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# Analysis of β-lactamases Among Multi Drug Resistant *Klebsiella pneumoniae* in Hilla city-Iraq.

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**ABSTRACT**

Among urinary tract infection (UTI) pathogen, *Klebsiella pneumoniae* rank second, after Escherichia coli and this may be due to seldom structural and physiological defences mechanisms like antiphagositic, anticomplement and serum resistance capabilities. Moreover the ability to form biofilm and their production to β-lactamases (such as ESBL) and carbapenemases (such as KPC) augment their resistance to the most common antibiotics used to get red UTI. During a period of 3 months, 135 mid-stream urine samplescollected from patients clinically diagnosed with cystitis who visit urology consultant clinic in Al-Hilla teaching hospital. All urine samples firstly checked for Pyuria with general urine examination and uriscan strip. Only samples with pyuria processed for bacteriological culture on MacConkey agar and eosin methylene blue (EMB) agar and then finally confirmed with VITECK 2 compact system using GN card. All *Klebsiella pneumoniae* isolates were tested for antibiotic susceptibility, ESBL production using both double disc synergy test and chromatic ESBL medium and tested for KPC production using MIC test and chromatic CRE medium test.The results revealed high percentage of *Klebsiella pneumoniae* isolation 23(31.9%) among cystitis patients and high sensitivity for amikacin 2(8.7%) norfloxacin 7(30.4%) and tobramycin 9(39.1%) while high percentage of resistance displayed to ampicillin 23(100%), ceftazidim 20(91.3%), cefotaxime 19(82.6%) and cefepime 17(73.9%) and ertapenem 10(43.5%). Investigation of ESBL and KPC were performed using DDST and chromatic ESBL medium for ESB, MIC test strip and chromatic CRE medium for KPC. Among *Klebsiella pneumoniae* 10 (43.5%) and 12(52.2%) positive DDS test and Chromatic ESBL medium respectively while 3(13%) and 4(17.4%) positive for KPC using MIC test and Chromatic CRE medium respectively. The current study conclude occurrence of ESBL and KPC among *Klebsiella pneumoniae* recovered from patients with cystitis.

**Keywords**: *K. pneumoniae*, ESBL, KPC, Cystitis, Chromatic.

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## INTRODUCTION

*Klebsiella pneumoniae*, accounts for a significant proportion of urinary tract infections and the principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the contaminated hands. The most important virulence of uropathogen is antibiotics resistance especially due to hydrolyzing enzymes rendering them hard to or untreatable. Until yet β-Lactam antibiotics represent the most common agents for treatment of most bacterial infections and at the same time regard the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria globally. The frequent exposure of bacterial isolates to a multitude of β-lactams induces vigorous, continuous production and mutation of β- lactamases in these bacteria, increasing their activity even against the newly developed β-lactam antibiotics. These enzymes are known as β-lactamases and the most important of them are Extended-spectrum β-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemase (KPC) [1, 2].

ESBLs are plasmid borne and capable of conferring bacterial resistance to the penicillins, first, second- and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems). They still the major challenge in clinical setups world over, conferring resistance to the expanded-spectrum cephalosporins by hydrolysis of these antibiotics. ESBLs producers are clinically resistant to many β-lactams, while non- ESBLs producers still keep a good sensitivity to most β-lactams [3, 4].As an uropathogen, *Klebsiella pneumoniae* have the capability to produce extended-spectrum ß-lactamases (ESBL) in large quantities and confer multiple drug resistance making urinary tract infection difficult to treat [5].

The presence of an ESBL is a good marker of the MDR phenotype. The drugs of choice for the treatment of infections caused by ESBLs were carbapenem and this may leads to emerging of carbapenem resistance isolates in certain areas [6]. At the first time the resistances to carbapenems are attributed to overproduction of AmpC until statement of the emergence of carbapenemases as another mechanism for carbapenem resistance. The most common carbapenemase in the United States is Klebsiella pneumoniae carbapenemase (KPC), an Ambler molecular class A enzyme that utilizes serine at the active site to facilitate hydrolysis of a broad variety of β-lactams. Both of them, ESBL and KPC, belong to the same class Ambler molecular class A (subgroup 2be for ESBL and subgroup 2f for KPC) and inhibited by clavulanic acid and boronic acid respectively [7]. The current study aims to investigate ESBL and KPC among *Klebsiella pneumoniae* isolated from patients with cystitis.

## MATERIALS AND METHODS

### Patients and Samples

Mid-stream urine samples were collected from 135 patients with ages (10-60 years) clinically diagnosed with cystitis. All patients visit urology consultant clinic in Al-Hilla teaching hospital during a period December - February 2014. The patientsAll urine samples firstly checked for Pyuria with general urine examination and uriscan strip. Only samples with pyuria processed for bacteriological culture on MacConkey agar and eosin methylene blue (EMB) agar.

### Bacterial Diagnosis

The mucoid pink to faint pink colonies on MacConkey agar were transferred to EMB agar plates. On the EMB agar plates the mucoid (with deep purple to black center) were primary suspected as *Klebsiella pneumoniae* which are further confirmed using VITECK 2 Compact system (Biomerieux/France) using GN card.

### Antibiotics Susceptibility Test (AST) and Double Disc Synergy Test

This test were done according to CLSI-2013[8], including selection of antibiotics with defined potency, test conditions and result interpretation. Double Disc Synergy Test (DDST) used to detect ESBL among *K. pneumoniae* isolates were performed also according to CLSI-2013[8]. This test consist of two step, the first one called initial screening step using third generation cephalosporin antibiotics such as cefpodoxime (10µg) or ceftazidim (30µg) or cefotaxime (30µg) or ceftriaxone (30µg) or monobactam antibiotics like aztreonam(30µg). When the K. pneumoniae isolates resist to one or more of the previously antibiotics will suspected as ESBL and must confirmed by second step called phenotypic confirmatory step which use combined antibiotic disc

consisted from (antibiotics+clavulanic acid). When the inhibition zone of the combined disc increased by ≥ 5mm than those disc tested alone the results were positive for ESBL. The current study uses Cefotaxime (30µg) and Ceftazidime (30µg) in the screening test and use Cefotaxime-clavulanic acid (30/10µg) and Ceftazidime-clavulanic acid (30/10µg) in the confirmatory test.

**Klebsiella pneumoniae Carbapenemase (KPC) Detection**

All *K. pneumonia* isolates resist to meropenem and ertapenem tested for KPC using MIC Test Strip for KPC detection using (ERTAPENEM / ERTAPENEM + BORONIC ACID) (ETP/EBO) (0.125-8 / 0.032-2 µg/mL). The results were interpreted as positive when the ration of ETP/EBO≥8 according to the instruction of manufacturer (Liofilchem/Italy).

**Chromatic Medium Test for ESBL and KPC**

Chromatic test used to check production of ESBL and KPC by *K. pneumoniae* isolates. This test includes inoculation of Chromatic ESBL and chromatic CRE medium with suspected colonies and incubated over night at 37˚C and the results were read as positive when blue-green or blue-purple colonies grown.

## RESULTS AND DISCUSSION

Isolation results record positive bacterial culture for 72(52.6%) of urine samples. *Klebsiella pneumoniae* isolates compile 23(31.9%) of total bacterial cultures and the others 49(68.1%) were diagnosed as gram negative bacilli figure (1). As urinary tract pathogen *K. pneumoniae* is second only to Escherichia coliand compile (6-35%) of all UTI. The high percentage of UTI caused by *K. pneumoniae* may be due to the unique virulence factor like uronic acid capsule (that inhibit complement activation, protect them from phagocytosis and empower biofilm formation). In addition to type I and type III fimbriae that play vital role in adhesion and biofilm formation, *K. pneumoniae* have siderophore and serum resistance. Moreover the ability to produce ESBL and KPC may augment their prevalence among uropathogens [9].

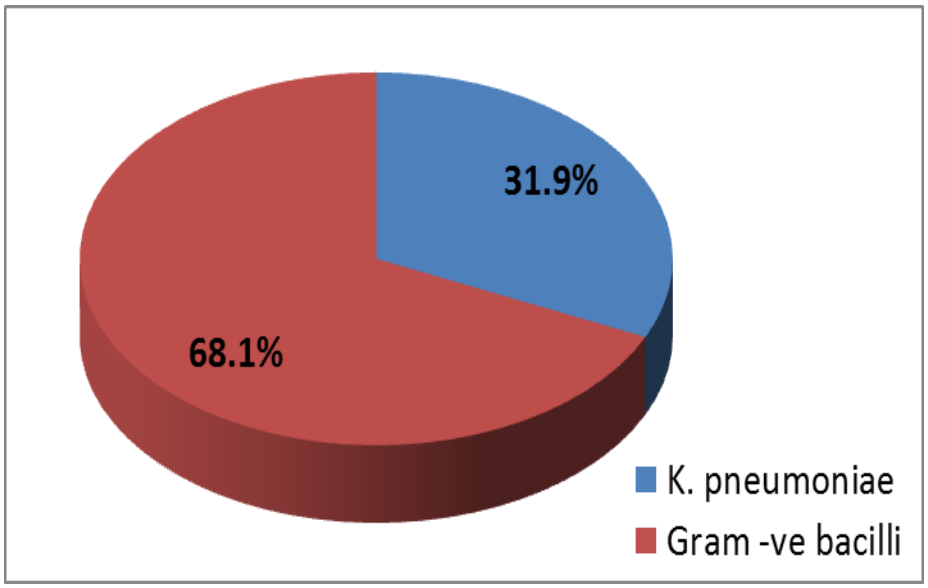


**Figure 1: Percentage of K. pneumoniae Isolation**

Antibiotics susceptibility results revealed high level resistance among *K. pneumoniae* isolates for ampicillin 23(100%), ceftazidim 20(91.3%), cefotaxime 19(82.6%), cefepime 17(73.9%) and ertapenem 10(43.5%) while low level of resistance displayed for norfloxacin 7(30.4%), tobramycin 9(39.1%) and amikacin 2(8.7%) figure (2). According to *in vitro* results, the amikacin and norfloxacin still active against Uropathogenic *K. pneumoniae*.

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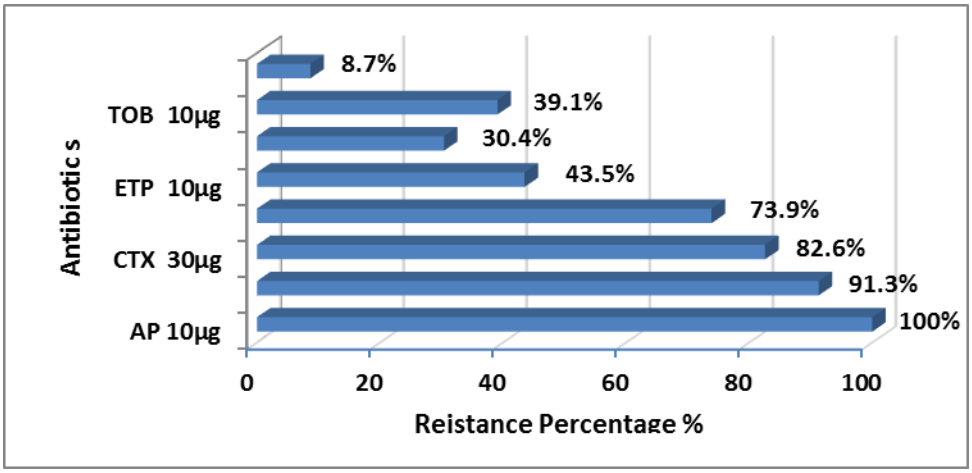


**Figure 2: Antibiotic Resistance among K. pneumoniae Isolates.**

The resistance to the β-lactams antibiotics mainly due to the production of β-lactamases which hydrolyze β-lactam ring rendering them inactive. The common β-lactamases among K. pneumoniae either ESBL (called group 2 or molecular class A) or AmpC (called group 1 or molecular class C) which can be differentiated according to their inhibition by clavulanic acid (ESBL inhibited while AmpC not inhibited). ESBL can hydrolyze oxyimino-β-lactam agents such as third-generation cephalosporins and aztreonam and the isolates may carry genes that confer resistance to other antibiotics including aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and tetracycline [10].

The results of ESBL and KPC detection using different tests displayed that, 10 (43.5%) and 12(52.2%) positive for ESBL using DDS test and Chromatic ESBL medium respectively while 3(13%) and 4(17.4%) positive for KPC using MIC test and Chromatic CRE medium respectively.

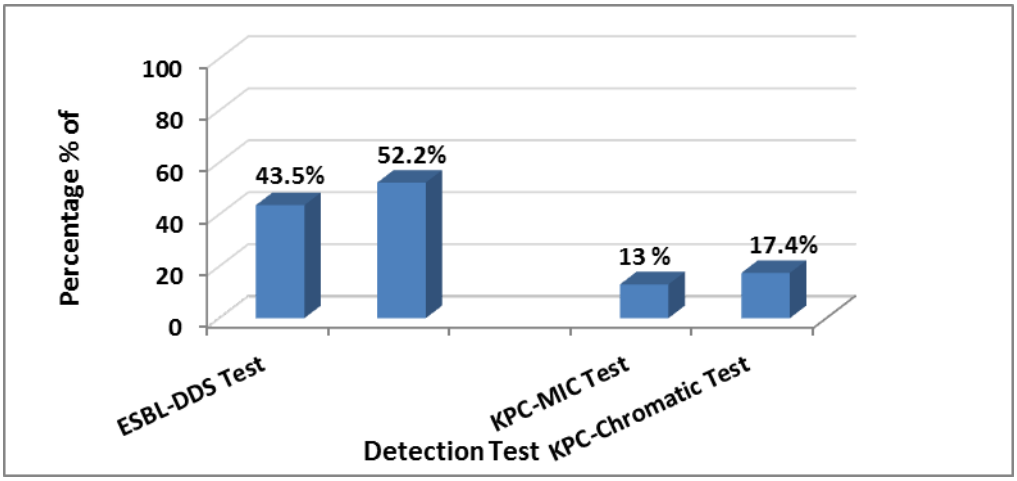


**Figure 3: Positive Results for ESBL and KPC among K. pneumoniae.**

The most common ESBL enzymes in clinical isolates of *K. pneumoniae* were SHV- and TEM-type ESBL enzymes while CTX-M type ESBL was infrequently reported [11, 12]. The CTX-M-14, which belongs to the CTX-M-9 group, is most common variant that is greatly prevalent in some Asian and European countries [13]. The carbapenemase enzyme produced by *Klebsiella pneumoniae* may cause an increase in spread of carbapenem-resistant Enterobacteriaceae (CRE) worldwide. Patel et al state that, there is risk for KPC acquisition or infection and found invasive infections with carbapenem-resistant *K. pneumoniae* were independently linked with organ transplantation, exposure to antimicrobials and receipt of automated ventilation, in comparsion with patients of carbapenem susceptible *K. pneumoniae* [14].The current study conclude occurrence of ESBL and KPC among *Klebsiella pneumoniae* recovered from patients with cystitis.

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