FrequencyofextendedSpectrum β-Lactamase(ESBLs)producing *Klebsiella* isolates among multi-drug resistant,causing infections in AL-Kindy hospital in Baghdad/Iraq.

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Abstract:

The antibiotic sensitivity pattern of extended spectrum beta lactamase (ESBL) producing *Klebiella* isolates was investigated .A total of 187 clinical isolates ,*Klebsiella* showed just 25 cause of infection isolated from urine, sputum and wounds .Primary sensitive screening of these isolates were carried out using disk diffusion by Kirby –Bauer, then tested for their ability to produce ESBL using disk approximation and standard rapid iodometric methods .The result of this study revealed that 25 isolates were distributed among 10(40%),8(32%),and7(28%)from urine ,sputum and wounds respectively .All these isolates showed resistance to antibiotic arranged between higher resistant 24(96%)to ampicillin and lower resistant 4(16%) to amikacin.,11(44%)isolates were ESBL and could resist more than 10 antibiotic used ,9 of them were ESBL when detected by disk approximation method but 5 only were ESBL when detected by standard rapid iodometric method .This study therefore ,do not only proclaim the presence of ESBL producing *Klebsiella* in al-kindy hospital, Baghdad but also emphasizes that they are multi-drug resistant.

تردد Klebsiella المنتجة للبيتا لاكتاميز واسع الطيف (ESBLs) من عزلات

بين المقاومة للعديد من الادوية والمسببة للاصابات في مستشفى الكندي في بغداد/العراق .

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الخلاصة:

تم التحقق من نمط الحساسية للمضادات الحيوية لعزلات *Klebsiella* المنتجة للبيتا لاكتاميز واسع الطيفESBL . من مجموع ١٨٧ عزلة سريريه عزلت من (الإدرار،البصاق و الجروح) أظهرت *Klebsiella* ٢٥ اصابة فقط .تم تنفيذ فحص الحساسية الرئيسي لهذه العزلات باستخدام انتشار الأقراص لكربي باور ،ثم اختبرت قدرتها لإنتاج ألبيتا لاكتاميز واسع الطيف ESBLباستخدام طريقتي اليود السريعة القياسية والأقراص المتاخمة .بينت نتائج الدراسة إن ٢٥عزلة لل*Klebsiella* توزعت بين ١٠(٤٠%)، ٨(٣٢%) و٧(٢٨%) من الإدرار،البصاق والجروح على التوالي. كل هذه العزلات أظهرت مقاومة للمضادات الحيوية ترتبت بين أعلى مقاومة٢٤(٩٦%)للامبسلين واقل مقاومة٤(١٦%)للاميكاسين. ١١(٤٤%) من العزلات كانت ESBLواستطاعت ان تقاوم أكثر من١٠ مضادات مستخدمة ،٩عزلات كانت منتجة لESBLعند التحري عنها بطريقة الأقراص المتاخمة ولكن ٥عزلات فقط كانت منتجة ل ESBLعند التحري عنها بطريقة اليود السريعة القياسية ،لذلك فأن هذه الدراسة لم تعلن عن وجود *Klebsiella*المنتجة لESBLفي مستشفى الكندي ،بغداد ولكن أيضا تؤكد بأنها مقاومة للعديد من الأدوية .

Introduction:

Extended beta-lactamase(ESBLs) have been found in many pathogenic gram negative bacteria ,but they are most common in nosocomial isolates of *Klebsiella* ,giving a proportion of 75% of SEBL-producing isolates(1).ESBLs were first recognized in a single strain of *K.pneumoniae* isolated in Germany by Knothe *et al*.,(1983).Since then several types of ESBLs have been described(2).Klebsiella causes many infections in humans (pneumonia beside lung),infections in the intraabdominal parts and urinary tract are also reported (3,4,5).

To be more precise ,*Klebsiella* is either hospital – aquired or community acquired ,both of them are highly lethal in patients who have been hospitalized ,typically after two days of hospitalization ,as a matter of fact: this hospital acquired *Klebsiella* infections tends to be very serious(6,7,8).

The ESBLs producing *Klebseilla* are increasingly causing infections both in hospitalized and out patients ,this is making therapy of these infections difficult and promoting greater use of expensive broad spectrum antibiotics(9).In the other side ,the development of antibiotic resistance can be viewed as a global problem in microbial genetic ecology ,it is a very complex problem to contemplate, let alone solve ,due to the geographic scale ,the variety of environmental factors and the enormous numbers and diversity of microbial participants., ESBLs continue to be a major problem in clinical setup worldwide, conferring resistance against extended spectrum cephalosporins., increasing resistance to third and fourth generation cephalosporins has become a cause of concern especially amongst enterobacteriaceae family which is one of the main cause of nosocomial infections., ESBLs are the derivatives of common b-lactamaces(TEM and SHV b-lactamases) that have undergone one or more amino acid substitutions near the active site of the enzyme, thus increasing their affinity and the hydrolytic activity against third generation cephalosporins and monobactams extensive use of newer generation, Cephalosporins has been the strong factor for the evolution of newer b-lactamase such as ESbLs.,ESbLs are encoded by transferable conjugative plasmids, which often code

resistance determinants to other antimicrobial agents such as aminoglycosides, these conjugative plasmids are responsible for the dissemination of resistance to other members of gram negative bacteria in hospitals and in the community(10,11,12,13).

Although *Klebsiella* isolates is one of the well known bacterial species in which the ESBLs have been most commonly reported around the world ,little or no studies are available in Iraq concerning ESBLs production in *Klebsiella* spp.To our knowledge, the present study is the first to investigate the occurrence *and* detection of ESBLs production *in Klebsiella* isolates in the Al-Kindy hospital in Baghdad /Iraq.

Materials and Methods:

1.Bacterial isolates:(187)isolates were taken from different clinical samples (urine, sputum wounds),these samples were collected from the main hospital in Baghdad(AL-Kindy hospital)during the period from June to September 2012.,isolates were cultured on enriched media (Blood and MacConkey agar) grown in aerobic condition at 37C^{o} for 24 hrs. then identified to *Klebsiella* from other bacteria by conventional biochemical tests(14,15)and with Api-*Klebsiella* system.

2.Antibiotic sensitivity: Antibiotic susceptibility testing was performed using eleven antibiotic disk ,these antimicrobial agents(disks) were tested by Disk diffusion method on Muller Hinton agar and reported as described by the Clinical and Laboratory Standards Institute(CLSI) (16).

3.ESBLs Detection : β-lactamase producing isolates were also tested for their ability to produce ESBLs, two methods were used for this purpose in the first method third generation cephalosporins(3GC) were used as representative of β-lactam antibiotics and clavulanic acid as β-lactamase inhibitor and as follows:

A. Disk approximation method :it was performed on Muller- Hinton agar plate inoculated with the test bacterial isolate, by placing disks containing 30µg cefotaxime and ceftriaxone ,15mm (edge to edge)from a disk of augmentin (20µg amoxicillin plus 10µg of clavulanic acid).,followed by incubation for 16-20 hr. at 35C^{o},any enhancement of the zone of inhibition between a β-lactam disk and augment in disk ,was indicative of the presence of an ESBL(17).

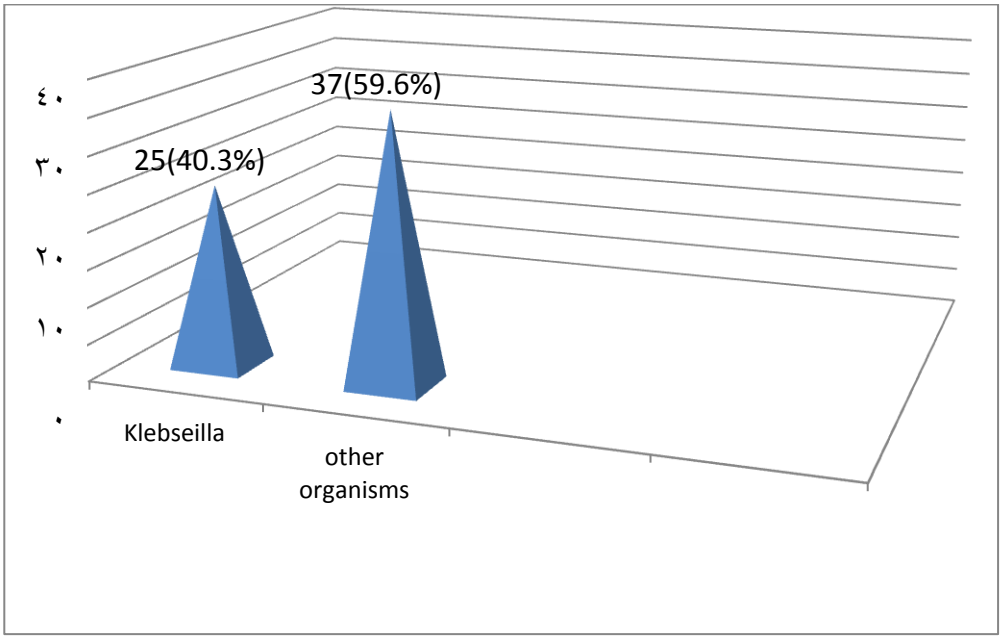
B. Standard rapid iodometric method:

The Standard rapid iodometric method was used to detect β-lactamase where the special solutions were used (phosphate buffer solution) according to the WHO,(1978)(18). Number of pure bacterial colonies with 24 hrs. old were quoted by bacteriology carrier to tubes containing 1000µL penicillin C .These tubes were preserved with 37C^{0} for 30 min. then starch solution 50 µL was added with a good mix with tube contents., after that 20 µL from iodine solution was added ,last ,the tubes were requested very well even the appearance of dark blue color .If the color of the solution was changed from dark blue to white that indicates to the positive test.

Results:

The total number of samples sent to the microbiology laboratory for diagnosis during the study period was 187,out of which 62 showed bacterial growth. The most common organisms isolated from these cultures were Gram-negative bacilli.

Among the isolated organisms, there were 25(40.3%) *Klebsiella* isolate and 37(59.6%) other bacteria (Figure 1).These 25 isolates from *Klebsiella* were distributed according to the source of isolation into7(28%),8(32%),10(40%)wounds, sputum, urine respectively(Figure 2).



Figure(1):Frequency of pathogens isolated in the study

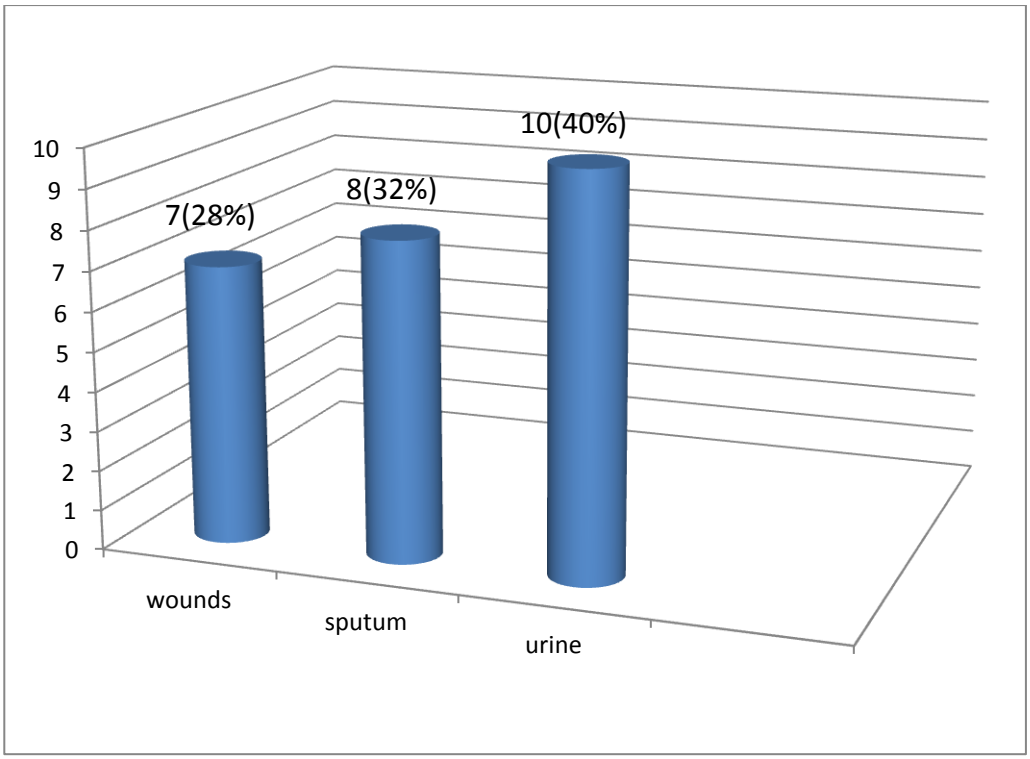


Figure 2:Distribution of *Klebsiella* isolates according to the source of isolation.

Antimicrobial drug resistance:

Primary screening of β-lactam resistant isolates showed that all 25 *Klebseilla* isolates testing for their antibiotic resistance against 11 of them ,100% of these isolates were found to be multi-drug resistant to least 4 antibiotics.

Table(1) summarize the antimicrobial susceptibility patterns of clinical *Klebsiella* isolates .Antimicrobial resistance was not distributed uniformly 24(96%) of *Klebsiella* isolates were resistant (highly resistant) to ampicillin and 23(92%) of them were resistant to cefotaxime ,nitrofurantion and cephalothin .Very low level of resistance was found 22,21(88%,84%) in isolates to chloramphenicol, trimethoprim respectively.

Results also showed that *Klebsiella* isolates were intermediate resistant to nalidixic acid 13(52%),doxycyclin 12(48%) and ciprofloxacin ,tobramycin 14,14(56%).,but they were highly sensitive to amikacin (just 4 (16%) isolates were resistant).

Table (1): Antimicrobial resistance patterns of *Klebsiella* isolates from clinical source

I.D	Antimicrobial resistance pattern										
	NA	CTX	AM	CL	AK	F	CIP	TOP	DO	KF	TMP
K1	R	R	R	R	S	R	R	R	S	R	R
K2	S	R	R	R	S	R	S	R	S	R	R
K3	S	R	R	R	S	R	S	R	S	R	R
K4	R	R	R	R	S	R	R	R	R	R	R
K5	S	R	R	R	S	R	S	S	R	R	R
K6	S	S	R	S	R	R	S	S	R	R	R
K7	R	R	R	R	S	R	R	R	S	R	R
K8	S	R	R	R	S	S	S	S	S	R	S
K9	R	R	R	R	R	R	R	R	R	R	R
K10	R	R	R	R	S	R	R	R	S	R	R
K11	S	R	R	R	S	R	R	R	R	R	R
K12	R	R	R	S	S	R	S	S	S	S	S
K13	S	R	R	R	S	R	S	R	R	R	R
K14	R	R	R	R	S	R	R	S	S	R	R
K15	S	R	R	R	R	R	S	R	R	R	R
K16	S	R	S	R	S	R	S	S	S	R	S
K17	S	R	R	S	S	R	R	S	S	S	S
K18	R	R	R	R	S	R	R	R	R	R	R
K19	S	R	R	R	S	R	S	R	S	R	R
K20	S	R	R	R	S	R	R	S	S	R	R
K21	R	R	R	R	S	R	S	R	R	R	R
K22	R	R	R	R	S	R	R	S	R	R	R
K23	R	R	R	R	R	R	R	R	R	R	R
K24	R	R	R	S	S	S	R	S	S	R	R
K25	R	S	R	R	S	R	R	S	R	R	R

I.D. :isolate designation

NA: nalidixic acid CIP:ciprofloxacin CTX:cefotaxime

AM: ampicillin CL:chloramphenicol AK:amikacin

F: nitrofurantion TOB:tobramicin DO:doxycyclin

KF: cephalothin TMP:trimethoprim

Two methods were used for detection of ESBLs production after they showed out of 25 *Klebseilla* isolates ,only 11(44%) were detected by screening test of (CLSI) and non ESBLs producing *Klebsiella* were 14(56%).Figure (3) and Table (2) shows Frequency and antibacterial resistance of ESBLs and non ESBLs producing *Klebsiella* isolates .

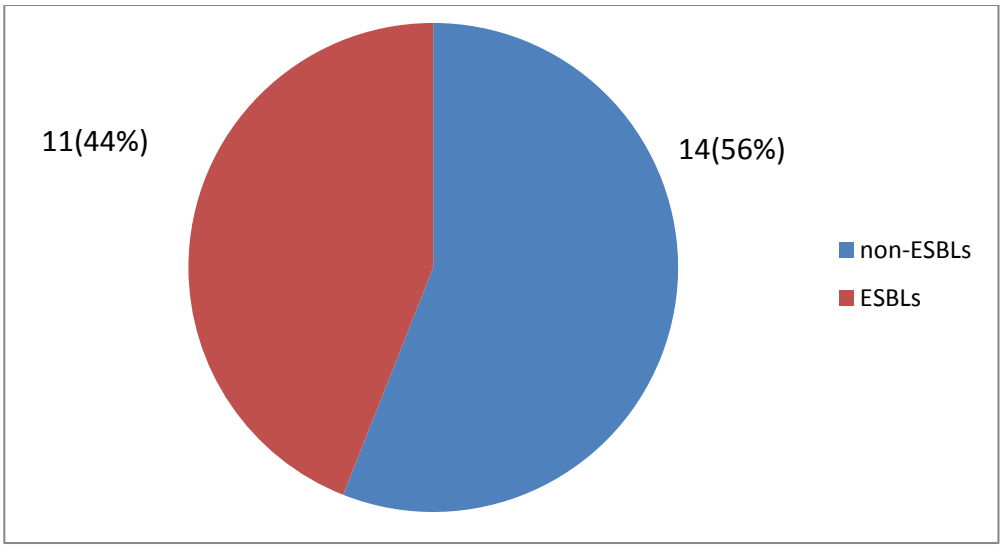


Figure (3):Frequency of ESBLs and non ESBLs *Klebsiella* isolates.

Table (2):Antibacterial resistance of ESBLs and non ESBLs producing *Klebsiella* isolates.

Antibiotic	*Klebseilla* isolates	
	ESBLs(11)	Non-ESBLs(14)
NA	8 (72.7%)	4 (28.5%)
CTX	11(100%)	12(85.7%)
AM	11(100%)	13(92.8%)
CL	11(100%)	11(78.5%)
AK	3 (27.2%)	0 (0%)
F	11(100%)	12(85.7%)
CIP	9 (81.8%)	5 (35.7%)
TOB	10(90.9%)	4 (28.5%)
DO	8 (72.7%)	4 (28.5%)
KF	11(100%)	12(85.7%)
TMP	11(100%)	10(71.4%)

In table (2) ESBLs producing *Klebseilla* isolates shows maximum resistance to cefotaxim,ampicillin ,chloramphenicol,nitrofurantoin,cephalothin,trimethoprim 11(100%) and minimum resistance was seen with amikacin 3(27.2%),while non ESBL producing *Klebseilla* isolates showed high resistance to ampicillin13(92.8%) and low resistance was seen with amikacin 0(0%).

In this study 9(36%) isolates were ESBL producing when detected by disk approximation method and 5(20%) isolates were ESBL producing when detected by standard rapid iodometric method. Figure (4) illustrates the production of ESBL in clinical isolate K23 ,by enhancement of the zone of inhibition between central disk of augmentin and the disks containing cefotaxime and ceftriaxone.

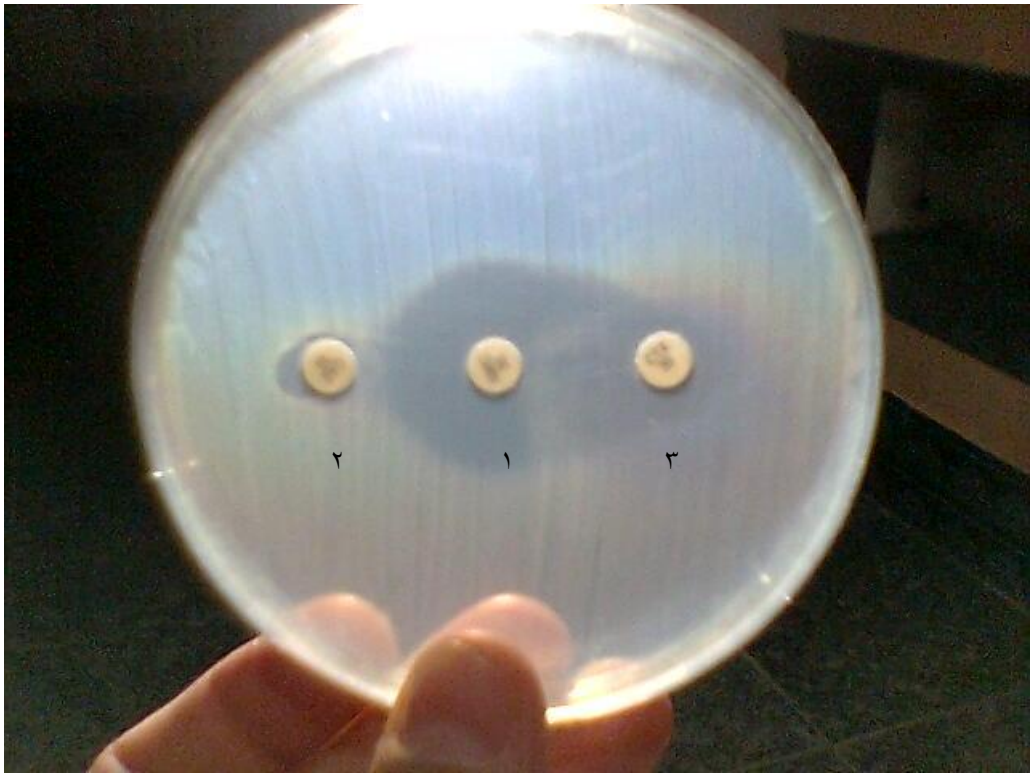


Figure (4):Detection of ESBL production in K23 by disk approximation method. 1.amoxicillin-clavulanate disk(20/10µg)

2.cefotaxime disk (30µg). 3.ceftriaxone disk (30µg).

Finaly, the results revealed that in the standard rapid iodometric method 5 isolates just was ESBL while the residue (4) isolates were not produced for ESBL in this method .Table (3) showed the differences between the ESBL producing isolates when using two methods for detection(Disc approximation and standard rapid iodometric).

Table(3): the differences between ESBL producing isolates when using two methods for detection(disc approximation and standard rapid iodometric).

Symbol of isolates	Detection method	
	Disc approximation	Rapid iodometric
K1	+	-
K4	+	-
K7	+	+
K9	-	+
K10	+	-
K11	+	+
K15	-	-
K18	+	-
K21	+	-
K22	+	+
K23	+	+

The isolates K7, K11, K22, K23 were remained productive for ESBL in the two methods, but K9 could not produce ESBL in the disc approximation while it could produce them ESBL in the standard rapid iodometric method .At the contrast ,the isolates K1, K4, K10, K18, K21 were able to produce ESBL in the disk approximation but they did not appear any production in the second method.

Discussion:

The high detection rate of clinical bacterial isolates in samples was expected in the hospital because these samples (urine , sputum ,wounds) provide an excellent growth conditions for these bacteria (19).The absence or the low ratios of recovered *Klebsiella* isolates in these samples could be due to the lower number of samples taken in this study in addition to the absence recovered *Klebsiella* isolates in environmental and clinical samples of blood and that of burn , skin and vagina swabs could be also due to the lower number of samples taken in this study .

The most common *Klebsiella* isolates in the present study were found in urine samples , the findings of the present study were supported by another study where *Klebsiella* were found as main culprits responsible for the urinary tract infection (UTI) among children in Pakistan (20,21) . On the other hand Patel *et al*.(2009)(22) showed that UTI is the most common hospital acquired infection and UTI is one of the most important causes of morbidity in general population and is the second most common cause of hospital visits and they can report high ratio of *Klebsiella* isolation from urine(13.14%) among other bacteria , so these frequencies of isolation of *Klebseilla* urinary pathogen were consistent with our study.

It should be noted that ,at least (32%,28%) of samples in the current study were also from clinically significant sources(wounds ,respiratory tract) and this agreed with the recently published studies that closely reflects the prevalence of *Klebseilla* isolates in similar setting reported elsewhere(23,24,25).

The production of β-lactamase by *Klebsiella* of β-lactam resistant isolates indicates that the enzymatic resistance was prevalent among more than half of β-lactam resistant *Klebsiella* isolates, results showed that all (5-9) β-lactamase –producing *Klebsiella* isolates were found to be multi-drug resistant a least 9 antibiotics .This is may be caused by the relatively high ratio of resistance to β-lactam antibiotics in primary screening was not attributed only to production of β-lactamase enzyme , but it could be also due to the decreased affinity of the target PBPs(Penicillin binding proteins) or decreased permeability of the drug into the cell (26).Antibiotic resistance among isolates of *Klebsiella* in the present study was comparable to reports from other parts of the world ,which also revealed multi-drug resistance among gram negative rods (18,27).

ESBL producing *Klebsiella* isolates showed higher resistance to cefotaxime (100%)while lower resistance was seen with amikacin (27.2%), these results are different from those of a study conducted in Pakistan where ESBL producing *Klebsiella* showed higher resistance to ceftazidime (100%) while lower resistance was seen with meropenem (3.6%) (18). In the same time our results were similar to results of Behroozi *et al*.(2012)(23) in Iran that showed ESBL producing *Klebseilla* isolates was to cefotaxime (100%) and all of isolates were also resistant to cephalothin and trimethoprim (100%).Mekki *et al*.(2012)(24) reported that ESBL producing *Klebsiella* isolates were maximum resistance to nalidixic acid and ciprofloxacin (100%) and minimum resistance to amikacin (39.4%),most of these results are in accordance with our study except that the nalidixic acid not showed the maximum resistance in our study .

Penicillin group of antibiotics are drug of choice for a wide variety of infectious disease, unfortunately these drugs are readily hydrolyzed by broad spectrum β-lactamases that are found with increasing frequency in clinical isolates of *Klebsiella* .

Our results with penicillin group were in corroboration with the one reported by other workers (22,28) that suggested an increased resistance to ampicillin (80-100).Other study was from western Nepal that reported high prevalence of resistance to ampicillin ,nalidixic acid and norfloxacin (28).Thus ,it is evident that β-lactamase producing *Klebsiella* isolates are resistant to monotherapy of penicillin .

However, in the present study all isolates were sensitive to amikacin relatively ,with non ESBL *Klebseilla* isolates (100%) and ESBL *Klebseilla* isolates (3%),the activity is excellent because of sensitivity of amikacin by *Klebseilla* isolates was higher when compared with doxycyclin (72.7%),ciprofloxacin(81.8%) ,tobramycin(90.9%). Similar observations have been reported that amikacin is demonstrated to be more active than others (21) ,also ,in our study it showed improved sensitivity against *Klebsiella* isolates ,our results correlate well the earlier published reports from india (22,29).

The prevalence rate of ESBL-mediated resistance to third-generation cephalosporins (3GC) using disk approximation method (36%) and this result is higher than those results reported by AL-Charraakh et al.,(2011) (30)who found that (10.5%) of *Klebsiella* isolates resistant to (3GC) antibiotics were ESBL-producers, but it is much lower than that reported by other studies(19,20).The low prevalence rate ,when they compared with our study ,can also be attributed to the fact that presence of ESBLs in a bacterial cell does not always produce a resistant phenotype when using the disk diffusion interpretive criteria published by the CLSI (3).The presence of the strong relationship between the resistance of those bacteria and their ability to produce the enzyme, used the second method to investigate the ability of these isolates to produce an enzyme which is the way standard rapid iodometric method to make sure match results, as is this method is the most appropriate to use with bacteria by launching enzymes outside the cell to the center,

as the tow isolates(K22,K23) was positive for enzyme production and change the blue color of the solution to the colorless (3 and 5) minutes respectively , With delayed each of the isolates (K7, K9, K11) by giving positive result after (8 and 10) minutes respectively, while the rest of the isolates were negative compared with negative control and treatment of these differences in the response may be due to the fact that isolates be productive enzyme but in small quantities don't appear in the detection test(30)

From the present investigation, we can say that there are increasing instances of the resistance to antimicrobials ,these increasing resistance surely due to the irrational and inappropriate use of antibiotics has been a major cause in development of drug –resistance .,therapeutic decisions in infections involve consideration of susceptibility-resistance patterns , pharmacokinetic profile , prophylactic /combined antibiotic therapy , host defense mechanisms , local factors and adverse reactions of the drug .There is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs " to minimize the misuse of available antimicrobials (31).

Conclusions & Recommendations:

In the present study ,ESBL producing *Klebsiella* isolates were found to be multi-drug resistant .In ESBL producing isolates ,high percentage of antibacterial resistance of non –β-lactam antibiotic is a serious matter of concern .Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients or administration requirement of larger doses or more expensive and toxic agents .ESBL producers are associated with increased morbidity and mortality ..the majority of ESBL-producing *Klebsiella* isolates were resistant to the common antibiotics used in the treatment of its infection. The early detection and reporting of suitable antibiotics can reduce the treatment failure in ESBL infections .

And based on our observations of the present study we recommended :

1- use of antimicrobials like ampicillin , amoxicillin and third generation cephalosporins (like cefotaxime and ceftriaxone) in combination with α-lactamase inhibitors (clavulanate) for the treatment ESBL *Klebseilla* infections in similar hospital settings .Further amikacin should be considered as reserved drugs for the treatment of severe nosocomial infections to avoid emergence of resistant strains .

2-The molecular characterization of ESBL producing bacteria in our country is required to understand the mechanisms of ESBL resistance.

3-This study is important for strict antibiotic policy implementation in hospitals ,to estimate the impact of increased drug resistance in bacteria and to take steps for reducing their resistance .

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