectively. High sensitivity was toward colistin and fosfomycin 35.9% and 19.9%, respectively. Of 50/123 carbapenem-resistant studied isolates, MHT and DDST showed that all studied isolates were carbapenemase producer. The presence of blaKPC, blaNDM-1 and blaOXA-48 was detected in 14 (10.9%), 13 (0.9%) and 6 (12%) of studied isolates, respectively. two co-producing were occurred between blaKPC with blaNDM-1 and blaKPC with blaOXA-48

Conclusion: Carbapenem-resistant K pneumoniae isolates harbored carbapenemase genes (blaNDM-1,bla KPC and blaOXA-48- types) with multiple drug resistance profiles are circulating in our setting, Iraq , namely bla KPC when compared with neighbored. Surveillance and reemphasis on infection control measures with rational use of antibiotics in Iraqi hospitals will minimize the spread of carbapenem resistance.

INTRODUCTION

Klebsiella pneumoniae belongs to the Enterobacteriaceae family and has the ability to cause various infections ranging from pneumonia to urinary tract infections as well as wound, surgical site infections, bloodstream infection, and meningitis (Cristina et al., 2016, Codjoe & Donkor, 2018).

The occurrence of infections caused by K pneumoniae strains that are resistant to extended-spectrum-lactam antimicrobial agents make clinicians to select the latest choices such as carbapenems to treat these resistant pathogens. However, over- and above misuse of carbapenems can variably induce different mechanisms of résistance. Infections in hospitalized patients with carbapenem-resistant K. pneumoniae isolates have recently become the most common infection with a pathogen that spread worldwide and caused an increase in mortality rates. Carbapenemases enzyme production with extended-spectrum beta-lactamase and high level AmpC are considered the major causes of the occurrence of resistance. There are many recognized carbapenemases types including: serine carbapenemases, such as K. pneumoniae carbapenemase (KPC)(class A), MBLs, like Verona integron-encoded MBL (VIM)) and New Delhi MBL (NDM), and imipenemase (IMP)(class B) and OXA

carbapenemase like blaOXA-48 (class D) and analysis showed that all beta-lactams are partly inhibited by clavulanic acid, tazobactam, and boronic acid (Codjoe & Donkor, 2018).

In our city Duhok, like elsewhere, owing to growing threat of infections due to carbapenem-resistant K pneumoniae strains and absence of inclusive indigenous evidence, reviewing the occurrence of tough genes and detecting suitable antibiotics is critical for the regulator of these kinds of treatments. The aim of the current study is to perform a molecular characterization of carbapenem resistance genes that mediated resistance mechanisms to carbapenem in K. pneumoniae isolates recovered from different clinical specimens among patients in different hospitals in Duhok City, Iraq.

MATERIAL AND METHODS

Bacterial isolates

A total of 281 K. pneumoniae isolates were collected from five main hospitals in Duhok City, Iraq during 2017. Bacteria recovered from clinical specimens such as blood, CSF, wounds, urine, sputum, and vaginal swab were identified using standard biochemical methods (Dennis et al., 2004) and on the Vitek 2 system computer using software version 5.04 (bioMerieux). The study was approved by the Regional Committee on Medical Research Ethics and was in accordance with the declaration of Helsinki.

Antibiotic susceptibility test

All isolates were phenotypically checked for their antibiotic susceptibility test by disk diffusion method "Kirby-Bauer method" toward diverse antimicrobial agents according to CLSI standards (Wayne, 2015). This test was performed on Muellar Hinton agar medium (Oxoid Ltd, England). A total of twenty-two antibiotic discs (Bio analyse) were used which are listed in Table 2.

Carbapenemase screening

All K. pneumoniae isolates were screened for determination of carbapenemase production by performing Modified Hodge test (MHT), then a double disk synergy test (DDST) was used to detect MBL (Metallo-beta-Lactames) production according to the CLSI recommendation and as described by (Hosseinzadeh et al., 2017).

To better study the carbapenem family genes encoding resistance, we selected and studied 50 out of 123 phenotypic carbapenem-resistant K. pneumoniae isolates recovered from clinical patients across many hospitals for further analysis of genomic determination targeting carbapenem resistant genes by PCR.

DNA Extraction

Preparation of crude DNA lysates was done by suspending a 1 µL loop-full of freshly cultured cells in 200 µL of sterile D.W. The suspension was incubated at 95□C for 5 min and 1 µL of supernatant from the centrifuged lysates used as template DNA for PCR (Ahmed and Dablool, 2017).

PCR confirmation Identification of K. pneumoniae isolates

All identified K. pneumoniae isolates were confirmed by PCR method, which was performed through the amplification of specific gene galacturonase (16 rRNA). Performing PCR was done through the amplification of 130 base pairs (bp) specific to K. pneumoniae. The primer used and PCR condition shown in Table 1.

PCR Analysis of the Carbapenem Resistance Genes

Carbapenemase gene families (bla NDM, bla KPC, and bla OXA-48-like) were determined according to Zowawi et al., 2014. The forward and reverse primers of target genes with their annealing temperatures are shown in Table 1. Concerning PCR master mix up to 50□l comprised of the following: 8 □l of primer for target genes, 25 □l hot-start premix , 4-5 □l of extracted DNA (30-100 ng/µl) and nuclease free water was added till to 50 □l. PCR amplification was performed in Gene Amp PCR system 7900 (applied biosystem). 2% agarose gel prepared from 1x TAE buffer plus red safe DNA staining solution (GeNetBio) was used for detection of amplification of PCR products.

Table 1

Primers used for detection of target genes in K pneumoniae isolates

Primer name	Sequence 5-3	Size (bp)	Optimum annealing temp (°C)	PCR cycle	References
bla KPC F bla KPC R	5'-TGT CAC TGT ATC GCC GTC-3' 5'-CTC AGT GCT CTA CAG AAA ACC-3'	61	56 59	Initial 94----------5 minute Initial 95----------45 sec Annealing 53-----45 sec 45 cycle Extension 72----------45 sec Final exte 72----------10 minute	Jena Bioscience GmbH , Germany
bla OXA 48 F bla OXA 48 R	5'- TTG GTG GCA TCG ATT ATC-3' 5'-GAG CAC TTC TTT TGT GAT-3'	744	58 59	Initial 94----------5 minute Initial 94----------45 sec Annealing 60-----45 sec 35 cycle Extension 72----------45 sec Final exte 72----------7 minute	
bla NDM F bla NDM R	5'-GCA GCT TGT CGG CCA TGC GGG C-3' 5'-GGT CGC GAA GCT GAG CAC CGC AT-3'	782	71 70	Initial 95----------5 minute Initial 95----------45 sec Annealing 66-----45 sec 35 cycle Extension 72----------1 min Final exte 72----------8 minute	
16 rRNA F 16 rRNA R	5'-TGG AGC ATG TGG TTT AAT TCG A-3' 5'-TGC GGG ACT TAA CCC AAC-3'	130	58 57	Initial 94----------5 minute Initial 95----------45 sec Annealing 57-----45 sec 45 cycle Extension 72----------45 sec Final exte 72----------10 minute	

RESULTS

Overall, of 281 identified K. pneumoniae isolates (125

inpatients and 156 out patients) there were 113 samples from male and 168 from female patients. The results of antibiotic susceptibility revealed that all the isolates exhibited absolute resistant to ampicillin, and then mostly resistant to ceftazidime, ceftriaxone and pipracellin, 76.9%, 74.0%, and 71.5 % respectively. The prevalence of carbapenem resistance in K. pneumoniae isolates was 43.8%. The highest sensitivity was toward cefoxitin, colistin, followed by Fosfomycin. 32.7%, 35.9% and 19.9% respectively. The full results of antibiotic resistance patterns of K. pneumoniae isolates are accessible in Table 2. All (123) carbapenem-resistant isolates were confirmed to be carbapenemase and MBL producers by modified Hodge test and DDST.

In PCR assay, all 50 selected phenotypically-identified K. pneumoniae isolates successfully produced amplified product targeted 16SrRNA gene. Out of 50 carbapenem-resistant isolates 33(66%) were producers for one and multiple carbapenem family genes. The carbapenemase gene bla KPC was detected in 14/50 (28%) isolates, followed by blaNDM-1 in 13/50 (26%), and bla OXA-48-like in 6 (12%). Four isolates were coproduction of carbapenem genes. Coproduction of bla KPC with blaOXA-48 occurred in 2 isolates and coproduction of bla KPC with bla NDM found in other 2 of carbapenem-resistant isolates. Over and above, one isolate carried all (bla KPC, blaNDM-1 and blaOXA-48) genes and recovered from the CSF of inpatient boy at Hevi Pediatric Hospital. Furthermore, the majority of the bla KPC gene originated from blood samples; while the majority of blaOXA-48 and blaNDM-1 were recovered from urine and wound samples respectively. Meanwhile, the two carbapenem-sensitive isolates from urine sample were negative in the phenotype carbapenemase test but contained a bla KPC gene when subjected to PCR assay. The distribution and characterization profile of bla KPC, blaNDM-1 and blaOXA-48 positive isolates among different hospitals and clinical specimens are shown in Tables 3 and 4. Sequencing results of the isolates harboring blaOXA-48 confirmed all of them as blaOXA-48 variant.

Table 2

Rates of antibiotic resistance in total and carbapenem-resistant K pneumoniae isolates

Antibiotic	Total N = 281 No. (%)	Carbapenem-resistant isolates N = 123 No. (%)
Emipenem	123 (43.8%)	-
Meropenem	123 (43.8%)	-
Ampicillin	281 (100%)	123 (100)
Amoxiclave	214 (76.2%)	123 (100)
Ceftazidime	216 (76.9%)	123 (100)
Ceftriaxone	208 (74.0%)	120 (97.5)
Cefotaxime	216 (76.9%)	122 (99.1)
Cefuroxime	214 (76.2%)	123 (100)
Aztreonam	210 (74.7%)	119 (96.7)
Cefixime	204 (72.6%)	119 (96.7)
Pipracillin	201 (71.5%)	123 (100)
Tetracyclin	211 (75.1%)	116 (94.3)
Cefepime	196 (69.8%)	117 (95.1)
Trimethoprim	184 (65.5%)	114 (92.6)
Ciprofloxacin	157 (55.9%)	70 (56.9)
Gentamicin	147 (52.3%)	107 (86.9)
Amikacin	134 (47.7%)	104 (84.5)
Ertapenem	133 (47.3%)	108 (87.8)
Nitrofurantoin	110 (39.1%)	70 (56.9)
Cefoxitin	092 (32.7%)	70 (56.9)
Colistin	101 (35.9%)	73 (59.3)
Fosfomycin	56 (19.9%)	29 (23.5)

Table 3

Frequency and distribution of Carbapenemase genes family among various clinical specimens

Specimen type	Frequency of Carbapenemase genes			No.
	bla KPC	*bla* NDM-1	*bla* OXA-48	
Blood	5	3	-	8
Urine	3	2	3	8
Wound	2	5	-	7
Sputum	2	1	1	4
CSF	1	2	1	4
HVS	1	-	1	2
Total	14	13	6	33

Table 4

Frequency and distribution of Carbapenemase genes family among different hospitals in the study area

Hospitals	Frequency of Carbapenemase genes			No.
	bla KPC	*bla* NDM-1	*bla* OXA-48	
Azadi	3	2	6	11
Heve	4	3	-	7
Emergency	2	-	-	2
Burn	1	4	-	5
Central Lab	4	4	-	8
Total	14	13	6	33

DISCUSSION

The results that obtained from characterizations of carbapenem-resistant K pneumoniae isolates using phenotypic and genotypic methods combined with their prevalence from various parts of the world is in fact emerging and to rise up. In the current study the prevalence of phenotypically carbapenem resistance K. pneumoniae isolates was increased markedly as 43.3% in this study

compared with 2.7% in another study from 2014 conducted in Iraq (Al-Obadi, 2014). The similar level of increase in carbapenems resistance was reported in China in the last decade with an increase from 10.5% to 48.1% in 2019 (Li et al., 2019). Meanwhile, in Turkey, the rate was 2.2% from 2004 till 2007 and increased to 5.8 % during 2017 (Celikbilek et al., 2017, Us et al., 2010). Similar findings were reported in the Countries of the Gulf Cooperation Council (Zowawi et al., 2014). In the USA, according to the Center for Disease Control CDC, the prevalence increased from 3.6% to 10.8% (Hidron et al.,2008). European antimicrobial resistance surveillance network (EARS-Net) data stated as 7.3% in Europe (European Center for Disease Control, 2014). By contrast, in Greece and Italy they had endemic problems with frequencies of 59.4% and 34.3%, respectively (European Antimicrobial Resistance Surveillance System, 2013). Basically, unrestricted carbapenems consumption joined with consequence of the widespread acquisition of carbapenemase genes may promote the increase of resistance trends (WHO Guidelines, 2017). A process of more effective curative measures, such as antimicrobial scientific stewardship and better-quality hospital infection control procedures should be promoted.

One of the most important finding of the current study is the high prevalence of bla KPC gene followed by blaNDM-1 among collected isolates from different hospitalized patients. To the best of our knowledge, this present study is the first report of K. pneumoniae carrying bla KPC gene followed by blaNDM-1 and blaOXA-48 in north of Iraq. Of 50/123 selected and studied carbapenem-resistant isolates, 14 (28%) and 13 (26 %) isolates were positive for bla KPC and bla NDM-1 respectively. Indication that bla KPC is dominance and is a major source of carbapenemases genes circulating in different medical institutions and hospitals in our setting is a serious concern. Recently, and dissimilar to our finding, bla KPC was infrequent in bordering and our neighboring countries such as Iran, Jordan, Turkey, Persian Gulf Cooperation Council , Egypt and just one isolate in Palestine (Hosseinzadeh et al. 2017; Aqel et al., 2017; Celikbilek et al., 2017; Zowawi et al., 2014; Barwa and Shaaban, 2017; Kattan et al., 2012), while a rapid dissemination of bla KPC was identified in distant states in Eastern part of the USA, China, Greece, Israel and India (Bratu et al., 2005; Cai et al., 2008; Giakoupi et al., 2009; Leavitt et al., 2007; Yong et al., 2009). The endemicity of bla KPC in Iraq may be attributed to international transfers and medical tourisms as large number of Iraqi civilians seeking the medical services in abroad hospitals. One of the striking findings in the present study was that 2 isolates were phenotypically sensitive to imipenem and meropenem, but contained bla KPC gene by PCR. This may be because of several mechanisms of controlling beta-lactamase gene expression and need to be checked again by real-time PCR (Singh, 2015).

In the current study, bla blaNDM-1 gene was detected in 13 (26%) isolates by PCR assay. Producers of blaNDM-1 have been identified mainly in the UK and in other studies (5, 8, 9, 11, 16), suggesting that those areas might also be reservoirs of NDM-1 producers and are being increasingly reported worldwide (25). The majority of K. pneumoniae isolates harboring bla NDM-1 gene were recovered from burn patients in our setting. High blaNDM-1 producers were recovered from blood cultures and rectal swabs in Turkey (5). Moreover, a case of blaNDM-1 producer was isolated from hospitalized an Iraqi injured male resident in Paris that already transferred from Baghdad for further health care (26). This indicates international spread of blaNDM-1 producers much wider than suspected and represent an additional clue that the Middle East might also be a reservoir for blaNDM-1 producers.

In the present study, blaOXA-48 producers were 12% and have been circulated and prevalent only in Azadi Teaching Hospitals in our region that could be associated with nosocomial infections in seriously ill patients. This emphasizes the need for optimized infection control in hospitals in this area. First identification of blaOXA-48 was in Turkey during 2003, and now it is considered an endemic area (27). Increasing numbers of reported cases across the different hospitals in Turkey was confirmed (28). Since we are border with Turkey, Iraqi population movement to Turkey for further health care may explain unceasing dissemination of this resistance gene in our setting. Also, blaOXA-48 producers have been found worldwide, in Iran, in Europe, southern and eastern parts of the Mediterranean Sea which became expanding problem (29). Indeed, such international transfer of blaOXA-48 gene has been confirmed in clinical cases of hospitalized patients between Saudi Arabia and Turkey, India, and Pakistan every year (30). In this study, coproduction of carbapenem genes was recorded with bla KPC and blaOXA-48 in 2 isolates and bla KPC with blaNDM occurring in other 2 isolates. Moreover, one isolate harbored all (bla KPC, blaNDM-1 and blaOXA-48) genes isolated from CSF of inpatient boy at

Hevi Pediatric Hospital. Since these carbapenem-resistant isolates containing multiple genes were almost resistant to all the tested antibiotics, maybe sophisticated treatment options are more worrisome in the near future as potential health threat. Many studies confirmed blaNDM-1 and blaOXA-48 coproduction (9, 11, 16). Meanwhile, there are no studies supports the coproduction of bla KPC with blaNDM and bla KPC with blaOXA-48 that is considered a most important findings and outcomes of this present study but need to be more evaluated. In the present study, we cannot find any of the targeted carbapenem resistance genes in 17/50 phenotypically carbapenem-resistant isolates, so other resistance mechanisms do not exclude Amp C beta lactamases, production of extended-spectrum beta-lactamases (ESBLs), decreased permeability of outer membrane, or efflux pumps activity (31).

As frequently observed for carbapenemase producers, multi-drug or pan-drug resistance was often associated (32). In our collection, all carbapenem-resistant isolates possessed a multi-drug resistance phenotype. Complete susceptible patterns were noted only to fosfomycin, colistin and ciprofloxacin somewhat thus leaving the health care workers with limited options for treatment of patients infected with those isolates. Many studies were accordance with our data (5,8, 9, 16). Recently, fosfomycin (susceptibility of 35.2%) has been recommended as a supplement in treating K. pneumoniae carbapenemase producer's infection, although the CLSI standards recommend it only for the treatment of urinary tract infections. In addition, the occurrence of fosfomycin resistance has obviously varied, from 0% to 97.2% due to the spreading of the fosA3 gene (33).

CONCLUSION

In conclusion, this study highlights that most studied carbapenem-resistant K pneumoniae isolates that were recovered from various clinical specimens harbored carbapenem gene(s). Molecular epidemiology identified bla KPC, blaNDM-1 and blaOXA-48- types with multiple drug resistance profiles in Iraq. Moreover, producer of bla KPC was common in our setting compared with neighboring countries and spreading of blaOXA-48 was only detected across Azadi teaching hospitalized patients in this area. Emergence of those carbapenemase producers in our country might be related to the close relationship between the Iraqi population and other countries in terms of patient exchange. The study emphasis on infection control and continuous surveillance processes of carbapenemase producers in Iraq to trace source of infection joined with careful and rational use of antibiotics are very important to minimize the spread of carbapenem resistance.

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