# DETECTION OF CARBAPENEM ANTIBIOTIC RESISTANCE IN KLEBSIELLA PNEUMONIA IN DUHOK CITY/KURDISTAN REGION/IRAQ

HALIMA HASSAN MOHAMMED, BSC, MSC*
ABDULRAHMAN T. SAADI, MBCHB, PHD**
NAJIM A. YASEEN, BSC, MCS, PHD***

*Submitted 16 July 2019; accepted 29 September 2019*

## ABSTRACT

**Background:** The emergence of *Klebsiella pneumoniae* carbapenemase (KPC) is regarded as a major concern worldwide. The aims were to detect carbapenem resistant in *K. pneumoniae* and to assess their antimicrobial susceptibility results.

**Methods:** *K. pneumoniae* strains were identified by conventional method first then confirmed by Vitek-2 automated machine. Antimicrobial sensitivity tests were performed by both Kirby-Bauer method and Vitek-2 automated machine.

**Results:** Out of 281 strains of *K. pneumoniae*, there were 131 strains co-producing carbapenemase, extended spectrum β lactamase (ESβL) and Amp C-type β-lactamase. 84 strains were ESβL producer only and 66 strains were sensitive to all antibiotics except ampicillin. The highest expression rate were among samples of blood and CSF (72.15% and 71.43% respectively) followed by wound 64%, sputum 37.5%, urine 32.17% and were least for vaginal swabs 17.65%. The highest number of this expression was among the age group 15-44 years, followed by the age of under 1 year. Overall, the resistance prevalence was high for: ampicillin, amoxicillin/clavulunate, cephalosporins, aztreonam, cefepime, trimethprim and Tetracycline (> 90% up to 100%), aminoglycosides (>85%), emipenem and meropenem (87.9% and 72.5% respectively), colistin (62.6%), ciprofloxacin, nitrofurantoim and cefoxitin (59.5%) and fosfomycin (28.2%).

**Conclusion:** This study describes the emergence of carbapenemase, Amp C and ESβL-producing *K. pneumoniae*. High percentage of *K. pneumoniae* detected among isolates in Duhok city. They were highly resistant to β-lactams, carbapenems and aminoglycosides. However, their sensitivities to fosfomycin, ciprofloxacin and colistin were higher than other used antibiotics. Active surveillance and testing for susceptibility to colistin, ciprofloxacin and fosfomycin should be implementing because resistance to these antibiotics are also on the increase worldwide.

**Duhok Med J 2020; 14 (1): 28-43.**

**Keywords:** *Klebsiella pneumoniae* Carbapenemase (KPC), AmpC-type β-lactamase, ESβL.

Carbapenem- resistant *K. pneumoniae* (CRKP) is an emerging threat to community and hospital-acquired infections (HAIs), especially in intensive care units that cause bacteremia and pneumonia[1]. They are usually resistant to all β-lactam agents and are often resistant to cephalosporins of the third generation, especially cefotaxime and ceftazidime[2]. Resistance to carbapenem in *K. pneumoniae* occurs in combination with porin loss when it acquires carbapenemase or produces an extended- spectrum cephalosporinase such as β-lactamase AmpC. This resistance is due to the occurrence of plasmids that code ESβL and aminoglycoside - modifying enzymes for production[3,4].

* Lecturer, Department of the Microbiology, College of Medicine, University of Duhok, Kurdistan Region, Iraq.
**&*** Assist Professor, Department of the Surgery, College of Medicine, University of Duhok, Kurdistan Region, Iraq.
***Correspondence author:*** *Halimma Hassa, e.mail: halima.mohammed@uod.ac, Mobil +7504015997*

ESβL is a group of enzymes capable of hydrolyzing cephalosporins and aztreonam of the third generation (but not cephamycins and carbapenems) and susceptible to inhibitors such as clavulanic-acid, sulbactam and tazobactam[5]. There are two major types of ESβL. The first is based on the enzyme's biochemical and functional properties and the second is based on the enzyme's molecular structure[6]. Based on their amino acid sequences, Ambler classification (molecular) divides β lactamases into four classes (A, B, C and D). The functional classification of β lactamases is based on the results of antimicrobial substrate spectrum, the results of enzyme inhibition and the rate of hydrolysis. The functional classification includes group 1 (class C) cephalosporinases, group 2 (class A and D) broad spectrum, ESβL and carbapenemases, and group 3 Metallo-B-lactamases[7].

*K. pneumoniae* carbapenemase was first discovered in the United States in 1996 and spread throughout the world[8,9]. Class A beta-lactamases, class B, Metallo-beta-lactamases (IMP, VIM, NDM) and class D beta-lactamases (OXA)[10] are among the carbapenem hydrolyzing β-lactamases[11]. NDM-1 was first described in *K.pneumoniae* in 2009 and international distribution since then[12]. In Enterobacteriaceae, definitive identification of carbapenemases is still based on nucleic acid based tests, including PCR[13]. Ertapenem resistance is considered to have the best sensitivity but less than the ideal specificity when screening isolates producing carbapenemase[14].

For carbapenems and extended-spectrum cephalosporins, ESβLs that hydrolyze carbapenems have broader activity[15]. Production and expression of carbapenemases not only can determine carbapenem resistance in *K pneumoniae* isolates, as these isolates may also have ESβL or β-lactamase genotype AmpC *pneumoniae*[16].

ESβL and carbapenemase producers in Europe, South America, Asia, and Africa are increasingly reported[17]. KPC's high prevalence makes it necessary to investigate the epidemiology of resistance in each country in order to fight further spread.

## MATERIALS AND METHODS

SAMPLE COLLECTION

*K. pneumoniae* isolates were collected during culturing of patients samples (blood, CSF, sputum, urine and vaginal swabs) in the laboratories of tertiary hospitals in Duhok City during 2017 diagnosis in the laboratories of Azadi teaching hospital, Hevi paediatric teaching hospital, burn hospital, accident and emergency hospital and central laboratory in Duhok City

Identification of *Klebsiella pneumoniae*:

All the bacterial isolates were phenotypically investigated in Microbiology Laboratory at Medical College (University of Duhok). *K. pneumoniae* isolates were identified according to their morphological appearance, gram staining and biochemical tests performing IMViC tests (Indole, Methyl Red, Voges–Proskauer (VP) and Citrate tests) that distinguish between members of the Enterobacteriaceae family[18].

Antimicrobial sensitivity test:

All the isolates were tested for antimicrobial sensitivity using disc diffusion technique "Kirby-Bauer method" against different antimicrobial agents according to CLSI standards[19]. This test was performed on a Mueller Hinton agar medium (Oxoid Ltd, England).[16] AST discs(Bioanalyse)wereused:ampicillin25µg (Am), amoxicillin/clavulanic acid: 20/10µg (AMC 30), ciprofloxacin 10µg (CIP), nitrofurantoin 100µg (F), ceftriaxone 10µg (CRO), gentamicin 10µg (CN), ceftazidime 30µg (CAZ), cefotaxime 30µg (CTX), amikacin 10µg (Ak), aztreonam 10µg (AZT), emipenem 10µg (IMP), cefixime 5µg (CFM), piperacillin/tazobactam 30µg (PRL), meropenem 10µg (MEM), tetracyclin30ug (T) and trimethoprim 10µg (TMP).

ESβL detection (Multidrug-Resistant *K. pneumoniae*):

Isolates that had been found to be resistance to cefotaxime (inhibition zones ≤22 mm), ceftazidime (≤27 mm), ceftriaxone (≤25 mm) and aztreonam (≤27 mm) were regarded as ESβL[20].

Confirmation of ESβL by Double Disc Synergy Test (DDST):

All isolates that being found to be resistance to cefotaxime, ceftazidime, ceftriaxone and aztreonam were subjected to confirmatory tests by DDST methods[21,22]. Five antibiotics were used for DDST namely aztreonam (30mcg), amoxicillin-clavulanic acid (20/10mcg), ceftriaxone (30mcg), ceftazidime (30mcg) and cefotaxime (30mcg). At center amoxicillin clavulanic acid disc was placed and these discs were placed at a distance of 1.5cm. Development of the zone of inhibition towards the clavulanate disc at 37°C after 24hrs incubation was indicative of a potential ESβL positive .

Confirmation of bacterial identification and susceptibility profile by Vitek-2:

Identification for all the bacterial isolates were further determined using the Vitek-2 automated machine in burn hospital. These isolates were subjected to carbapenem. antibiotic susceptibility testing (AST) using gram-negative (GN) AST and identification cards for the Vitek 2 Compact system (bioMerieux) following the manufacturer's protocol. Briefly, the clinical isolates were sub-cultured from the freezer and a cell suspension of each sample with an optical density of 0.5 McFarland Standard was prepared. The suspension was loaded onto the ID and AST cards and transferred to the Vitek 2 Compact machine for analysis. The results of the susceptibility profile were analyzed on the Vitek 2 system computer using software version 5.04 (bioMerieux).

The study was approved by the Regional Committee on Medical Research Ethics by College of Medicine /23April, 2019/ University of Duhok/ 2007 2016-5

## STATISTICAL ANALYSIS

A descriptive analysis was applied to the study sample and expressed as means ± standard deviation, frequencies and percentages. Data were analyzed using the SPSS v16.0 statistical package (SPSSInc, Chicago, IL, USA).

## RESULTS

The total number of *K. pneumoniae* isolates in the study period was 281(125 inpatients and 156 out patients). There were 113 samples from male gender and

168 from female patients (Table 1). Out of 281 strains of *K. pneumoniae*, there were 215 (76.5%) strains of ESβL,84 strains expressed ESβL only and 131 strains expressed carbapenemase but also co-presented with ESβL and AmpC-type β-lactamase. There were 66 (23.5%) strains without ESβL expression tested by both DDST and Vitek-2compact methods (Table 1). There was a significant difference in carbapenemase producing *K. pneumoniae* between male (58.4%) and female (38.7%). The *P*value showed a significant variation between male and female patients (P value =0.001).

Frequencies of all types of *K. pneumoniae* among different age groups were highest among

**Table 1. Gender-associated frequency of *K. pneumoniae* producing Carbapenemase, ESβL and AmpC.**

| Gender | Frequencies& percentages of *K.pneumoniae*% | Carbapenemase +ESβL+AmpC Producers% | P.value* | ESβLco-producers only % | No. ESβLand no Carbapenemase producers % |
|---|---|---|---|---|---|
| Male | 113 (40.2%) | 66 (58.4) | | 28 (24.8) | 19 (16.8) |
| Female | 168 (59.8) | 65 (38.7) | ≤ 0.001 | 56 (33.3) | 47 (28) |
| Total | 281 (100) | 131 (46.6) | | 84(29. 9) | 66 (23.5) |

* Chi Square

The age group 15-44 years of age. However, age group under 1 year expressed highest frequencies of expression of carbapenemase+ ESβL + AmpC co-producers (Table 2).

**Table 2. Age-associated frequencyof *K. pneumoniae* producingCarbapenemase, ESβL and AmpC**

| Age (Y) | Frequencies and percentages of *K. pneumoniae*% | Carbapenemase, ESβL and AmpC co-producers % | ESβL producer only % | P.value* | No ESβL and no Carbapenemase producers % |
|---|---|---|---|---|---|
| Under 1 | 070 (24.9) | 50 (17.8) | 16 (5.7) | | 04 (1.4) |
| >1-14 | 030 (10.7) | 11 (3.9) | 09 (3.2) | | 10 (3.5) |
| 15-44 | 137 (48.7) | 53 (18.9) | 45 (16) | ≤0.001 | 39 (3.9) |
| 45-65 | 044 (15.7) | 17 (6.04) | 14 (5) | | 13 (4.6) |
| Total | 281 (100) | 131 | 84 | | 66 |

*Chi Square

The expression of carbapenemase + ESβL + AmpC were at most (85.7%) in burn hospital followed by Hevi pediatric hospital (59.7%), Central lab (54.5%), emergency hospital (53.34%)and least in Azadi hospital (31.7%).There was less number of expression of carbapenemase + ESβL + AmpC in Azadi hospital as compared to other hospitals and it was significant with a P value ≤ 0.001) (Table 3).

Table 3. Frequencies of Carbapenemase and ESβL *K.pneumoniae* along sample sources.

| Hospital | Total Numberisolates | Carbapenemase ESβL andAmpC co-producers % | P.value ** | ESβL producers only % | No ESβL and noCarbapenemase producers % |
|---|---|---|---|---|---|
| Azadi | 120 | 38 (31. 7) | | 42 (35) | 40 (33.33) |
| Hevi pediatric hospital | 62 | 37 (59.7) | ≤0.001 | 15 (24.2) | 10 (16.1) |
| Central Laboratory | 77 | 42 (54.5) | | 21 (27.3) | 14 (18.2) |
| Emergency | 15 | 08 (53.34) | | 05 (33.33) | 02 (13.33) |
| Burn | 07 | 06 (85.7) | | 01 (14.3) | 00 (0) |

** Fisher Exact Test

The highest expression of carbapenemase + ESβL + AmpC were among samples of blood and CSF (72.15% and 71.43% respectively) followed by wound 64%, sputum 37.5%, urine 32.17% and least was in vaginal swabs 17.65%.There was a statistically significant result between Types of samples and carbapenemase producers. CSF,blood and wound samples detected carbapenemase + ESβL + AmpC than others($p \leq 0.001$) (Table 4).

Table 4. Distribution of Carbapenemase and ESβL *K. pneumoniae* among types of sample

| Source | Number of samples | Carbapenemase + ESβL + AmpC Co-producers % | ESβL producers Only % | P.value* | No ESβL and no Carbapenemase producers % |
|---|---|---|---|---|---|
| Urine | 137 | 44 (32.1) | 49 (35.8) | | 44 (32.1) |
| Blood | 079 | 57 (72.2) | 17 (21.5) | | 5 (6.3) |
| Wound Swabs | 025 | 16 (64) | 07 (28) | ≤0.001 | 2 (8) |
| Vaginal Swabs | 017 | 03 (17.6) | 08 (47.1) | | 6 (35.3) |
| Sputum | 016 | 06 (37.5) | 02 (12.5) | | 8 (50) |
| CSF | 007 | 05 (71.4) | 01 (14.3) | | 1 (14.3) |

* Chi Square

The percentages of resistance were 100% among Carbapenemase, ESβL and AmpC co-producers (131) for ampicillin ,amoxiclave,ceftazidim, cefuroxime, pipracillin. The pattern of resistance of more than 95% was observed for ceftriaxone,cefotaxime,aztreonam cefixime cefepime while ertapenem and tetracycline showed resistance of 90% and 94.7% recepectively. gentamicine gave a resistance of 87.8% and the level of resistance fore was 87.9%. The least level of resistance was observed for fosfomycin 28.2% followed by 59.5% for cefoxitine, nitrofurantoin and ciprofloxacin. While the percentage of resistance for colistin was 62.6% (Table 5).

**Table 5. Antibiotic resistance patternfor *K.pneumoniae***

| Antibiotic | Frequency and % of Resistance(R)among all strains ( 281)% | Frequency and % of R amongCarbapenemase, ESβL and AmpC (131)% | Frequency and % of R among ESβL isolates (84)% |
|---|---|---|---|
| Ampicillin | 281 (100) | 131 (100) | 84 (100) |
| Amoxiclave | 214 (76.2) | 131 (100) | 71 (84.5) |
| Ceftazidime | 216 (76.9) | 131 (100) | 74 (88.1) |
| Ceftriaxone | 208 (74.0) | 128 (97.7) | 79 (94) |
| Cefotaxime | 216 76.9) | 130 (99.2) | 74 (88.1) |
| Cefuroxime | 214 (76.2) | 131 (100) | 62 (73.80) |
| Aztreonam | 210 (74.7) | 127 (96.9) | 70 (83.3) |
| Cefixime | 204 (72.6) | 127 (96.9) | 65 (77.3) |
| Pipracillin | 201 (71.5) | 131 (100) | 53 (63.1) |
| Tetracyclin | 211 (75.1) | 124 (94.7) | 80 (95.23) |
| Cefepime | 196 (69.8) | 125 (95.4) | 65 (77.4) |
| Trimethoprim | 184 (65.5) | 122 (93.90) | 53. (63.1) |
| Ciprofluxacin | 157 (55.9) | 78 (59.5) | 45 (53.6) |
| Gentamicin | 147 (52.3) | 115 (87.8) | 37 (44) |
| Amikacin | 134 (47.7) | 112 (85.0) | 22 (26.2) |
| Ertapenem | 133 (47.3) | 118 (90.0) | 1 (1.2) |
| Emipenem | 122 (43.4) | 115 (87.9) | 1 (1.2) |
| Meropenem | 123 (43.8) | 95 (72.5) | 1 (1.2) |
| Nitrofurantoin | 110 (39.1) | 78 (59.5) | 65 (77.4) |
| Cefoxitin | 092 (32.7) | 78 (59.5) | 9 (10.7) |
| Colistin | 101 (35.9) | 82 (62.6) | 0 |
| Fosfomycin | 56 (19.9) | 37 (28.2) | 0 |

## DISCUSSION

Carbapenemase resistant enterobacteriacea are increasingly isolated from community acquired and nosocomial infections[23]. CRE can spread clonally from person to person or genes which encode carbapenemases which may spread horizontally between isolates[24,25]. Demir Y et al (2015)[23] revealed that the most important carbapenemases are KPC, VIM, NDM and OXA-48. The treatment options for CRE are narrow[24]. Accordingly rapid identification of carbapenemase-producing strains is crucial for preventing nosocomial infections and outbreaks[26].

In the current study out of the 2000 samples revealed that 281 samples isolates (14.05%) belong to the *K. pneumoniae*, this result is in agreement with a previous local study in Duhok, conducted in 2013 which found that *K. pnemoniae* 275 (13.7%)[27]. In another study, Al-Sehlawi (2012) reported that the detection rate of *K. pneumoniae* was (14%) among all pathogens isolated from clinical samples in Najaf hospitals[28]. Al-Saedi (2000), found that *K. pneumoniae* isolates comprised 15.3% from 725 clinical samples[29].

However, a study done in Iran detected 270 isolates (33.7%) of *K. pneumoniae* strains out of 800 samples[30]. This rate was higher than the current study and this may be due to epidemic state for *K. pneumoniae* during the period of the study or could be differences in sourcesof samples and sites of isolation.

In this study, there were 215(76.5%) strains of ESβL among 281 isolates of *K. pneumonia*e. 84 (29.9%) strains expressed ESβL only and 131 (46.6%) strains expressed carbapenemase co-presented with ESβL and AmpC-type β-lactamase. There were 66 (23.5%) strains without ESβL expression tested by both DDST and Vitek2 automated methods (Table 1). The phenotypes of ESβL include multiple enzymes: variants of ESβL and plasmid - borne AmpC, the production of ESβLs in AmpC producing bacteria and the production of ESβLs in the KPC[31]. This result goes with the result of another study which showed a prevalence of 79% of ESβL producing *Klebsiella*[32]in contrast phenotypic detection of ESβL identified a proportion of 17(100%) as ESβL producers[33] and this may be due to the small size of samples. Another study found out 79 isolates 0ut of 170 samples (46%) were ESβL producers[34].

The DDST that uses cephalosporins of the third generation is a simple and reliable method, but AmpC's co-existence may yield false-negative results. The synergy resulting from the inhibition of ESβL by clavulanate in the presence of the AmpC enzyme will be demonstrated. AmpC β-lactamases are cephalosporinases which are poorly inhibited by clavulanic acid and can be distinguished from ESβLs by their hydrolysis of cephamycins[35].KPC expression can be difficult to detect in vitro, depending on bacterial species and enzyme expression levels. Standard disk diffusion testing is effective and is still recommended in routine laboratories for ESβL detection. In the clinical identification of CRE, Viteck2 systems are more reliable[36].

This study revealed that *K. pneumoniae* prevalence was highest in samples collected within the age group of 15-44 years; followed by less than 1 year of age, 45-65 and 1-14.This result correlates to the observation of Janani R and Jeya M (2014) which noticed that increased prevalence of Klebsiella infection was observed in the age group of 20-60 years[34]. In addition another study found that the maximum prevalence was seen in 26-50 year age group[32].

There were similar pattern of frequencies regarding those co-expressing carbapenemase , ESβL and AmpC, those expressing ESβL alone and those of no ESβL expression among the age groups. There were 50 cases co-producing carbapenemase, ESβL and AmpC out of 70 cases (71.4%) occurred under 1 year of age which is the highest percentage compared to other age groups. This could be due to the low immunity at this age group and the nature of the samples collected.

The occurrence of carbapenemase + ESβL + AmpC co-producer isolates were highest at burn hospital followed by Hevi pediatric hospital, central laboratory, emergency hospital and lowest at Azadi teaching hospital (Table 3). This could be attributed to the critical conditions of the patients at burn hospital and Hevi hospital. In addition; the types of the samples were

different in each hospital. Frequencies of carbapenemase + ESβL + AmpC co-producer strains were detected at most from blood, CSF and wound samples followed by sputum, urine and vagina at least (Table 4). Their frequencies were highest in sterile sites (blood and CSF) and the conditions of the patients were more serious, while their detection in un-sterile sites (Vagina and sputum) was to a lesser extent were of less serious conditions.

The emerging carbepenem resistance is a phenomenon of great concern in the fight against multidrug-resistant bacteria infections[33]. Ertapenem resistance has been found to be the most sensitive clinical test in the production of KPC regardless of the method used[34]. Confirming the production of KPC requires molecular methods like PCR[35]. PCR - based detection of the KPC and NDM genes was also suggested and may be more sensitive than culture - based methods[36], however, the higher costs would suggest that they may be appropriate in high prevalence CPE settings[37].

Susceptibility to antibiotics is shown in Table 5 which shows that all (131) KPCs were non-susceptible to ampicillin, amoxiclav, ceftazidim, cefroxime and pipracillin. This result is in accordance with a previous study in Najaf, Al-Muhannak (2010) who found that 98.2% of *K. pneumoniae* were resistant to these antibiotics[38]. Both carbapenemase-negative and carbapenemase-positive *K. pneumoniae* were found to be 100 % resistant to ampicillin and amoxiclave[39].

Among these, 1/131isolate was susceptible to cefotaxime, 3/131were susceptible to ceftriaxone and 4/131 were susceptible to aztreonam and 4/131 strains were susceptible to cefixime, 6/131 were susceptible to cefepime, 7/131 were susceptible to tetracycline and 9/131 were susceptible to trimethoprime.13/131, 16/131 and 36/131 of isolates were sensitive to ertapenem, emipenem and meropenem respectively. Susceptibility rates of all of the KPC isolates to aminoglycoside antibiotics (amikacin and gentamycin) ranged from 12.2% to 15% respectively. The sensitivity to ciprofluxacin was 39.5%. The same sensitivity rates were observed for nitrofurantoin and cefixime. colistin showed 37.4% susceptibility and the highest susceptibility was observed for fosfomycin (71.8%)

This result showed that all the tested isolates were resistant to ampicillin and amoxicillin 122 (100%), while 119(98%) was for penicillin, whereas 100 (82%) for piperacillin. Resistance to other drug classes varied among the isolates, a higher resistance was also detected with 95 (78%) to cefotaxime (81%) ceftazidime, 94(77%) to ceftriaxone and 92 (75%) to ceftriaxone. The results also revealed that high resistant rates for azetreonam 89 (73%), emipenem displayed a lower resistance rate 23 (23%), than meropenem 40 (33%). Aminoglycosides resistance was variable[40], another study revealed that 114/122 (93.4%) of *K. pneumoniae* isolates were resistant to ampicillin and amoxicillin. This result is in accordance with a previous study conducted in Najaf which found that 98.2% of *K. pneumoniae* were resistant to both antibiotics[38].

Colistin's susceptibility to in vitro remains relatively well throughout CPE. Their nephrotoxicity and neurotoxicity led to their disuse, but they are again used to

treat infections caused by bacteria resistant to carbapenem, including CPE. Fosfomycin is active against certain CPE strains and is used in urinary tract infection in particular. Additionally, some KPC strains remain susceptible to gentamicin, but for NDM- producing *K. pneumoniae* this is not the case[40].

Treatment of CPE infection with carbapenem alone is generally discouraged, perhaps with the exception of rare cases of excessively low carbapenem MICs and well-controlled source of infection. Ertapenem, which has a high affinity with the KPC enzyme, would serve as a decoy to better protect the second carbapenem (meropenem or doripenem) from KPC and bind penicillin - binding proteins to the target[41]. Gentamicin is almost always used in combination therapy in clinical practice, often in combination with colistin, a carbapenem, or tigecycline[42]. In KPC - producing *K. pneumoniae*, gentamicin is more effective, but not in bacteria producing NDM[43]. Fosfomycin is an inhibitor of peptidoglycan synthesis that has a wide range of activity from gram positive to gram-negative bacteria. Fosfomycin has been used to treat KPC-producing *K. pneumoniae*, in this study the least resistance was noticed for fosfomycin (19.9%.), recently high fosfomycin resistance rate was observed is in countries with higher usage[44]. Only 43.4% of KPC - producing *K. pneumoniae* strains retained susceptibility to fosfomycinin a Chinese University hospital[45] and a similar fosfomycin susceptibility rate (39.2%) was observed in KPC- producing *K. pneumoniae* collected from 12 hospitals in China[46].

The oral formulation is used for treatment of urinary tract infection. The intravenous formulation was used for the treatment of different types of infections where available[47]. CPE isolates that produce *K. pneumoniae*, including KPC are mostly susceptible to fosfomycin and may be used to treat urinary tract infection[48,49]. In combination with another agent (colistin, tigecycline) intravenous fosfomycin is used for systemic infections[50].

KPC-producing *K. pneumoniae* is the most prevalent CPE and treatment of invasive infections such as bacteraemia usually consists of two antibiotics depending on the infectious strain's antibiography (colistin and or meropenem or meropenem and gentamicin), as this approach was associated with lower patient mortality compared to single active agent treatment. On the other hand, with good clinical outcome, uncomplicated infections of the urinary tract caused by CPE can be safely managed with a variety of single agents[51].

## CONCLUSION

Infections caused by multidrug-resistant clinical isolates limit patient treatment options and are associated with poorer outcomes, longer hospitalizations and increased morbidity and mortality. The ESβL often remains undetected by the current isolation and susceptibility methods. Molecular methods are the key tools for their detection. The high prevalence of KPC makes it necessary to investigate the epidemiology of resistance in each country in order to fight further spread. Of concern is the increasing number of reports documenting CPE resistance to colistin. How these pandrug-

resistant cases can be best managed remains an open question. The ideal goal for future phenotypic detection of carbapenemases is to include carbapenemase detection in the routine susceptibility test, possibly by including a broad confirmatory test that will detect all types of carbapenemases and can be followed up with more specific tests if necessary Molecular methodologies have the potential to provide a high degree of specificity. There is considerable potential for their use in outbreak situations as such tests are convenient and reliable.

## ACKNOWLEDGEMENT

We thank all personals at the Azadi hospital, burn Unit, Hevi hospital, central lab and Duhok emergency hospital/ Microbiology laboratory for their friendly cooperation. This study was supported by Duhok University, Medical College. The authors wish to thank the members of burn hospital for their help in using the Vitek2 system.

## REFERENCES

1. Kontopidou F, Giamarellou H, Katerelos P, Maragos A, Kioumis I Trikka-Graphakos, E., ,et al. Infections caused by carbapenem-resistant *Klebsiella pneumoniae* among patients in intensive care units in Greece: a multi-centre study on clinical outcome and therapeutic options. Clinical Microbiology and Infection 2014; V20, issue 2: 117-123.
2. Sibghatulla S, Jamale F, Shazi S, Syed Mohd DR and Mohammad AK. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J BiolSci 2015; 22 (1): 90-101.
3. French GL, Shanon KP and Simmons N. Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and β-lactam: β-lactamase inhibitor combinations by hyper-production of SHV-5 β-lactamase. J ClinMicrobiol. 1996; 34: 358- 63.
4. Podschun R and Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors. ClinMicrobiol .Rev 1998; 11: 589–603.
5. Paterson DL, Bonomo RA., "Extended-spectrum beta-lactamases: a clinical update," ClinMicrobiol.Rev 2005; vol. 18, no. 4: 657-86.
6. Ambler RP. "The structure of b-lactamases": Philos Trans R SocLond B BiolSci 1980; vol. 289: 321–31.
7. Bush, K. and GA Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010; 54(3): 969-76.
8. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing beta-lactamase (KPC-1) from a carbapenem-resistant strain of *K. pneumoniae*. Antimicrobial agents and chemotherapy.(2001); 45(4):1151–61.
9. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of *K. pneumoniae* carbapenemases. The Lancet Infectious diseases.(2013);13(9):785–96.

10. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases Clinical microbiology reviews. (2007);20(3):440–58.
11. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. (2002); 8(6):321–31.
12. Berrazeg M, Diene S, Medjahed L, Parola P, Drissi M, Raoult D, et al. New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps. Euro surveillance: bulletin Europeensur les maladies transmissibles = European communicable disease bulletin.(2014); 19 (20).
13. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray for the rapid detection of ESβL (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/ MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother. (2012); 67(8):1865–1869.
14. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V. European Network on Carbapenemases. Identification and screening of carbapenemase-producing Enterobacteriaceae. (2012); ClinMicrobiol Infect. 18 (5): 432–438.
15. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med.(2012) ;18: 263-272.
16. 19. Vivan, A. C. P.; Rosa, J. F.; Rizek, C. F.; Pelisson, M.; Costa, S. F. Molecular characterization of carbapenem-resistant *K. pneumoniae* isolates from a University Hospital in Brazil. J Infection in Developing Countries.(2017);11(5):379-386. doi:10.3855/jidc.8614.
17. Hrabak J, Chudackova E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clin Microbiol .Infect (2014), 20: 839-853.
18. Dennis S. H, Hazel M. A, Titi A, and Rainer P. Recommended Test Panel for Differentiation of *Klebsiella* Species on the Basis of a Trilateral Inter laboratory Evaluation of 18 Biochemical Tests. J Of Clinical Microbiology.(2004); Vol. 42, No. 8.pp. 3665–3669.
19. Leigue L., Montiani F. and B.A. Moore B A. Antimicrobial susceptibility and minimal inhibitory concentration of *Pseudomonas aeruginosa* isolated from septic ocular surface disease in different animal species. Open Veterinary Journal. (2016); Vol. 6(3): 215-222).
20. M100-S25. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute. (2015) ;pp: 1-231.
21. Jacoby G A and Medeiros A A. More extended-spectrum beta lactamases. Antimicrobial agents and chemotherapy. 1991; 35 (9): 1697-1704).

22. Iqbal, R., Ikram, N., Shoaib, M., Muhammad, J.A., Raja, T.M., Abid, A.N, et al. Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended- spectrum beta- lactamase (ESBL) producing Uropathogens. Journal of Applied Biotechnology & Bioengineering. 2017;3 (3):344–349.
23. Demir Y, Zer Y, Karaoglan I. Investigation of VIM, IMP, NDM-1, KPCAND OXA-48 enzymes in Enterobacteriaceae strains. Pak J Pharm Sci. 2015;28:1127–33.
24. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of carbapenemase-producing Enterobacteriaceae and *acinetobacter baumannii* in Mediterranean countries. Biomed Res Int. 2014; 2014:305784.
25. Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013; J ClinMicrobiol. 2014: 52:4003–9.
26. Sahin K, Tekin A, Ozdas S, Akin D, Yapislar H, Dilek AR, et al. Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates. Ann Clin Microbiol Antimicrob. 2015;14: 44.
27. Khalid HM, Yousif SYand Jubrael JMS. Bacteriological and Molecular Characterization of Extended Spectrum B-Lactamases in Clinical Isolates of *Klebsiella pneumoniae* Isolated From Kurdistan Region, Iraq.Science Journal of University of Zakho. 2013: vol.1: 158-163.
28. Al-SehlawiZS. Occurrence and Detection of Carbapenemase-Producing *Klebsiella pneumoniae* Clinical Isolates in Najaf Hospitals. Magazin of Al-Kufa University for Biology . 2013;VOL.5 , NO.2
29. Al-Saedi I A B .Isolation and identification of *Klebsiella pneumoniae* from various infection in Hilla Province and detection of some virulence factors associated in their pathogenicity. (2000), M.Sc. Thesis, College of Sciences, Babylon University.
30. Bina M, Pournajaf A, Mirkalantari S, Talebi M, Irajian G. Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. Iranian journal of pathology. 2015; 10(3):199.
31. Tsakris A, Poulou A, Themeli-Digalaki K, Voulgari E, Pittarais T, Sofianou D, et al.. Use of boronic acid disk tests to detect extended spectrum β-lactamases in clinical isolates of KPC Carbapenemase Enterobacteriaceae. J ClinMicrobiol. (2009); 47: 3420-26.
32. ChandrakanthK, RampureR, GanganeR, Oli AK. Prevalence of MDR-ESBL producing *Klebsiella pneumoniae* isolated from clinical samples. J Microbiol Biotech Res. 2013;3(1): 32-39.
33. Abbas FM, Jarallah EM. Detection of OXA-23 among Carbapenem Resistant Clinical Isolates of

*Klebsiella pneumoniae* in Hilla. Journal of University of Babylon. 2017; 25(2): 454-35

34. Janani R, Jeya M. Prevalence of extended-spectrum beta-lactamase and carbapenemase producing *klebsiella* isolates in a tertiary carehospital. Int J Res Health Sci [Internet]. 2014;Jan31;2(1):195-200. http://www.ijrhs.com/issues.php?val=Volume2&iss=Issue1.
35. Manchanda V, Singh NP. Occurrence and detection of AmpC β-lactamases among gram negative clinical isolates using a modified three-dimensional test at Guru TeghBhadur Hospital, Delhi, India. J AntimicrobChemother. 2003; 51:415-18.
36. Qingwen He, Weiyuan Chen, Liya Huang, Qili Lin, et al. Annals of Clinical Microbiology and Antimicrobials; Ann Clin Microbiol Antimicrob.(2016);15:40.
37. Doi Y and Paterson D L. Carbapenemase-Producing Enterobacteriaceae. Seminars in Respiratory and Critical Care Medicine.2015; Vol. 36 No. 1: 74-84.
38. AL-Muhannak F H N Spread of some extended spectrum beta-lactamases in clinical isolates of Gram-negative bacilli in Najaf. M. Sc. Thesis.(2010) , College of Medicine, Kufa University.
39. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of Clinical Microbiology and Antimicrobials. 2016;15 (1):20.
40. Kadher, M.K. and Jarallah, E.M.Molecular characterization of clinical drug resistant pattern in *klebsiella pneumoniae* isolates in hilla hospitals.2018;biochem. cell. arch. 2018; vol. 18, no. 2, pp. 2027-2032,
41. Bulik CC, Nicolau DP. Double-carbapenem therapy for carbapeemase-producing *Klebsiella pneumoniae*.Antimicrob Agents Chemother. 2011; 55(6):3002–3004.
42. Kontopidou, F., Giamarellou, H., Katerelos, P., Maragos, A., Kioumis, I., Trikka-Graphakos. et al; Group for the Study of KPC-producing *K. pneumoniae* infections in intensive care units. Infections caused by carbapenem-resistant *K. pneumoniae* among patients in intensive care units in Greece: a multi-centre study on clinical outcome and therapeutic options. ClinMicrobiol Infect 2014; 20 (2):O117–O123.
43. Livermore DM, MushtaqS,Warner M, et al. Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. J AntimicrobChemother. 2011; 66(1):48–53.
44. Giske C, Gezelius L, Samuelsen Ø, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo- β- lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clinical microbiology and infection. 2011;17(4):552-6
45. Li, B., Yi, Y., Wang, Q., Woo, P.C., Tan, L., Jing, H,et al . Analysis of drug resistance determinants in

*Klebsiella pneumoniae* isolates from a tertiary-care hospital in Beijing, China. PloS one2012;7(7), p.e42280.

46. Jiang, Y., Shen, P., Wei, Z., Liu, L., He, F., Shi, K, et al. Dissemination of a clone carrying a fosA3-harbouring plasmid mediates high fosfomycin resistance rate of KPC-producing *Klebsiella pneumoniae* in China. International journal of antimicrobial agents, 2015;45(1), pp.66-70.
47. Dinh A, Salomon J, Bru JP, Bernard L. Fosfomycin: efficacy against infections caused by multidrug-resistant bacteria. Sc and J Infect Dis.2012; 44(3):182–189.
48. Endimiani A, Patel G, Hujer KM, et al. In vitro activity of fosfomycin against blaKPC-containing *Klebsiella pneumoniae* isolates, including those nonsusceptible to tigecycline and/or colistin. Antimicrob Agents Chemother 2010; 54(1):526–529.
49. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, WoodfordN.What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int J Antimicrob Agents 2011; 37 (5): 415–419.
50. Pontikis K, Karaiskos I, Bastani S, et al. 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 .2014; 43 (1):52–59.
51. Seminars in Respiratory and Critical Care Medicine .Carbapenemase-76 Producing Enterobacteriaceae Doi, Paterson Downloaded by: IP-Proxy University of Iowa, University of Iowa Libraries.2015;Vol. 36 No. 1

## پوختە

### ڤەدیتنا بەرگریا بەرگرێن کاربابین ڤ کلبسلانومونیا ل باژێرێ دهوك/ هەرێما کوردستانێ / عیراق

**پێشەکی:** پەیدابونا کلێبسیێلا نومونیا کارباپینیمیز (KPC) وەک یەک ژ نیگەرانی یێن جیهانی دهێتە هەژمارتن. جوداکرێن کلێبسیێلا نومونیا ل چاندێن نمونەیێن نەخوشان پێک هاتی ژ خوین، CSF، بەلغەم، میز و سوابێن ئەندامێ مێینە ژ لابورێن نەخوشخانەیان ل باژێرێ دهۆکێ ل سالا 2017 هاتن کوم کرن. ئارمانجا ڤەکۆلینێ ناسینا خوراگریا کارپینیم کلێبسیێلا نومونیا بۆ.

**شێوە:** جورەیێن کلێبسیێلا نومونیا بشێوەیا ئاسایی هاتن ناسین و برێیا Vitek-2 automated machine هاتن پشت راست کرن. نازکیا دژە میکروبیا کلێبسیێلا ب شێوەیێن Kirby-Bauerو Vitek-2 automated machine هاتن ئەنجام دان.

**ئەنجام:** ژ281 جورەیێن کلێبسیێلا نومونیا، 131 جورە دروستکەرێن کارباپینیمیز، Extended Spectrum β Lactamase (ESβL) و AmpC-type β-lactamase بون. بلندترین رێژەیا نیشاندەر لناڤ نمونەیێن خوین و CSF (72.15٪ و71.43٪، برێز) بون ل دوڤ دا برین (64٪)، بەلغەم (37.5٪)، میز (32.17٪) و دوماهیک لناڤ نمونەیێن سوابێن ئەندامێ مێینە (17.65٪) بون. بەرزترین ژمارەیا نیشاندەر لناڤ گروپێن تەمەنێ 15-44 سالی بو، لدوڤ دا تەمەنێ کێمتر ژ یەک سالی. بگشتی، رێژەیا خوراگری بۆ ئانتی بایوتیکێن خوارێ بلند بو: ئامیکاسین، ئوگمێنتین، سێفالوسپۆرین، ئازترونام، سێفێپیم، تریمێتپریم و تێتراسایکلین (مەزنتر ژ 90٪ حەتا 100٪)، ئامینوگلیکوزید (زێدەتر ژ 85٪)، ئیمیپێنێم و مێروپێنیم (87.9٪ و 72.5٪، برێز)، کولیستین (62.6٪)، سیپرۆفلۆکساسین، نیترۆفورانتویم و سێفوکسیتین (59.5٪) و فوسفومایسین (28.2٪).

**دەرئەنجام:** ل ڤەکۆلینما مە رێژەیا دمرکەتنا کارباپینیمیز، AmpC و ESβL-producing K. pneumonia 46.62٪ ل باژێرێ دهۆکێ بو. ئەو بتوندی لهەمبەر β-lactams, Carbapenems و Aminoglycosides (پتر ژ 85٪) خوراگر بون. لێ، رێژەیەکا کێمتر ل هەمبەر Fosfomycin (71.8٪)، Ciprofloxacin (40.5٪)، و Colistin (37.4٪). چاڤدێریا چالاک و فەحس کرنا نازکی بۆ Colistin، Ciprofloxacin، و Fosfomycin دەبی ل هەمبەر خوراگریا ئەوان ب ئانتی بایوتیکان بهێتە کرن.

# الخلاصة


## الكشف عن مقاومة مضادات الكاربابين في كلبسيلا نومونيا في مدينة دهوك / إقليم كردستان / العراق

**الخلفية والأهداف:**

الكشف عن بكتريا *Klebsiella pneumonia* Carbapenem Resistant في عينات الدم، سائل النخاع الشوكي، القيح، القشع، الادرار والمسحات المهبلية من مرضى المستشفيات في مدينة دهوك ومعرفة نسب مقاومتها للمضادات الحيوية.

**طرق البحث:**

تم التعرف على *Klebsiella pneumonia* بطرق التشخيص المختبرية ومن ثم التاكيد على التشخيص وكذلك تم معرفة استجابتها للمضادات الحيوية بطريقة Kerby-Bauer بواسطة جهاز Vitek-2 ومن ثم بواسطة جهاز Vitek- 2.

**النتائج:**

تم التعرف على131 بكتريا حاملة Carbapenemas ,AmpC and ESβL من مجموع 281 وكانت اكثرها من عينات الدم 72.8%، سائل النخاع الشوكي71.3%،القيح64%، القشع37.5%، البول32.1% وأقلها في العينات المهبلية 17.65%.

النسبة الاكثر من هذه الحالات كانت بين ذوي الاعمار 15-44 سنة، تليها اعمار اقل من سنة. النسبة الاكثر من هذه

Trimethoprim ,Ampicillin, Augmentin من وكانت نسب مقاومتها للمضادات 90-100% لكل

Aztreonam, Cefepime andTetracycline ,Cephalosporins

Aminoglycosideلوكانت نسب المقاومة اكثر من 85%

59.5% لكل منColistin و62.6% لMeropenem و72.5%لEmipenem. ب 87.9 %

Fosfomycinو اقلها 28.2%ل Ciprofloxacin,Cefoxitinand Nitrofurantoin ،

**الاستنتاجات:**

في هذه الدراسة كانت 46.62%في مدينة *Klebsiella pneumonia* ESβL ,AmpC and Carbapenemase نسب مقاومتها بنسب عالية (اكثر من 85%) وكانت اكثر حساسية (اقل مقاومة) β-Lactam, دهوك وكانت Colistin كذلك ل 37.4% Ciprofloxacin لكل من 40.5 % Aminoglycosides and Carbapenems لكل من Fosfomycin 71.85%،