# Molecular detection of CTX-M extended spectrum beta-lactamase among carbapenem- resistant Klebsiella pneumoniae from Al-Hillah Teaching Hospital environment, Babylon Province, Iraq

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# Molecular detection of CTX-M extended spectrum beta-lactamase among carbapenem- resistant Klebsiella pneumoniae from Al-Hillah Teaching Hospital environment, Babylon Province, Iraq

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**Abstract.** This study analyze the prevalence of *bla*$_{\text{CTX-M}}$ containing *Klebsiella pneumoniae*. During the period from October,2017 to the end of January, 2018, a total of one hundred swab samples were collected from environment of Al-Hillah Teaching Hospital / Hillah city. Thirteen (13%) isolates were identified as *K.pneumoniae*. All *K.pneumoniae* isolates were subjected to antibiotic susceptibility testing using Kirby-Bauer disk diffusion method. Higher resistance rates were observed for penicillin antibiotics (ampicillin and cloxacillin) with resistance rate of (84.61%) and (69.23%), respectively. Extended spectrum beta-lactamase (ESBL) production was assayed phenotypically using disk combination method. Five (38%) isolates were screen-positive. Carbapenem resistance was detected in 2 isolates of *K.pneumoniae*, these were checked further by Polymerase Chain Reaction (PCR) method for the presence of *bla*$_{\text{CTX-M}}$ gene, 1 (50%) isolate gave positive result.

**Keywords** : *Klebsiella pneumoniae*, Carbapenem resistance, ESBL, *bla*$_{\text{CTX-M}}$ gene, PCR.

## Introduction

Resistance to beta-lactam antibiotics is associated with production of β-lactamases which mainly inserted into plasmid, transposons or integron and rapidly spread among different clinical isolates[1]. One of the most common types of β-lactamases are extended spectrum beta-lactamases (ESBLs) which are mutant forms of broad spectrum β-lactamases such as TEM-1 ,TEM-2 and SHV-1 enzymes [2].

Nowadays, microbial resistance through ESBL has been documented worldwide and pose a serious threat for public health since infections caused by these agents are associated with high morbidity, mortality and healthcare costs [3,4]. Recently, non TEM (Temoneira) or/and SHV(sulphydryl variable) plasmid mediated ESBLs have been recognized mainly of the CTX-M (cefotaxime-hydrolysing β-lactamase) enzyme [5]. CTX-M enzyme was detected for the first time in Germany in 1989 and documented in *Escherichia coli* and *Klebsiella pneumoniae* as well as other genera of *Enterobacteriaceae* family [3,6]..Until now, over 69 different types of enzyme have been characterized worldwide [7].

This study was performed to determine the prevalence of *K. pneumoniae* isolated from environment of Al-Hillah Teaching Hospital / Hillah city, detect their resistance profiles and identify $bla_{CTX-M}$ gene by phenotypic and genotypic, Polymerase Chian Reaction (PCR) method among carbapenem- resistant isolates.

## Materials and Methods

### Bacterial isolates

Over a period of four months from October, 2017 to end of January, 2018, a total of 100 swabs were taken from environment of Al-Hillah Teaching Hospital / Hillah city including : floors, walls, doors, beds, tables, windows, sink, medical equipment and cleaning tools. All samples were cultured on different selective and differential media such as Blood agar, MacConkey agar (Himedia, India) and Eosin methylen blue agar (Biolife, Italy). The species identification was performed following the standard methods described by Holt *et al*.[8],Collee *et al.* [9] and MacFaddin [10].

### Antimicrobial susceptibility assay

The antibiotic susceptibility testing of bacterial isolates was done against a range of 11 antibiotics from 6 different classes using the standard, Kirby-Bauer disk diffusion method on Mueller- Hinton agar plates (Oxiod, England) [11]. The following antibiotic disks were tested : ampicillin (10µg), cloxacillin (10 µg), amoxicillin- clavulanic acid (10 µg) , cefotaxime(30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) , cefoxitin (30 µg), aztreonam ( 30 µg), imipenem (10 µg), meropenem (10µg), and levofloxacin (5 µg). After 18 hrs of incubation at 37 $C^{o}$, the zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. *Escherichia coli* ATCC 25922 (College of Medicine, University of Kufa) was used as quality control.

### Phenotypic detection of extended spectrum β-lactamase production, Recommended by CLSI [12].

Detection of ESBL producing isolates was assayed phenotypically by disk combination method as previously described [13].

### Molecular analyses of CTX-M gene

### DNA preparation

DNA of cultured bacterial isolates was prepared following the protocol described by Pospiech and Neuman [14] with some modifications and used directly for PCR as DNA template.

### PCR amplification

PCR assay was used for detection $bla_{CTX-M}$ gene using the following sets of primers (Bioneer, Korea) CTX-M /F (5`- CGCTTTGCGATGTGCAG `3`) and CTX-M /R (5`- ACCGCGATCGTTGGT `3`) (550bp), in a 25 µl reaction volume using 12.5 µl Go Taq Green Master Mix 2X (Promega, USA), 5 µl DNA template, 2.5 µl of 10 pmol/ µl of specific up stream primers and, 2.5 µl of 10 pmol/ µl of specific down stream primers and 2.5 µl nuclease-free water. The cycling parameters consisted of : an initial denaturation at 94 C˚ for 30 sec, followed by 35 cycles of denaturation at 94 C˚ for 30 sec, anneling at 60 C˚ for 1 min, extension at 72 C˚ for 1 min and a final extension step of 72 at 10 min [15]. The PCR product was analyzed by electrophoresis at 70 volts for 2-3 hrs in 1.5% agarose gel containing ethidium bromide 0.5 µg/ml , the product was visualized under UV-Transilluminator, then

photographed with Gel documentation system. 100 bp DNA Ladder (Bioneer, Korea) was used to assess PCR product size.

## Results and Discussion

Over the period of four months, from October, 2017 to end of January, 2018, a total of 100 swabs were taken from environment of Al-Hillah Teaching Hospital / Hillah city. Thirteen (13%) isolates were identified as *K.pneumoniae*, 6(6%) isolates were obtained from floors, 4(4%) isolates from tables and 3(3%) isolates from beds. (Table-1).In lines with other studies, Tan *et al*. [16] recorded 14 isolates as *K.pneumoniae* recovered from door handle of hospital environment. One study documented 4 *K.pneumoniae* isolates from sink of burn unit in a tertiary care hospital [17]. Also, previous studies conducted in Hillah city reported the presence of *K.pneumoniae* from different environmental and clinical samples [18,19].

**Table(1):**Numbers and percentages of *K.pneumoiae* isolates recovered from environment of Al-Hillah Teaching Hospital.

| Sample source | No. of samples | No.(%) of *K.pneumoiae* isolates |
|---|---|---|
| **Floors** | 20 | 6(6%) |
| **Walls** | 15 | 0(0%) |
| **Doors** | 5 | 0(0%) |
| **Beds** | 13 | 3(3%) |
| **Tables** | 12 | 4 (4%) |
| **windows** | 3 | 0(0%) |
| **Sink** | 5 | 0(0%) |
| **Medical equipment** | 17 | 0(0%) |
| **Cleaning tools** | 10 | 0(0%) |
| **Total** | 100 | 13(13%) |

The detection rates for walls, doors, windows, sink, medical equipment and cleaning tools were 0(0%). (Table-1).This may be explained by contamination with other type of microorganism. However, most nosocomial infections result from transmission of pathogenic bacteria from one person to person, from hospital environment, equipment and materials to patient or may be from the endogenous flora of the patient [20]. It has been suggested that the hands of the nurses were more frequently contaminated by coliform bacteria like *Escherichia coli* and *Klebsiella* and seems to be the major sources for epidemic strain transmission in clinical settings [21].

Results of antibiotics susceptibility testing revealed higher resistance for penicillin antibiotics (ampicillin and cloxacillin) with resistance rate (84.61%) and (69.23%), respectively (Table-2). Similar results were documented by Al-Hilli [22] who found (81%) and (100%) resistance rate for penicillin antibiotics by *Klebsiella* spp. isolated from Merjan hospital environment, Hillah city .

However, the lowest rates of resistance were observed for meropenem (15.38%) and imipenem (7.69%) (Table-2). Mohapatra *et al*[17] detected zero resistance for imipenem , meropenem and ertapenem antibiotics among *K.pneumoniae* isolated from tertiary care hospital.

**Table(2):**Antibiotics resistance profile of *K.pneumoniae* isolates against different antibiotics ( n=13).

| Antibiotic class | Agent used | No.(%) of resistant isolates |
|---|---|---|
| **Penicillins** | ampicillin | 11 (84.61%) |
| | cloxacillin | 9(69.23%) |
| **β –lactams/β-lactamase inhibitor combinations** | amoxicillin-clavulanic acid | 6(46.15%) |
| **Cephems** | cefotaxime | 8(61.53%) |
| | ceftazidme | 7 (53.84%) |
| | cefriaxone | 7(53.84%) |
| | cefoxitin | 6(46.15%) |
| **Monobactams** | azteronam | 5 (38.46%) |
| **Penems** | imipenem | 2(15.38%) |
| | meropenem | 1 (7.69%) |
| **Quinolones** | levofloxacin | 3 (23.07%) |

ESBL production was determined by disk combination method, 5 (38%) isolates were screen positive, (Table-3).One study carried out by Veena Krishnamurthy *et al*. [5] identified 28 *K.pneumoniae* isolated from clinical specimen as ESBL producers by this method.

Phenotypic tests can be used for primary screening, However molecular methods such as PCR are more reliable techinques for detection β-lactamase producing isolates [23].

**Table (3):**Frequency of ESBLs producing *K.pneumoniae* using disk combination method.

| No. of *K.pneumoniae* isolates | No. (%) of ESBL-positive isolates | No. (%) of ESBL-negative isolates |
|---|---|---|
| 13 | 5(38%) | 8(62%) |

According to PCR assay, 1(50%) carbapenem- resistant *K.pneumoniae* isolate containing $bla_{\text{CTX-M}}$ gene .(Fig-1). The existence of $bla_{\text{CTX-M}}$ gene among *K.pneumoniae* isolates was proved by Al-Hilli [22] in Merjan hospital environment. Pérez-Etayo *et al* [24] identified $bla_{\text{CTX-M}}$ genes among *E.coli* isolated from different environmental sources in Navarra, Spain.

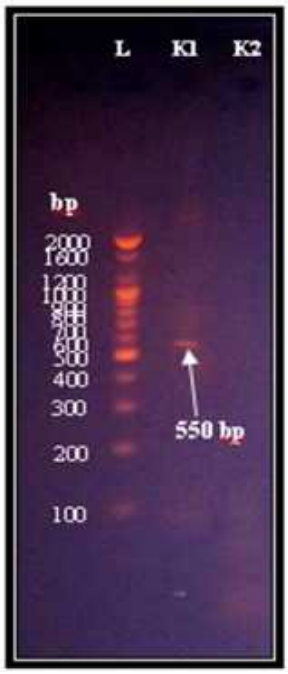


**Figure (1):** Agarose gel electrophoresis for *bla*$_{CTX-M}$ gene (550bp) in carbapenem-resistant *Klebsiella pneumoniae* isolates. Lane (L), DNA moleculer size marker (100- bp Ladder). Lane (1) of *K. pneumoniae* isolate showing positive result for *bla*$_{CTX-M}$ gene.

## Conclusions

This study report the existence of *K.pneumoniae* harboring CTX-M β-lactamase gene in environment of Al -Hillah Teaching Hospital. This finding emphasize the need for careful disinfection and implementation of surveillance and effective infection control programs to prevent dissemination of resistant isolates.

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