

## Detection of Carbapenem-Resistant *Klebsiella pneumonia* using two Sensitivity Methods

Ghsoon Ali Abdulhasan\*, Suzan Yousif Jasim\*\* and Hula Younis Fadhil\*

\*Department of Biology, College of Science, University of Baghdad.

\*\* Department of Clinical laboratory science, College of Pharmacy, University of Al-Mustansriyah.

E-mail: suzanyousif78@yahoo.com

### Abstract:

Carbapenems, potent  $\beta$ -lactams antibiotics, were initially used as last-resort treatments for severe Gram-negative bacteria such as *Klebsiella pneumonia*. These bacteria were considered one of Enterbacteriaceae family associated with pneumonia and urinary tract infection. In the current research, Carbapenems were studied to identify the resistant *K. pneumonia* isolates and identifying the relation with some  $\beta$ -lactam resistance to avoid their abuse for treatment.

A urine sample got from 80 infected female with chronic urinary tract infection, these samples tested for *K. pneumonia* and their carbapenemase production. The procedure was based on disc diffusion and Landman *et al.* methods.

The results pointed out that *Klebsiella pneumonia* is significantly present in patients with urinary tract infection, the carbapenem resistance in disc diffusion and Landman procedure was 100% and 88.5% for meropenem (MEM), while it was 57.2% and 65.4% for imipenem (IPE), respectively. Regarding the carbapenem resistance in same isolate for both (MEM and IPE), 69.23% (18/26) were coincidence and 30.77% (8/26) were mismatch in both methods. In addition, the Landman procedure more accurate than disc diffusion for carbapenem resistance and there is a strong association with Beta-lactam resistance.

It can be conclude that there is a high incidence of carbapenem resistance in local isolates of *Klebsiella pneumonia*. We recommend to use the supercarba media in diagnoses of bacteria routine work because it is more specific along with disc diffusion method for patients with urinary tract infection and the use of other treatments to decrease the level of resistance.

**Keywords:** Carbapenem resistance, *Klebsiella pneumonia*.

### التحري عن بكتريا *Klebsiella pneumonia* المقاومة لمجموعة الكاربابنيم

#### باستخدام اختبار حساسية

غصون علي عبد الحسن\*, سوزان يوسف جاسم\*\* و حلى يونس فاضل\*

\* قسم علوم الحياة, كلية العلوم, جامعة بغداد.

\*\* فرع العلوم المخبرية السريرية, كلية الصيدلة, الجامعة المستنصرية.

#### الخلاصة :

تعد مجموعة الكاربابنيم من مضادات البيتا لاكتام القوية المستخدمة كخيار اخير لعلاج البكتريا السالبة لصبغة كرام *Klebsiella pneumonia*. تنتمي هذه البكتريا الى عائلة البكتريا المعوية المسببة لإصابات المجاري البولية وذات

الهدف من هذه هو تشخيص عزلات بكتريا *Klebsiella pneumonia* المقاومة لهذه المجموعة من ات وعلاقتها بمقاومة مضادات البيتا لاكتام لتجنب الاستخدام الخاطيء في العلاج. 80 عينة درار من نساء مصابات بالتهاب المجاري البولية المزمن. تم التحري فيها عن عزلات بكتريا *Klebsiella pneumonia* المقاومة لمجموعة الكاربابنيم (الميروبنيم والاميبينيم). تم اختبار مقاومة البكتريا باستخدام طريقتي انتشار الاقراص وطريقة لاندمان.

أظهرت ن بكتريا *Klebsiella pneumoniae* هي المسبب لالتهاب المجاري البولية بصورة معنوية وكانت حساسية البكتريا باستخدام طريقة انتشار الاقراص وطريقة لاندمان بنسبة 100% 88.5% للميروبيم بينما كانت 57.2% 65.4% للاميبينيم على التوالي وعند الاخذ بنظر الاعتبار مقاومة العزلات نفسها للمضادين المذكورين. 69.23% (18/26) من العزلات مقاومة لكلا المضادين بينما كانت 30.77% (8/26) غير متطابقة في حساسيتها للطريقتين ظهرت النتائج ن طريقة لاندمان كانت كثر دقة من طريقة الكاربابنيم كما ن هنالك ارتباط بين مقاومة العزلات المدروسة لهذه المجموعة والمقاومة للبيبتاكتام. نستنتج من هذه الدراسة أن هنالك انتشار كبير لبكتريا *Klebsiella pneumoniae* المقاومة لمجموعة الكاربابنيم supercarba (تشجع نمو البكتريا المقاومة لمجموعة الكاربابنيم) في الشخيص الروتيني لهذه البكتريا طريقة الإ خرى لالتهاب المجاري البولية للتقليل من

الكلمات المفتاحية: *Klebsiella pneumoniae*، مقاومة الكاربابنيم.

## Introduction:

*Klebsiella pneumoniae*, Gram negative, non-motile, encapsulated, lactose fermenter, facultative an-aerobic, rod shapes bacteria, although as a normal flora of the mouth, skin and intestine<sup>[1]</sup>.

Members of *Klebsiella* genes typically express two types of antigens on their cell surface. *Klebsiella* organisms are often resistant to antibiotics. Current evidence implicates plasmids as the primary source resistance genes<sup>[2]</sup>. It also has the ability to produce extended-spectrum beta-lactamases (ESBLs) as a resistant mechanism to many classes of antibiotics. The most frequent resistance includes resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and trimethoprim/ sulfamethoxazole<sup>[3]</sup>.

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is resistant to almost all available antimicrobial agents, and infections with CRKP may cause high rates of morbidity and mortality in the particular among persons with prolonged hospitalization and those critically ill patients, and extended to invasive devices (e.g. ventricatheters or central venous catheters)<sup>[4]</sup>.

Imipenem and Meropenem are the first line agents for the treatment of serious urinary tract infections caused by multidrug resistant *Enterobacteriaceae* clinical isolates<sup>[5]</sup>. From January 2005 to March 2010, one hundred non duplicate *Klebsiella pneumoniae* isolates which are

resistance to Ertapenem were collected<sup>[6]</sup>. Also, carbapenemase producing *Enterobacteriaceae* has been assessed in the report from the antimicrobial surveillance program<sup>[7]</sup>. Tigecycline also showed excellent activity against *Klebsiella pneumoniae* producing ESBLs and plasmid mediated AmpC-type beta lactamase, or both<sup>[8]</sup>.

In *Enterobacteriaceae*, carbapenemases are diverse problem, these belong to molecular class B (imipenem (IMP), Verona integron-encoded metallo- -lactamase (VIM) or New Delhi metallo- -lactamase (NDM)); class A *Klebsiella pneumoniae* carbapenemase (KPC); and class D oxacillinase (OXA -23 and OXA-48) enzymes, as well as those combining AMPC enzymes or an extended-spectrum B-lactamase (ESBL)<sup>[9,10]</sup>.

There are several methods to detect carbapenem resistant of *Enterobacteriaceae* including: modified hodge test, biochemical tests (NP), CHROM agar-KPC medium and molecular detection of carbapenemase genes<sup>[11]</sup>.

CHROM agar KPC is a commercially prepared chromogenic solid medium supplemented with agents that inhibit the growth of carbapenem-sensitive bacteria following 24 h of incubation. Carbapenem-resistant *Enterobacteriaceae* colonies appear with different colonies according to their specific enzymatic properties: *Escherichia coli* appear as red colonies, *Klebsiella* spp. and *Citrobacter* spp. appear

as metallic blue and *Pseudomonas* spp. appear as translucent cream colonies<sup>[12]</sup>.

Supercarba medium is a Drigalski agar-based culture medium containing a low concentration of ertapenem and cloxacillin to prevent the growth of non-carbapenemase producing carbapenem-resistant isolates. This medium might be used for the detection of not only KPC producers but also OXA-48 producers that do not co-express ESBLs. Supercarba medium exhibits higher sensitivity and specificity than other media and is useful for the specific selection of carbapenemase producers in stools, as this medium inhibits the growth of ESBL producers<sup>[11]</sup>.

Thus, this phenomenon was studied in the current study to identify *K. pneumonia* isolates that carbapenem resist and the relation with some  $\beta$ -lactamase resistance to avoid using it for treatment.

## **Materials and Methods:**

### **Sample collection and identification:**

Eighty urine samples were collected from females with chronic urinary tract infection, ranging from 19-25 years old. Culturing 100 $\mu$ l of sample on MaCconkey agar was incubated overnight at 37°C. Lactose fermenting colonies are taken to diagnose *Klebsiella pneumonia* by using Analytical Profile Index (API) system in the national center health laboratories. Twenty eight isolates of the lactose fermenting colonies were diagnosed as *Klebsiella pneumonia*, stored and selected for carbapenemase detection.

### **Detection for carbapenem resistance:**

#### **1 - Disc diffusion testing method:**

Carbapenem disc (Meropenem-10  $\mu$ g and Imipenem with EDTA-10  $\mu$ g) and other related antibacterial discs (Aztreonam-30  $\mu$ g, Piperacillin-100  $\mu$ g and Cefepime-30  $\mu$ g) were used in disc diffusion method. This was done on Muller-Hinton agar and tested the *Klebsiella pneumonia* isolates by taking 100  $\mu$ l ( $1 \times 10^6$  CFU/ml) of bacterial

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broth, then spreading on surface media and overnight incubated at 37°C. These zones size criteria were related for standardized disk diffusion method as described in Clinical and Laboratory standards Institute (CLSI), in 2009.

#### **2 - Landman et, al. Procedure:**

Selected 26 isolates was diluted to  $1 \times 10^4$  CFU/ml to initiate inoculum in this procedure. Briefly, 100 $\mu$ l for bacterial culture was placed in 5ml of tryptic soya broth containing a 10 $\mu$ g disk of imipenem with EDTA (IPE) and another tube containing 10  $\mu$ g disc of meropenem (MEM). Following an overnight incubated at 37°C, 100 $\mu$ l was streaked on to MaCconkey agar and incubated overnight, the presence of lactose fermenting Gram negative rods was then recorded<sup>[13]</sup>.

### **Statistical analysis:**

All data were tabulated and analyzed using the SPSS IBM version 20. The Chi-Square and crosstab test were done to investigate significant comparison between percentages. Values were considered statistically significant when  $P < 0.05$ .

### **Results and Discussion:**

Results of the current study showed that 35% (28/80) of bacterial isolates pointed out that *K. pneumonia* a significantly present in patients with chronic urinary tract infection ( $X^2=7.2$ ,  $P<0.05$ ). This can be explained by their resistance towards wide range of antibiotics, by production of enzymes, and the ability to change its genes like the emergence of MBL activity by acquisition of *bla*VIM-1 gene following carbapenem therapy<sup>[14]</sup>.

In current study, 100% (28/28) of *K. pneumonia* was resist to meropenem (MEM) and 57.2% (16/28) for imipenem with EDTA (IPE) in disc diffusion method. This high percentage of resistance to MEM may be attributed to small number of *K.*

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*pneumonia* isolates included in the current study, also may be due to abuse of this antibiotic in Iraqi patients. Moreover, isolates resistance for other antimicrobial agents were 100% for piperacillin (PRL) and 96.43% for each cefepime (FEP) and aztreonam (ATM), as shown in figure-1(A+B). Although the bacterial resistance for (IPE) was lower than other studied antimicrobial agents, there is no significant statistical differences between them ( $X^2=3.27$ ,  $P=0.07$ ). There has been shown a strong association between carbapenemase antibiotics and beta-lactamase antibiotics because the resistance rate is very close in these antibiotics, as mention in previous

study<sup>[6]</sup>. On the other hand, the produced carbapenemase by *Klepsiella pneumonia* (KPC) belongs to class A that is resistant to Penicillin, extended- spectrum Cephalosporins, Carbapenem and Monobactam<sup>[15]</sup>. Carbapenem resistant enterobacteriaceae (CRE) are difficult to detect, because some strains that harbor *blaKPC* have minimal inhibition concentrations (MICs) that are elevated but still within susceptible range for carbapenem. Because these strains are susceptible to carbapenem, they are not defined as potential clinical or infection control risks using standard susceptibility testing guidelines<sup>[16]</sup>.

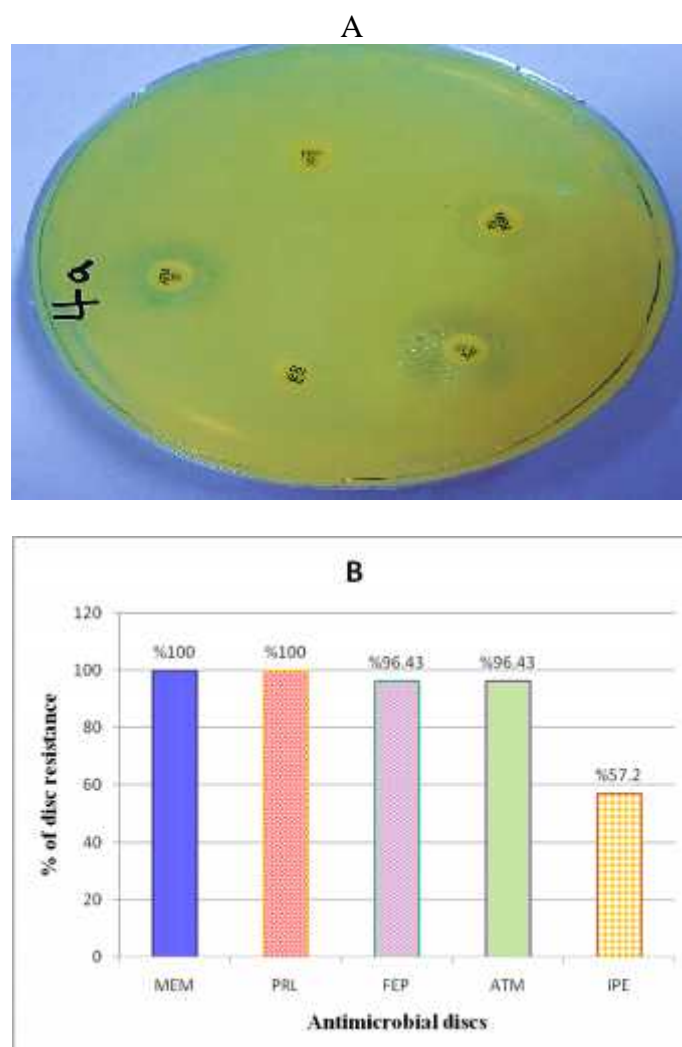


Figure-1 (A+B): Percent of bacterial resistance for five types of antimicrobial agents in disc diffusion method.

Landman *et, al.* procedure was applied on 26 *K. pneumonia* isolates (two isolates were lost during cultivation), and the results of MEM and IPE resistance were observed [88.5% (23/26) and 65.4% (17/26), respectively]. Percentage of

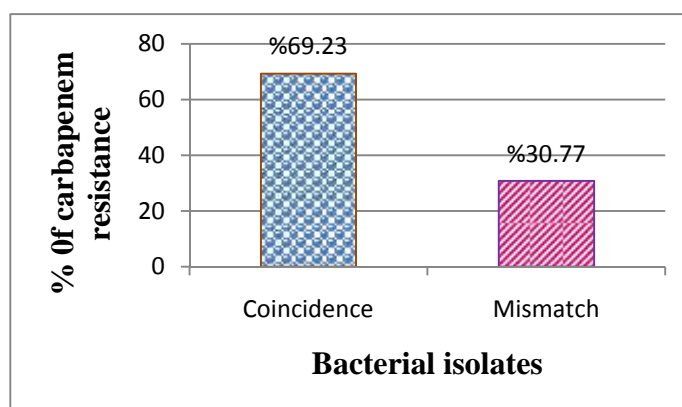
carbapenem resistance for IPE increased compared with disc diffusion method, as shown in table-1. There was no statistically significant difference between the two methods ( $X^2=0.296, P>0.05$ ).

**Table-1: Observation of bacterial resistance to carbapenem agents using disc diffusion and Landman procedure in 26 isolates of *K. pneumonia*.**

Carbapenem disc	Number of bacteria that resist and sensitive to carbapenems			
	Disc diffusion		Landman procedure	
MEM	+ (26)	- (0)	+ (23)	- (3)
IPE	+ (15)	- (11)	+ (17)	- (9)
$X^2$ -test= 0.296, (P>0.05)				

In disc diffusion method: (+) = resistance, (-) = sensitive or intermediate according to CLSI (M07-A9).

In Landman procedure: (+) = resistance, (-) = sensitive (no growth on MaCconkey agar).



**Figure-2: Coincidence of bacterial resistance in the same isolates to carbapenem agents in both disc diffusion and Landman procedure.**

From the results above, the bacterial carbapenem resistance for MEM was more than IPE. This may be attributed to the EDTA conjugation with imipenem, which increased the toxicity for bacteria by chelating with  $zn^{2+}$ , but inhibit the hydrolyzing enzyme [7]. The resistance to MEM was more because EDTA associated with IPE act as chelating agent to free ions ( $Ca^{2+}$  and  $Mg^{2+}$ ), thus, it will lead to decrease the level of resistance towards IPE because these isolates used the hydrolyzing method in resistance to MEM but other isolates had no significant action towards IPE [6,17].

In comparison between methods, the carbapenem resistance (MEM and IPE) in the same isolates was consist in two test

69.23% (18/26), while 30.77% (8/26) was mismatch (figure-2) which distributed to 19.23% (5/26) sensitive or intermediate in disc diffusion method, then converted to resistance (growth on MaCconkey agar), while 11.54% (3/26) lose the resistance (no growth on MaCconkey agar) in Landman procedure.

In general, the current study finds that *Klebsiella pneumonia* isolates were more resist to meropenem compared with imipenem and this were consistence with the results mentioned by a study which reported that *Klebsiella pneumonia* resist to almost all beta-lactams except imipenem and designated this strains as ISMRK (imipenem-susceptible meropenem-resistant *Klebsiella*). This unique suscep-

tibility phenotype to beta-lactams is due to the double production of a metallo-beta-lactamase, and the extended-spectrum beta-lactamase (ESBL) by *Klebsiella*<sup>[19]</sup>.

The most important mechanism of resistance by CRKP is the production of carbapenemase enzyme. *bla*-KPC enzyme is carried on mobile piece of genetic material (a transposon; the specific transposon involved is called Tn4401), which increase the risk for dissemination. The gene that is responsible for production of carbapenemase is OXA-48 gene that was carried on the plasmid (IncL/M) and it has been spread into multiple sequences types of *Klebsiella pneumoniae*<sup>[16]</sup>. Hence, these bacterial isolates may lose their CR gene upon sub-culturing.

Furthermore, the results of Landman procedure displayed carbapenem resistance best than disc diffusion method, and there is slightly statistical significant difference between them ( $X^2=3.85$ ,  $p=0.05$ ). Because disc diffusion method results may be intermediate with CLSI, Landman was used to confirm which isolates is resistance and produce carbapenemase enzyme. However, the local isolates have high carbapenem resistant in clinical cases; hence, a recent study mentioned that polymyxins and other antimicrobial therapies may have similar efficacy in the treatment of infections caused by carbapenemase producing enterobacteriaceae<sup>[20]</sup>.

### **Conclusion:**

It can be conclude that there is a high incidence for carbapenem resistance in local isolates of *Klebsiella pneumoniae*. so it could be recommended to use the supercarba media in diagnoses of bacteria routine work because it is more specific along with disc diffusion method for patients with urinary tract infection and the use of other treatments to decrease the level of resistance. Moreover, the Landman procedure was more accurate than disc diffusion for carbapenem resistance and

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there is a strong association with Beta-lactamase.

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