Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(1)

Klebsiella pneumoniae in Hospital Acquired and Community Acquired Urinary Tract Infections in an Iraqi Cohort: Frequency, Antibiotic Susceptibility and the Percentage of bla KPC Resistance Gene

Heba H. Nasser[1] *MSc*, **Thanaa R. Abdulrahman**[2] *PhD*, **Arif S. Malik**[3] *FICMS*

[1]Medical City Complex, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq,[3]Dept. of Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background *Klebsiella pneumoniae* (*K. pneumoniae*) is an important pathogen in hospital and community-acquired urinary tract infections (HAUTI and CAUTI). It is showing a high resistance towards many antibiotics

Objective This study aims to estimate the percentage of *K. pneumoniae* in HAUTIs and CAUTIs, antibiotic resistance, extended spectrum bata lactamase (ESBL), and detect the beta lactamase *K. pneumoniae* carbapenemases (bla KPC) resistance gene and presence of mutation in this gene.

Methods A total of 200 urine samples were collected randomly; *K. pneumoniae* was cultured and isolated on MacConkey agar, antibiotic sensitivity testing and ESBL detection were performed by Vitek 2 system, then DNA was extracted, polymerase chain reaction and sequencing for KPC gene were done.

Results There was 7% (14 isolates) of *K. pneumoniae* (50%) HAUTI and (50%) CAUTI. ESBL was positive in 3 isolates (21.43%). Percentage of positive bla KPC gene was 42.8%. KPC gene sequencing showed high rate of mutation in positive isolates reaching to 50 different nucleotides.

Conclusion This work has increased the knowledge on *K. pneumoniae* causing HAUTI and CAUTI in Iraqi patients.

Keywords *Klebsiella pneumoniae*, hospital acquired urinary tract infection, community acquired urinary tract infection, ESBL, bla KPC gene.

Citation Nasser HH, Abdulrahman TR, Malik AS. *Klebsiella pneumoniae* in hospital acquired and community acquired urinary tract infections in an Iraqi cohort: Frequency, antibiotic susceptibility and the percentage of bla KPC resistance gene. Iraqi JMS. 2022; 20(1): 121-131. doi: 10.22578/IJMS.20.1.16

List of abbreviations: , ANOVA = Analysis of variance, bla KPC gene = beta lactamase *Klebsiella pneumoniae* carbapenemase gene, CAUTI = Community acquired urinary tract infection, CLSI = Clinical and laboratory standard institute, DDT = Disc diffusion test, ESBL = Extended spectrum beta lactamase, *K. pneumoniae* = *Klebsiella pneumoniae*, HAUTI = Hospital acquired urinary tract infection, KPC = *Klebsiella pneumoniae* carbapenemases, MSAV = Multiple sequence alignment viewer, OD = Optical density, WHO = World Health Organization

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a significant pathogen in nosocomial infections [(1)], also it is the second most common etiological agent involved in community-acquired urinary tract infections (CAUTI) [(2)]. Nosocomial health-care-associated infection is described as an infection rising in a patient hospitalized for more than 48 hours

before the onset of signs and symptoms consistent with the infection [3]. Non-nosocomial health-care-associated infection is explained as an infection detected within 48 hours of admission in an outpatient with extended healthcare contact [4].

K. pneumoniae is showing a high resistance to a broad spectrum of antibiotics including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides [5,6]. This resistance is leading to arising worldwide problem with the choice of the proper antibiotic treatment for hospital-acquired infections [7].

KPC gene (Klebsiella pneumoniae carbapenemase) belongs to the Ambler class A, Bush subgroup 2f, serine based carbapenemases, which are active against all beta-lactams, including the carbapenems [8].

This study aimed to estimate the percentage of *K. pneumoniae* in HAUTI and CAUTI, study antibiotic resistance and extended spectrum beta lactamase (ESBL), detect the resistance gene (bla KPC) and determine the presence of mutation in bla KPC resistance gene.

Methods

Subjects

A total of (200) urine samples (100 from HAUTI and 100 CAUTI) from (one day to 80 years old) patients with UTI were collected randomly from the hospital laboratory of Al-Imamein Al-Kadhimein Medical City Baghdad, Iraq. The UTI patients were diagnosed as HAUTI or CAUTI by the specialists in a period from October 2020 to February 2021. This study was approved by the by the Institutional Review Board of the College of Medicine, Al-Nahrain University (No.1451) and conducted in the Microbiology Department of this College. All collected urine samples were cultured on MacConkey Agar medium (Biolab, Hungary), incubated at 37°C for 24 hours in aerobic conditions. The isolated bacteria were recognized according to morphological characteristics and the identification of the grown colonies was confirmed by the VITEK-2 system.

Antimicrobial susceptibility tests

Resistance patterns of Klebsiella isolates to 14 different antibiotics were detected by disk diffusion test (DDT) and by VITEK 2 System according to Clinical and Laboratory Standards Institutes (CLSI) 2016 [9].

Detection of ESBL by double disk diffusion method

A disk containing amoxicillin/clavulanate (AMC) is placed in proximity to a disk containing ceftazidime (CAZ) or another oxyimino-cephalosporin. The clavulanate in the amoxicillin-clavulanate disk diffuses through the agar and inhibits the lactamase surrounding the ceftazidime disk. Enhancement of the zone of the ceftazidime disk on the side facing the amoxicillin/clavulanate disk is interpreted as a positive test [10].

Molecular study

DNA Extraction

DNA was extracted according to the manufacture instructions by using Gram negative bacteria Wizard genomic DNA purification Kit. DNA concentration and purity was determined by Nano-drop system.

Polymerase chain reaction (PCR) screening bla KPC resistance gene

Approximately 1.5 µl from both forward and reverse primers sequence (Table 1) were apply to yield a DNA fragment of (340) bp using conventional PCR in a total volume 20 µl of reaction mixture. Thermal profile of reaction was showed in table (2).

Agarose gel electrophoresis for detection of PCR products

Five µl of each PCR product and negative control was subjected into 1.5% (wt/vol) agarose gel electrophoresis with ethidium bromide (0.5 µg /ml; Promega, USA) at 7 V/cm for 1.5 hr. Five microliters of the 100bp DNA ladder was included as a marker during PCR products electrophoresis. Amplicon visualization was performed using an UV light

trans illuminator and then photographed by mobile device camera (iPhone) [13].

Table 1. Oligonucleotide's primers and product size of bla KPC resistance gene

Gene		Nucleotide sequences (5' ⟶ 3')	Products *bp*	Reference
***bla*KPC**	**F**	TGTTGCTGAAGGAGTTGGGC	**340**	[11]
	R	ACGACGGCATAGTCATTTGC		

Table 2. Polymerase chain reaction program for amplification of bla KPC resistance gene by thermal cycler

No.	Steps	Temperature	Time	No. of cycles
1	Initial denaturation	95˚C	5 minutes	1
2	Denaturation	95˚C	1 minute	
3	Annealing	56˚C	1 minute	35
4	Extension	72˚C	1 minutes	
5	Final extension	72˚C	5 minutes	1

PCR product of bla KPC resistance gene

In the conventional PCR were sequenced in forward direction using the same primers used in the PCR reactions; according to the Macrogen, Inc. protocol (Korea) Results were compared according to data base of Gene bank.

Statistical methods

The statistical analysis system program includes Fisher's exact test that used to analyze data of this study. Entry of data into Excel systems and the exact tests were achieved by statistical package for social sciences (SPSS) version 20 (2020).

Results

The percentage of *K. pneumoniae* isolates was 7% (14 isolates out of 200 urine samples). Regarding gender distribution, the percentage of females was 42.9% (6 isolates) and males were 57.1% (8 isolates). In the current study, the percentage of hospital acquired urinary tract infection patients were 50% and community acquired urinary tract infection patients were 50%.

Antibiotic resistance of *K. pneumoniae* isolates

The results of the antimicrobial susceptibility test showed that all *K. pneumoniae* isolated from HAUTI and CAUTI patients were complete resistant (100%) to Amoxicillin/clavulanic acid; while there was a high rate of resistance (71.4%) to Aztreonam, trimethoprim/sulfamethoxazole and Trimethoprim in HAUTI and CAUTI, also resistance to Cefotaxime and Ceftazidime were (57.1% and 71.4%), whereas Ceftriaxone were (42.8% and 71.4%) in both HAUTI and CAUTI. The resistance was moderate to Ciprofloxacin (57.1%), Gentamicin (57.1% and 28.5%), Norfloxacin (42.8%) for both, Cefoxitin and Amikacin (42.8% and 14.2%), Meropenem (28.5% and 42.8%) and low to Imipenem (28.5% and 28.5%) in HAUTI and CAUTI respectively. Antibiotic resistance to ceftriaxone, meropenem, ceftazidime and cefotaxime is higher in CAUTI, while antibiotic resistance to cefoxitin, gentamicin and amikacin is higher in HAUTI as shown in the table (3).

Table 3. The percentages of resistance of *Klebsiella pneumoniae* isolates to different antibiotics by disc-diffusion method in HAUTI and CAUTI

	ATM	CRO	NOR	CTX	FOX	CIP	AMC	SXT	CN	AK	IPM	MEM	CAZ	TMP
% of resistance in HAUTI	71.4	42.8	42.8	57.1	42.8	57.1	100	71.4	57.1	42.8	28.5	28.5	57.1	71.4
% of resistance in CAUTI	71.4	71.4	42.8	71.4	14.2	57.1	100	71.4	28.5	14.2	28.5	42.8	71.4	71.4

Aztreonam (ATM), Ceftriaxone (CRO), Norfloxacin (NOR), Cefotaxime (CTX), Cefoxitin (FOX), Ciprofloxacin (CIP), Amoxicillin/Clavulanic acid (AMC), Trimethoprim/sulfamethoxazole (SXT), Gentamicin (CN), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Ceftazidime (CAZ), Trimethoprim (TMP)

Detection of Extended Spectrum Beta Lactamase (ESBL)

The percentage of positive ESBL in this study was 21.43% (3 isolates). The percentage of ESBL was (28.5%) in CAUTI (2 isolates) and (14.28%) in HAUTI (1 isolate). The ESBL positive *K. pneumoniae* isolates, were resistant to all 14 antibiotics used in this study except one isolate that was resistant to ciprofloxacin, Norfloxacin, Amoxicillin/Clavulanic acid only.

Polymerase chain reaction screening for bla KPC resistance gene

Sequence amplification of Klebsiella pneumoniae bla KPC resistance gene was done by PCR technique with product size 340 bp as shown in figure (1). Current study revealed that percentage of bla KPC gene was 42.8% (6 isolates).

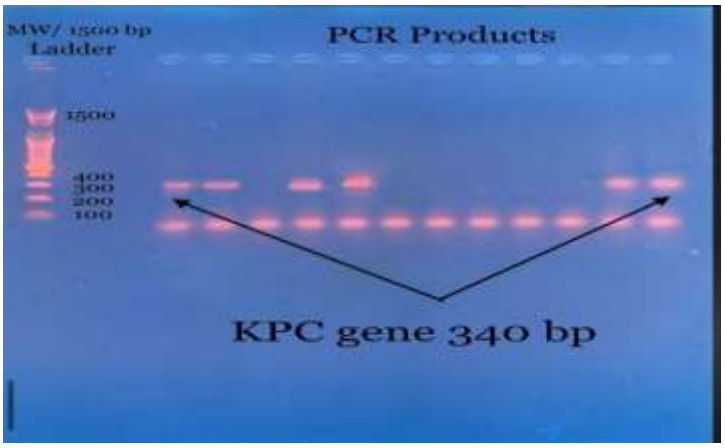

Figure 1. Gel electrophoresis of PCR products (340 bp) for bla KPC resistance gene. Lane 1: 100bp ladder. Lanes 2-13: PCR products of Klebsiella pneumoniae isolates. (1.5% agarose, 7 v/cm, 45 min)

Study KPC resistance gene

The results of antimicrobial susceptibility test showed that all Klebsiella pneumoniae isolates were completely resistant (100%) to Amoxicillin/clavulanic acid whether KPC gene positive or negative; KPC negative isolates showed a higher rate of resistance to Aztreonam (87.5%), Ceftriaxone (62.5%), Norfloxacin (50%), Cefotaxime (75%), Ciprofloxacin (62.5%), Trimethoprim/sulfamethoxazole (75%), Imipenem (37.5%), Meropenem (37.5%), and Trimethoprim (75%). While KPC positive isolates showed a slightly higher resistance for Cefoxitin (33.3%), Gentamicin (50%), Amikacin (33.3%), and Ceftazidime (66.6%) as shown in table (4).

Association Between ESBL and KPC gene

In current study there is no significant association between ESBL and KPC gene as shown in table (5).

Table 4. Association between percentages of resistance bla KPC gene negative and bla KPC gene positive with different antibiotics

	ATM	CRO	NOR	CTX	FOX	CIP	AMC	SXT	CN	AK	IPM	MEM	CAZ	TMP
% of resistance in KPC gene negative	87.5	62.5	50	75	25	62.5	100	75	37.5	25	37.5	37.5	62.5	75
% of resistance in KPC gene positive	66.6	50	33.3	50	33.3	50	100	66.6	50	33.3	16.6	33.3	66.6	66.6

Aztreonam (ATM), Ceftriaxone (CRO), Norfloxacin (NOR), Cefotaxime (CTX), Cefoxitin (FOX), Ciprofloxacin (CIP), Amoxicillin/Clavulanic acid (AMC), Trimethoprim/sulfamethoxazole (SXT), Gentamicin (CN), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Ceftazidime (CAZ), Trimethoprim (TMP)

Table 5. Association between ESBL and KPC gene

ESBL		KPC_gene		Total
		Absent	Present	
Negative	Count	6	5	11
	% within ESBL	54.5%	45.5%	100.0%
	% within KPC gene	75.0%	83.3%	78.6%
Positive	Count	2	1	3
	% within ESBL	66.7%	33.3%	100.0%
	% within KPC gene	25.0%	16.7%	21.4%
Total	Count	8	6	14
	% within ESBL	57.1%	42.9%	100.0%
	% within KPC gene	100.0%	100.0%	100.0%

KPC resistance gene sequencing

Gene sequencing is backed by automated DNA sequencing methods and computer software (BLAST which means Basic Local Alignment Search Tool) to assemble the enormous sequence data.

This gene is highly diveresed. Despite it is chromosomal gene; but exposed to highly mutation. So it might affect antibiogram and selective pressure of mutated strain. Z1, Z2, Z3, Z4 and Z5 are blasting with gene bank. The closest strain to Z1 is E16KP0102 which has been submitted to Gene bank with 98% identities and 1% Gaps. The types of mutation are insertion and transition as seen in figure (2).

The closest strain to Z2 is KPN1344 which has been submitted to Gene bank with 94% identities and 2% Gaps. The types of mutation are insertion, transition and transversion as seen in figure (3).

The closest strain to Z3 is E17KP0052 which has been submitted to Gene bank with 86% identities and 3% Gaps. The types of mutation are insertion, transition and transversion as shown in figure (4).

The closest strain to Z4 is KPN236 which has been submitted to Gene bank with 99% identities and 0% Gaps. The type of mutation is insertion as shown in figure (5).

The closest strain to Z5 is E17KP0052 which has been submitted to Gene bank with 98% identities and 1% Gaps. The types of mutation are insertion and transversion as shown in figure (6).

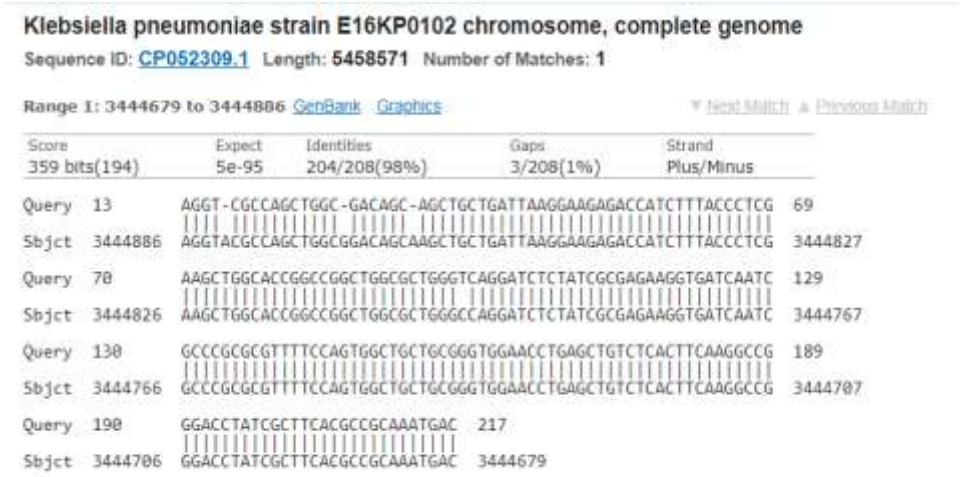

Figure 2. Gene blasting of Z1: Closest strain in gene bank is E16KP0102 within 98% Identities. Query means the studied strain, while subject means strain in the gene bank

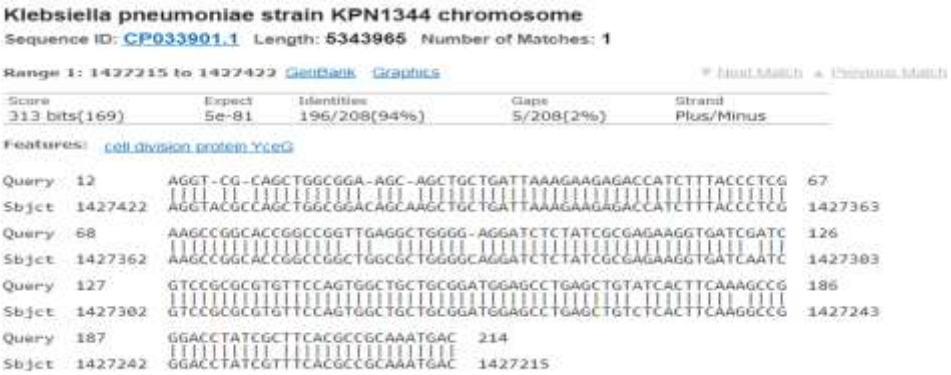

Figure 3. Gene blasting of Z2: Closest strain in gene bank is KPN1344 within 94% Identities. Query means the studied strain, while subject means strain in the gene bank

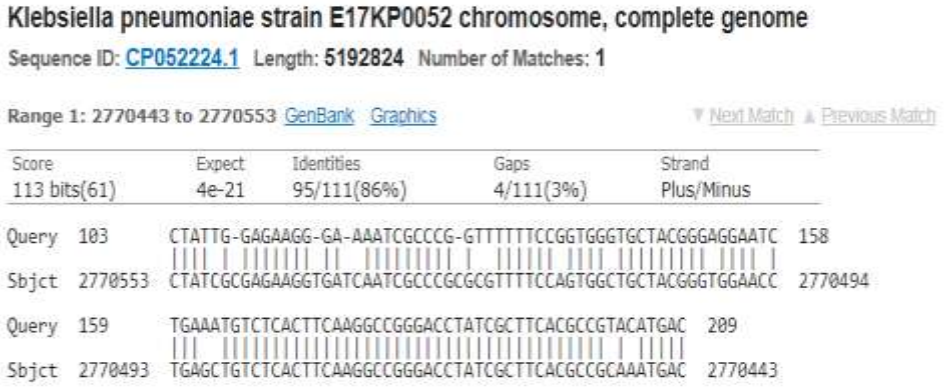

Figure 4. Gene blasting of Z3: Closest strain in gene bank is E17KP0052 within 86% Identities. Query means the studied strain, while Subject means strain in the gene bank

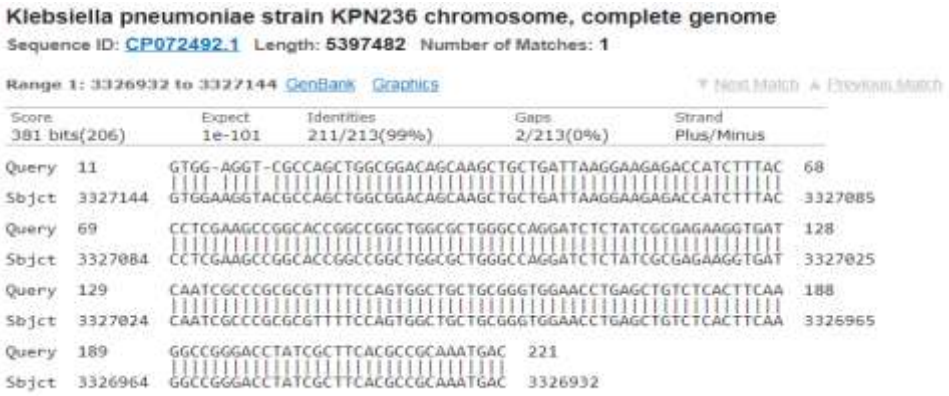

Figure 5. Gene blasting of Z4: Closest strain in gene bank is KPN236 within 99% Identities. Query means the studied strain, while Subject means strain in the gene bank

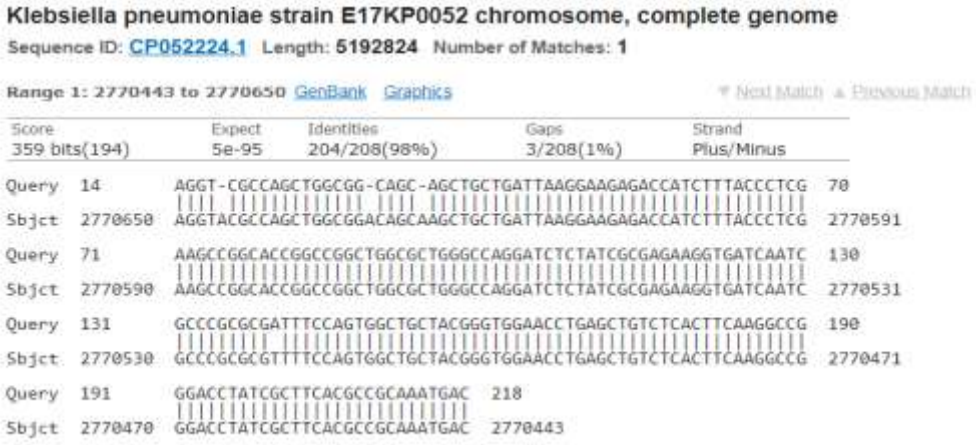

Figure 6. Gene blasting of Z5: Closest strain in gene bank is E17KP0052 within 98% Identities. Query means the studied strain, while Subject means strain in the gene bank

Discussion

In the present study the percentage of *K. pneumoniae* in HAUTI and CAUTI were 50% and 50% respectively. In a study published in Moroco by El Bouamri et al. in 2015 [(14)], the prevalence of *K. pneumoniae* causing UTI was (22%) (321 isolates), in which (82%) (263 isolates) of isolated strains of *K. pneumoniae* caused CAUTIs. Whereas in study conducted in Portugal by Caneiras et al. in 2019 [(15)], the frequency of total *K. pneumoniae* isolates was 81 (50 CAUTI and 31 HAUTI). This difference in the prevalence *of K. pneumoniae* in the current study and previous studies could be due to the larger sample size included in mentioned studies, differences in geographical areas, in addition to the differences of methods used in each study and the skill of researchers.

Emergence of antibiotic resistance is an important process due to its capacity to resist and acquire various resistance mechanisms against antibacterial drugs.

The results of this study showed that 100% of *K. pneumoniae* isolates were resistant to Amoxicillin/clavulanic acid in both HAUTI and CAUTI. Similar findings in a study conducted in Iran by Ranjbar et al. in 2020 [(16)], who reported that, the high rate of resistance to Amoxicillin/clavulanic acid was (95.65%) making this antibiotic a poor choice for

treatment of infections with Klebsiella pneumoniae that causes hospital acquired urinary tract infection.

The current study included high rate of resistance (71.4%) to Aztreonam, trimethoprim/sulfamethoxazole and Trimethoprim in HAUTI and CAUTI. In a study conducted in Iran by Ranjbar et al. in 2020 [16], that percentage of resistance to Aztreonam was (74.78%,) which is compatible with the current results. In study that done in South Africa by Vasaikar et al. in 2017 [17], showed high percentage of resistance to Trimethoprim/Sulfamethoxazole (70.8%) and aztreonam (62.4%), which agree with the current one.

In the present study resistance to Cefotaxime and Ceftazidime were (57.1% and 71.4%), whereas Ceftriaxone was (42.8% and 71.4%), Ciprofloxacin (57.1% and 57.1%) in HAUTI and CAUTI respectively. This study is in compatible with the study done in north-central Iran by Yazdansetad et al. in 2019 [18], who reported that the percentage of resistance of Cefotaxime (40%), Ceftazidime (55%) and Ciprofloxacin (60%) in HAUTI.

The study conducted in Bobo-Dioulasso by Sagna et al. in 2019 [19], found the resistance to Cefotaxime was (56.4%), Ceftazidime (49.1%), Ceftriaxone (1.8%), which disagree with the current results.

In the present study the resistance was moderate – to - low to Gentamicin (57.1% and 28.5%), Norfloxacin (42.8% and 42.8%), Cefoxitin and Amikacin (42.8% and 14.2%), Meropenem (28.5% and 42.8%) and Imipenem (28.5% and 28.5%) in HAUTI and CAUTI respectively. The current study shows a kind of compatibility with a study published in South Africa by Vasaikar et al. in 2017 [18], the resistance to Amikacin (5%), Imipenem (0%), Meropenem (0%), Norfloxacin (1.5%), Cefoxitin (8.9%), Ciprofloxacin (29.7%) were low. Also, this study agrees with the study published in north-central Iran by Yazdansetad et al. in 2019 [18], the resistance of Gentamicin was (30%), Norfloxacin (65%), Amikacin (50%) and Imipenem (15%) in nosocomial UTI.

In the current study, the percentage of the positive ESBL was 21.43%. (28.5% positive in CAUTI and 14.28% positive in HAUTI). While in a study that published in Kurnool by Sarojamma et al. in 2011 [20], ESBL was positive in (28%) of the hospital isolates compared to (6%) of community isolates.

Antibiotic resistance has been increased in Iraq because these antibiotics are taken without prescription (abuse of antibiotics). Also, the bad mark that imported, bad storage of antibiotics leads to develop MDR (multidrug resistance) and XDR (extensively drug resistance) strains.

The current study exhibited a percentage of positive ESBL as 21.43%. (28.5% in CAUTI and 14.28% in HAUTI). Prevalence of ESBL producers in any hospital depends upon various factors like antibiotic policy, the carriage rate among the hospital personnel, and the type of disinfection used especially in ICU [21]. In a study conducted in in Africa by Sirot et al. in 1987 [22], the percentage of positive ESBL was 22.8% in which is compatible with the current results. In a study done in China by 23. Rodríguez-Martínez et al. in 2011 [23], positive ESBL was 49.2% which is not compatible with the present results. This differences in prevalence of high resistance rate of antimicrobials and high prevalence of ESBL producing *K. pneumoniae* strains may be attributable to the differences degrees in virulence strains, antimicrobial stewardship program, geographic differences and infection control practices [24,25].

In this study, bla KPC resistance gene was identified in *K. pneumoniae* isolates using PCR technique. Current study revealed that the percentage of bla KPC gene by conventional PCR was 42.8%. Whereas in study that conducted in Brazil by Ferreira et al. in 2018 [26], all isolates (100%) were positive for the bla KPC gene which is not compatible with the current results.

The results of antimicrobial susceptibility test showed that all Klebsiella pneumoniae isolates were complete resistant (100%) to Amoxicillin/clavulanic acid regardless of whether KPC gene positive or KPC gene negative; while there is high rate of resistance (87.5%) to Aztreonam, (75%) to each cefotaxime, Trimethoprim/Sulfamethoxazole

and Trimethoprim alone in KPC gene negative. While resistance to these mentioned antibiotics were moderate in KPC gene positive. Also, the resistance was moderate - to- low to Ceftazidime (62.5 % and 66.6 %), Ceftriaxone and Ciprofloxacin (62.5 % and 50 %), Gentamicin (37.5 % and 50 %), Norfloxacin (50 % and 33.3 %), Cefoxitin and Amikacin (25 % and 33.3 %), Meropenem (37.5 % and 33.3 %) and Imipenem (37.5 % and 16.6 %) within each KPC gene negative and KPC gene positive respectively.

In study conducted in Iran by Bina et al. in 2015 [27], 14.65% of the *K. pneumoniae* strains were resistant to carbapenems. The antibiotic susceptibility test results exhibited that the highest resistance to the antibiotic were related to Gentamicin and Cefepime [28], whereas, the lowest rate was related to Imipenem, Meropenem, Ertapenem and Ceftazidime [29]. In the MHT (modified Hodge test), 80.5% strains were positive for KPC. All 41 carbapenem resistant *K. pneumoniae* isolates were negative for bla-KPC gene.

While, other studies in the USA [30], China [31] and Italy [32] confirm the presence of the bla-KPC gene in carbapenem resistant *K. pneumoniae* isolates by PCR. This difference can be due to decreased susceptibility to at least one extended-spectrum Cephalosporin [33] and another mechanism such as of carbapenem resistance as a result of a combination of an ESBL or AmpC-type enzyme with porin loss [34,35].

In the current study, there was no significant association between ESBL and bla KPC gene. While in a study done in Brazil by Peirano et al. in2019 [36], the isolates that were positive for KPC were ESBL phenotype production. ESBL-encoding gene bla KPC-2 (six isolates) carbapenemase was detected among isolates. Most KPC producers are ESBL producers as well which is not compatible with the current results [37].

The present study observed that KPC gene is highly diverse. Despite it is a chromosomal gene; it is highly mutating. This might affect antibiogram and selective pressure of mutated strain. As Study that was published in Virginia by Sheppard et al. in 2016 [38], explained that KPC leads to multidrug resistance, with KPC-producing Enterobacteriaceae becoming increasingly widespread in nosocomial infections. This gene revealing a high level of genetic diversity, with the most prevalent lineage being ST941 [39].

The power of the current study was finding Imipenem as the most effective antibiotic can be used for treatment of UTI caused by *K. pneumoniae*. Also, finding different types of mutation of bla KPC gene which have positive effect in antibiotic susceptibility of *K. pneumoniae* isolates.

While the limitations in the present study was as in most empirical studies, it was limited by the time, Sample size, location of study, sampling technique used and variables selected.

Acknowledgement

All authors contributed to this manuscript. Dr. Abdulrahman: design, interpreted and arranged this manuscript. Nasser: performed all laboratory work, implementation and progress of this study. Dr. Malik: helped by giving clinical notes in collection of samples of the study.

Author contribution

All authors contributed to this manuscript. Dr. Abdulrahman: design, interpreted and arranged this manuscript. Nasser: performed all laboratory work, implementation and progress of this study, Dr. Malik: helped by giving clinical notes in collection of samples of the study.

Conflict of interest

There is no conflict of interest.

Funding

No financial support to this study.

References

1. Lin WH, Wang MC, Tseng CC, et al. Clinical and microbiological characteristics of Klebsiella pneumoniae isolates causing community-acquired urinary tract infections. Infection. 2010; 38(6): 459-64. doi: 10.1007/s15010-010-0049-5.

2. Tacconelli E, Magrini N. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO. 27 February 2018. URL: https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf
3. Venditti M. Definitions and challenges in every-day - practice: how to establish that an infection is healthcare associated, Medicine May issue, 2009.. URL: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjuqerT0sz2AhVzQvEDHVU7C0wQFnoECA4QAQ&url=https%3A%2F%2Fwww.who.int%2Fgpsc%2Finformation_centre%2Fvenditti-mario.pdf%3Fua%3D1&usg=AOvVaw1Cre0RrGs9JEUH8O2mjXTe.
4. Ku YH, Chuang YC, Chen CC, et al. Klebsiella pneumoniae isolates from meningitis: Epidemiology, virulence and antibiotic resistance. Sci Rep. 2017; 7(1): 6634. doi: 10.1038/s41598-017-06878-6.
5. Dsouza R, Pinto NA, Hwang I, et al. Panel strain of Klebsiella pneumoniae for beta-lactam antibiotic evaluation: their phenotypic and genotypic characterization. PeerJ. 2017; 5: e2896. doi: 10.7717/peerj.2896.
6. Russo R, Kolesnikova I, Kim T, et al. Susceptibility of Virulent Yersinia pestis bacteria to predator bacteria in the lungs of mice. Microorganisms. 2018; 7(1): 2. doi: 10.3390/microorganisms7010002.
7. Okoche D, Asiimwe BB, Katabazi FA, et al. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLoS One. 2015; 10(8): e0135745. doi: 10.1371/journal.pone.0135745.
8. Tzouvelekis LS, Tzelepi E, Tassios PT, et al. CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. Int J Antimicrob Agents. 2000; 14(2): 137-42. doi: 10.1016/s0924-8579(99)00165-x.
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100- S26. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
10. Drieux L, Brossier F, Sougakoff W, et al. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect. 2008; 14 Suppl 1: 90-103. doi: 10.1111/j.1469-0691.2007.01846.x.
11. Mlynarcik P, Roderova M, Kolar M. Primer evaluation for PCR and its application for detection of carbapenemases in Enterobacteriaceae. Jundishapur J Microbiol. 2016; 9(1): e29314. doi: 10.5812/jjm.29314.
12. Malekjamshidi MR, Zandi H, Eftekhar F. Prevalence of extended-spectrum β-lactamase and integron gene carriage in multidrug-resistant Klebsiella species isolated from outpatients in Yazd, Iran. Iran J Med Sci. 2020; 45(1): 23-31. doi: 10.30476/IJMS.2019.45334.
13. Sambrook J, Russell DW. Molecular cloning: A laboratory manual, 3rd ed. Cold Spring Harbor NY, Cold Spring Harbor Laboratory Press; 2001.
14. El Bouamri MC, Arsalane L, El Kamouni Y, et al. Antimicrobial susceptibility of urinary Klebsiella pneumoniae and the emergence of carbapenem-resistant strains: A retrospective study from a university hospital in Morocco, North Africa, African. J Urology. 2015; 21(1): 36-40. doi: http://dx.doi.org/10.1016/j.afju.2014.10.004.
15. Caneiras C, Lito L, Melo-Cristino J, et al. Community- and hospital-acquired Klebsiella pneumoniae urinary tract infections in Portugal: Virulence and antibiotic resistance. Microorganisms. 2019; 7(5): 138. doi: 10.3390/microorganisms7050138.
16. Ranjbar R, Fatahian Kelishadrokhi A, Chehelgerdi M. Molecular characterization, serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the Klebsiella pneumoniae strains isolated from different types of hospital-acquired infections. Infect Drug Resist. 2019; 12: 603-611. doi: 10.2147/IDR.S199639.
17. Vasaikar S, Obi L, Morobe I, et al. Molecular Characteristics and antibiotic resistance profiles of Klebsiella Isolates in Mthatha, Eastern Cape Province, South Africa. Int J Microbiol. 2017; 2017:8486742. doi: 10.1155/2017/8486742.
18. Yazdansetad S, Alkhudhairy MK, Najafpour R, et al. Preliminary survey of extended-spectrum β-lactamases (ESBLs) in nosocomial uropathogen Klebsiella pneumoniae in north-central Iran. Heliyon. 2019; 5(9): e02349. doi: 10.1016/j.heliyon.2019.e02349.
19. Sagna T, Somda WDN, Koné AC, et al. Antibiotic susceptibility of Escherichia coli and Klebsiella pneumoniae strains, urinary tract infections cases in Bobo-Dioulasso, Burkina Faso; EC Microbiology 15.3 (2019): 172-8.
20. Sarojamma V, Ramakrishna V. Prevalence of ESBL-producing Klebsiella pneumoniae isolates in tertiary care hospital. ISRN Microbiol. 2011; 2011: 318348. doi: 10.5402/2011/318348.
21. Ananthan S, Subha A. Cefoxitin resistance mediated by loss of a porin in clinical strains of Klebsiella pneumoniae and Escherichia coli. Indian J Med Microbiol. 2005; 23(1): 20-3. doi: 10.4103/0255-0857.13867.
22. Sirot D, Sirot J, Labia R, et al. Transferable resistance to third-generation cephalosporins in clinical isolates of Klebsiella pneumoniae: identification of CTX-1, a novel beta-lactamase. J Antimicrob Chemother. 1987; 20(3): 323-34. doi: 10.1093/jac/20.3.323.
23. Rodríguez-Martínez JM, Cano ME, Velasco C, et al. Plasmid-mediated quinolone resistance: an update. J

Infect Chemother. 2011; 17(2): 149-82. doi: 10.1007/s10156-010-0120-2.
24. Collee JG, Fraser AG, Marmion BP, et al. Mackie and McCartney Practical medical microbiology. 14th ed. London, UK: Elsevier; 1996. p: 263-98.
25. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. London, UK: Lippincott Williams & Wilkins; 2000. p. 912.
26. Ferreira RL, da Silva BCM, Rezende GS, et al. High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-Lactamase encoding genes in a Brazilian Intensive Care Unit. Front Microbiol. 2019; 9: 3198. doi: 10.3389/fmicb.2018.03198.
27. Bina M, Pournajaf A, Mirkalantari S, et al. Detection of the Klebsiella pneumoniae carbapenemase (KPC) in K. pneumoniae Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. Iran J Pathol. 2015; 10(3): 199-205.
28. Cury AP, Andreazzi D, Maffucci M, et al. The modified Hodge test is a useful tool for ruling out Klebsiella pneumoniae carbapenemase. Clinics (Sao Paulo). 2012; 67(12): 1427-31. doi: 10.6061/clinics/2012(12)13.
29. Bradford PA, Bratu S, Urban C, et al. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin Infect Dis. 2004; 39(1): 55-60. doi: 10.1086/421495.
30. Munoz-Price LS, Hayden MK, Lolans K, et al. Successful control of an outbreak of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae at a long-term acute care hospital. Infect Control Hosp Epidemiol. 2010; 31(4): 341-7. doi: 10.1086/651097.
31. Chen S, Hu F, Xu X, et al. High prevalence of KPC-2-type carbapenemase coupled with CTX-M-type extended-spectrum beta-lactamases in carbapenem-resistant Klebsiella pneumoniae in a teaching hospital in China. Antimicrob Agents Chemother. 2011; 55(5): 2493-4. doi: 10.1128/AAC.00047-11.
32. Mosca A, Miragliotta L, Del Prete R, et al. Rapid and sensitive detection of bla KPC gene in clinical isolates of Klebsiella pneumoniae by a molecular real-time assay. Springerplus. 2013; 2(1): 31. doi: 10.1186/2193-1801-2-31.
33. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 20th informational supplement. Wayne, PA: CLSI; 2010.
34. Kaczmarek FM, Dib-Hajj F, Shang W, et al. High-level carbapenem resistance in a Klebsiella pneumoniae clinical isolate is due to the combination of bla(ACT-1) beta-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin phoe. Antimicrob Agents Chemother. 2006 Oct; 50(10): 3396-406. doi: 10.1128/AAC.00285-06.
35. Crowley B, Benedí VJ, Doménech-Sánchez A. Expression of SHV-2 beta-lactamase and of reduced amounts of OmpK36 porin in Klebsiella pneumoniae results in increased resistance to cephalosporins and carbapenems. Antimicrob Agents Chemother. 2002; 46(11): 3679-82. doi: 10.1128/AAC.46.11.3679-3682.2002.
36. Peirano G, Seki LM, Val Passos VL, et al. Carbapenem-hydrolysing beta-lactamase KPC-2 in Klebsiella pneumoniae isolated in Rio de Janeiro, Brazil. J Antimicrob Chemother. 2009; 63(2): 265-8. doi: 10.1093/jac/dkn484.
37. Anderson KF, Lonsway DR, Rasheed JK, et al. Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase in Enterobacteriaceae. J Clin Microbiol. 2007; 45(8): 2723-5. doi: 10.1128/JCM.00015-07.
38. Sheppard AE, Stoesser N, Sebra R, et al. Complete Genome squence of KPC-Producing Klebsiella pneumoniae strain CAV1193. Genome Announc. 2016; 4(1): e01649-15. doi: 10.1128/genomeA.01649-15.
39. Mathers AJ, Stoesser N, Sheppard AE, et al. Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae at a single institution: insights into endemicity from whole-genome sequencing. Antimicrob Agents Chemother. 2015; 59(3): 1656-63. doi: 10.1128/AAC.04292-14.

Correspondence to Heba H. Nasser
E-mail: hebeh.hussein@gmail.com
Received Oct. 24th 2021
Accepted Mar. 13th 2022