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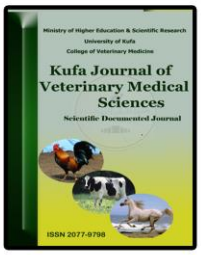

Molecular expression of blaCIT and blaFOX in *Klebsiella spp*. isolates from Al-Hussein teaching hospital in Al-Muthanna Province-Iraq

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Abstract :

klebsiella is the most common pathogen responsible for acquired infections in the hospitals and because of the extensive ability to produce β-lactamases made this bacteria resistance to many antimicrobial agents. This study was done to determine *Klebsiella pneumoniae* isolates containing both blaFOX and blaCIT by multiplex polymerase chain reaction . Out of fifty one *Klebsiella* isolates , 31 (60.8%) *K. pneumoniae* isolates were resistant to cefoxitin by disk diffusion method . The production of molecular class C β-lactamase tested through modified three dimension method in all cefox resistance isolates and observed 14 (45.2%) isolates were producing for AmpC β-lactamase . The cefoxitin resistance isolates further screened by AmpC disk method and the result was 11 (35.5%)isolates were producing for AmpC β-lactamase . Multiplex PCR technique showed 8/31 (25.8%) *K. pneumoniae* isolates possess *AmpC* β- lactamase gene as well as this technique also revealed that FOX gene was exists in (37.5%) and CIT gene (62.5%)of the isolates .

Keywords: *Klebsiella pneumoniae*, AmpC, CIT , FOX and PCR.

التعبير الجزيئي لكلا الجينين CIT و الجين FOX في بكتريا الكليبسيلا المعزولة من مستشفى الحسين التعليمي في محافظة المثنى

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الخلاصة:

بكتريا الكليبسيلا هي من أكثر العوامل الممرضة و المسؤولة عن الأمراض المكتسبة في المستشفيات وبسبب قدرتها الواسعة على أنتاج أنزيمات البيتا لاكتاميز أصبحت هذه البكتريا مقاومة للعديد من المضادات الحياتية , هذه الدراسة أجريت لتحري عن وجود كل من الجينين CIT وFOX في بكتريا الكليبسيلا وبواسطة تقنية سلسلة تفاعل إنزيم البلمرة المتعدد , حيث جمعت 120 عينة سريرية من مستشفى الحسين التعليمي في المثنى وخلال الفترة من 2016/3/1 ولغاية 2016/7/1وكان عدد عزلات الكليبسيلا هو 51(42.5%) عزلة ثم اجري لها اختبار الحساسية الدوائية تجاه 21 مضاد حيوي وبطريقة نشر الأقراص حيث وجد أن 31(60.8%) عزلة من *K. pneumoniae* كانت مقاومة للسيفوكسيتين , بعد ذلك تم التحري عن إنتاج أنزيمات البيتا لاكتاميز في جميع العزلات المقاومة للسيفوكسيتين مختبريا بواسطة طريقتين هما طريقة الأبعاد الثلاثة المحور MTDT وطريقة الأقراص AmpC حيث كانت النتيجة أن 14(45.2%) عزلة أعطت النتيجة الموجبة بواسطة طريقة الأبعاد الثلاثة المحور و 11(35.5%) عزلة أعطت النتيجة الموجبة بواسطة طريقة الأقراص AmpC , كما تم البحث عن الجينات المشفرة لإنتاج أنزيمات البيتا لاكتاميز بواسطة تقنية سلسلة تفاعل أنزيم البلمرة المتعدد وباستخدام

بوادئ متخصصة حيث وجد أن 8(25.8%) من عزلات الكليبسيلا كانت تمتلك جين AmpC من بين هذه العزلات, 3 (37.5%)عزلات كانت تمتلك Fox جين و5(62.5%)عزلات كانت تمتلك

1-Introduction

Klebsiella is the most common among the intestinal bacteria , the german scientist Edwin Klebs (1834–1913) suggested the naming of these bacterium and Trevisan gave a full description of these bacteria in 1885 [1] . The typical natural place of this microorganisms is the intestinal tract of human and animal, but may be move to another places causing extensive variety of infections for example, burned skin, lacerations areas of the body , lung and bladder infections ; the increased ability of Klebsiellae to oppose distinctive sorts of drugs this was the cause of the difficulty of dealing with such infections [2]. There are two types of AmpC β- lactamase resistance are chromosomal mediated ampC β-lactamase and plasmide mediated ampC β- lactamase resistance, the latter resistance generated by movement of the chromosomal genes to the plasmid and are exists significantly in *K. pneumonia ,E.coli , Enterobacter aerogenes, Salmonella Spp., proteus mirabilis, Citrobacter freundii* [3]. The enzyme that possesses activity against cephalosporin in *Klebsiella. pneumoniae* is molecular class C β-lactamase (AmpC) , the ampicillin resistance genes commonly carried on plasmid with other β-lactamase genes such as Temoneria gene (TEM-1), Sulfhydryl sulfate gene (SHV), Brazilian extended spectrum gene (BES) and Cefotaxime gene (CTX-M3) [4]. The antibiotic can be used for the detection of β- lactamase enzymes but emergence of new types of β-lactamases became antibiotic susceptibility test undependable therefore, it is necessary to develop new ways to help in reaching the right diagnosis such as determination isoelectric point of enzymes [5] . The naming AmpC β- lactamases relied on its resistance to Cefamycin (CMY), latamoxef(LAT) , moxalactam (Mox) , Cfoxitin (Fox), or depending on the discovery location such as ; Miriam Hospital in Providence (MIR), Dhahran Hospital in Saudi Arabia (DHA), or the patient's name Bilal (BIL) , so as depending on the similarities in gene sequence and origin collected to the six families such as DHA (Originating from *Citrobacter freundii*), DHA(Originating from *Morganella morgannii*), ACC(Originating from *Hafnia alvei*), EBC(Originating from *Enterobacter cloaca*) , Fox (unknown) and Mox (unknown) [6]. The present study aimed to determine the prevalence of AmpC β- lactamase producing *klebsiella Spp.* and knowing its resistance to β- lactam antibiotics and comparing the detection of AmpC β- lactamase by phenotypic tests and molecular technique.

2-Materials and Methods

2-2- Sample collection:

Collected 120 clinical samples from Al-Hussein teaching hospital in Al-Muthanna province during the period from 1/ 3/2016 to 1/7/2016 . The samples were arranged as follows : urine (30) , wound swabs (35) , burns (20) and sputum (35) and then cultured of samples on differential media and incubated for 24hour at 37^{o}C after that diagnosed by biochemical test according to [7]. Then confirmed

diagnosis by analytical profile index kit (API-20E, BioMérieux, France) .

2-3- Antibiotic susceptibility Testing :

The distinguish bacterial resistance in the laboratory related with molecular class C β- lactamase was accomplished by using disc diffusion method where the use of 21 antibiotics according to NCCLS[8]. Antibiotics that are used in the laboratory Ampicillin, Imipenem, Gentamicin and Tobramycin(10 µg for each) ; Amoxicillin (25µg); Amoxi -clav, ceftriaxone, cefotaxime, Cefoxitin, Ceftazidime, Cefepime and Cefixime (30 µg for each);Cefixime (5 µg);Carbenicillin and Piperacillin (100 µg for each) ; Aztreonam , Amikacin, Nalidixic acid and Tetracycline (30 µg for each); Ciprofloxacin, Trimethoprim and Norfloxacin (5 µg for each). The bacterial resistant to cefoxitin antibiotic considered (≤18mm inhibition zone diameter) are supposed as primarily molecular class C β- lactamase producers [9] , and then confirmed by two methods. These methods included:

2-3-1-Modified Three Dimensional method :

This method is accomplished as described by bacteriologist Parveen *et al*. [10].

2-3-2-AmpC Disk method :

This method also completed as recommended by bacteriologist Basak *et al.*[11] Parveen *et al.*[10].

2-4- DNA extraction:

The method of separate genetic meterial from Klebsiella cells achieved by salting out technique as recommended by researcher [12], with some of the following changes : Prepared tubes containing 5 mL of bacterial culture and deposited by centrifugation at speed (1000 run per minute for 10 minutes) , and then washed three times by Tris-EDTA buffer , While the pellet suspended again in 5 ml Tris-EDTA buffer . Subsequently, we added 600 microliters of 25 percent sodium dodecyl sulfate freshly made and incubated for 5 minutes at 55˚C . After that, added to the lysate 2 ml of 5 mueller sodium chloride solution and well mixed then allowed to cool 37˚C. Then added to the lysate 5 ml from phenol and chloroform and isoamylacohol in the Volumetric ratio (25: 24: 1) and mixed for 30 minutes. It was precipitate by centrifuge 4500 rpm for 10 minutes. Then the lysat was pour to a fresh tube, After that added 0.6 microliter of isopropanol and well mixed . Then DNA was transferred by pipette and added a 5 ml of 70 percent ethanol and DNA extract saved in the freezer after dissolved in 300µl Tris-EDTA buffer until use.

2-5- Prepare of primer solution

Spinning down the lyophilized primers before dissolving with TE buffer depending on the company's instructions in the leaflet as stock solution , While primer working solution was prepared according to the manufactures instructions**.**

2-6- Detection of Fox and CIT genes

The investigation of blaCIT and blaFOX genes accomplished by the use of Multiplex PCR technique with certain primers (table 1). The total volume of the PCR mixture 20 microliter includes : 5 microliter of PCR PreMix (Bioneer , korea) and 5 picomol / µl for each primer and 5microliter of DNA template

Table (1): The primer sequences used in PCR technique for *klebsiella pneumonia* .

primer	Primer sequence (3'-5')	Size	Reference
FOX	**Forward:** AAC ATG GGG TAT CAG GGA GAT G **Reverse :** CAA AGC GCG TAA CCG GAT TGG	**190bp**	Perez-Perez and Hnson, [41]
CIT	**Forward:** TGG CCA GAA CTG ACA GGC AAA **Reverse:** TTT CTC CTG AAC GTG GCT GGC	**462bp**	

3-Results :

The current results revealed the number of *Klebsiella spp*. was 51(42.5%) isolates out of the (120) clinical isolates , the isolates were taken from various infections which include : 8(15.7%) isolates from burns , 13(25.5%) isolates from urine , 19(37.3%) isoletes from sputum , 11(21.6%) isolates from wounds infections , as well the current results showed that *Klebsiella pneumonia*e subsp. *pneumoniae* was (58.8%) of the total number of isolates , whereas *Klebsiella. oxytoca* was (11.8%) , *K.pneumoniae* subspecies *aerogenes* was (23.5%) , *K.pneumoniae* subsp. *rhinoscleromatis* was (2%) and *K.pneumoniae* subsp. *ozanae* was (2%) , showed in table (2 and 3) .

Table (2): Division of *Klebsiella* spp. according to infection sites:

sample type		subspecies			
		rhinosclero matis	***Ozanae***	***pneumoniae***	***aerogenes***
Urine	**Count**	1	0	7	3
	Probability Count	.3	.3	7.8	3.1
	% among sample	7.7%	0.0%	53.8%	23.1%
	% among subspecies	100.0%	0.0%	23.3%	25.0%
	% Of the whole	2.0%	0.0%	14.0%	6.0%
Burns	**Count**	0	0	5	3
	Probability Count	.2	.2	4.8	1.9
	% among sample	0.0%	0.0%	62.5%	37.5%
	% among subspecies	0.0%	0.0%	16.7%	25.0%
	% Of the whole	0.0%	0.0%	10.0%	6.0%
Sputum	**Count**	0	0	12	4
	Probability Count	.4	.4	11.4	4.6
	% among sample	0.0%	0.0%	63.2%	21.1%
	% among subspecies	0.0%	0.0%	40.0%	33.3%
	% Of the whole	0.0%	0.0%	24.0%	8.0%
Wounds	**Count**	0	1	6	2
	Probability Count	.2	.2	6.0	2.4
	% among sample	0.0%	10.0%	60.0%	20.0%
	% among subspecies	0.0%	100.0%	20.0%	16.7%
	% Of the whole	0.0%	2.0%	12.0%	4.0%
Total	**Count**	1	1	30	12
	Probability Count	1.0	1.0	30.0	12.0
	% among sample	2.0%	2.0%	60.0%	24.0%
	% among subspecies	100.0%	100.0%	100.0%	100.0%
	% Of the whole	2.0%	2.0%	60.0%	24.0%

Table (3): Division of *Klebsiella* spp. according to infection sites:

sample type		subspecies *oxytoca*	Total
urine	Count	2	13
	Probability Count	1.6	13.0
	% among sample	15.4%	100.0%
	% among subspecies	33.3%	26.0%
	% Of the whole	4.0%	26.0%
burns	Count	0	8
	Probability Count	1.0	8.0
	% among sample	0.0%	100.0%
	% among subspecies	0.0%	16.0%
	% Of the whole	0.0%	16.0%
sputum	Count	3	19
	Probability Count	2.3	19.0
	% among sample	15.8%	100.0%
	% among subspecies	50.0%	38.0%
	% Of the whole	6.0%	38.0%
wounds	Count	1	10
	Probability Count	1.2	10.0
	% among sample	10.0%	100.0%
	% among subspecies	16.7%	20.0%
	% Of the whole	2.0%	20.0%
Total	Count	6	50
	Probability Count	6.0	50.0
	% among sample	12.0%	100.0%
	% among subspecies	100.0%	100.0%
	% Of the whole	12.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.060a	12	.698
Likelihood Ratio	8.978	12	.705
Linear-by-Linear Association	.000	1	.990
N of Valid Cases	50		

a. 17 cells (85.0%) have expected count less than 5. The minimum expected count is .16.

various antibiotics have been used to determine the resistant rate of fifty one *Klebsiella* isolates as shown in (Fig. 1). The isolates were considered multidrug resistant if it is resist at least three classes of antibiotics .

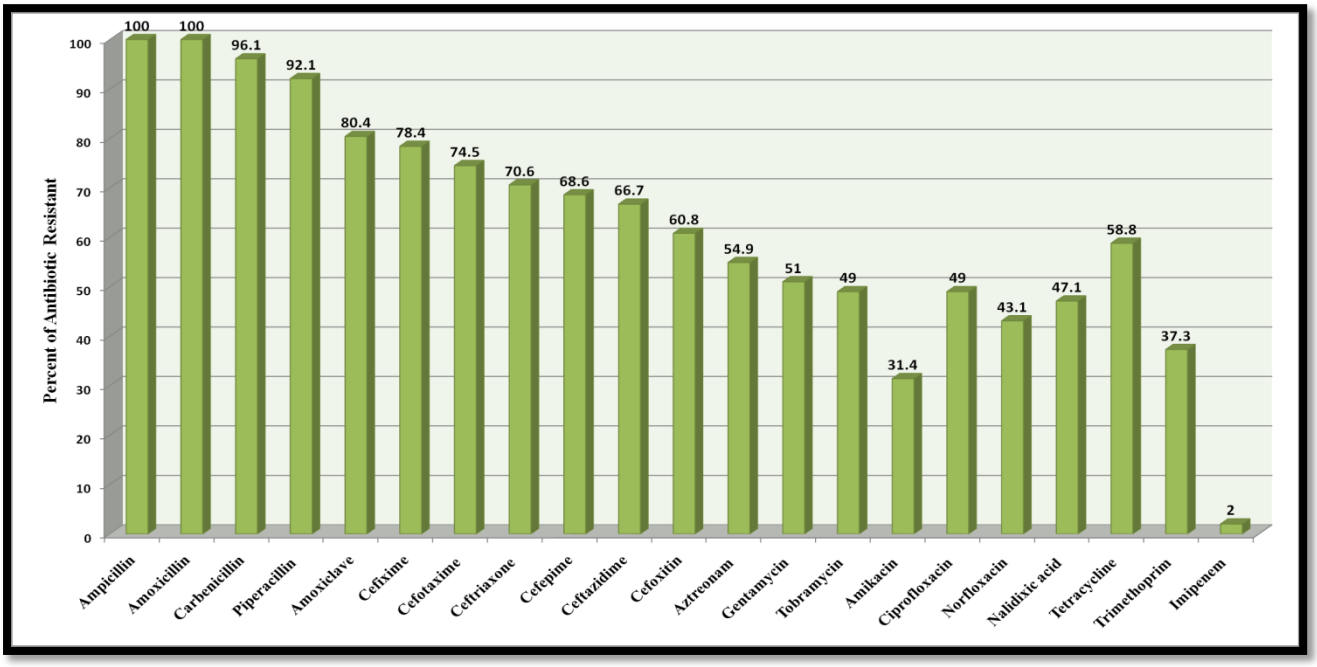


Figure (1): Antibiotics susceptibility profile for Klebsiella isolates (number=51).

The investigation of production AmpC β- lactamases among resistant *K. pneumoniae* isolates used two methods , the results showed that 31 (60.8%) *K. pneumoniae* isolates were cefoxitin resistant by the disk method and believed as AmpC β-lactamase producers, the production of molecular class C β-lactamase tested via modified three dimension way in all cefoxitin resistant isolates and observed 14 (45.2%) out of 31isolates were producing for AmpC β-lactamase . The cefoxitin resistance isolates further screened by AmpC disk method and the result was 11 (35.5%) out the 31 isolates were producing molecular class C β-lactamase as illustrated in(table 4) .

Table (4): AmpC β-lactamases production in *Klebsiella* isolates .

susceptibility		status		Total
		positive	negative	
*MTDT	Count	14	17	31
	Probability Count	12.5	18.5	31.0
	% among susceptibility	45.2%	54.8%	100.0%
	% among status	56.0%	45.9%	50.0%
	% Of the whole	22.6%	27.4%	50.0%
AmpC disk test	Count	11	20	31
	Probability Count	12.5	18.5	31.0
	% among susceptibility	35.5%	64.5%	100.0%
	% among status	44.0%	54.1%	50.0%
	% Of the whole	17.7%	32.3%	50.0%
Total	Count	25	37	62
	Probability Count	25.0	37.0	62.0
	% among susceptibility	40.3%	59.7%	100.0%
	% among status	100.0%	100.0%	100.0%
	% Of the whole	40.3%	59.7%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.603[a]	1	.437		
Continuity Correction[b]	.268	1	.605		
Likelihood Ratio	.604	1	.437		
Fisher's Exact Test				.605	.303
Linear-by-Linear Association	.594	1	.441		
N of Valid Cases	62				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.50. b. Computed only for a 2x2 table

* **Modified three dimensional test**

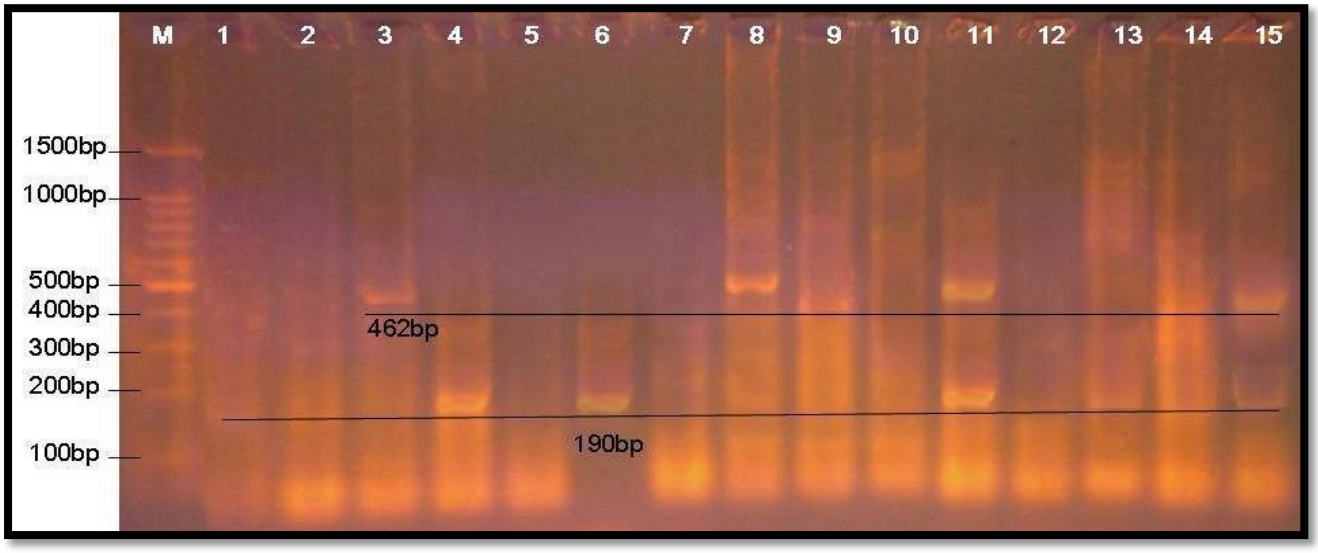
Among the 31 β-lactam resistant isolates that were molecular screened by PCR technique with *bla*$_{AmpC}$ gene and only 8 (25.8%) *K. pneumoniae* isolates contained *bla*$_{AmpC}$ gene (table- 5) . Afterward, various AmpC genes tested were (CIT and FOX) by the multiplex PCR technique and the results showed that presence of *AmpC* genes in all the study isolates were a varying . AmpC genes which returning to CIT family were found in 5 (62.5%) isolates while, AmpC genes which returning to FOX family were found in 3 (37.5%) isolates as showed in (Fig. 2) .

Table (5): Distribution of AmpC genes which returning to CIT and FOX families among blaAmpC positive Klebsiella isolates .

Genes		status		Total
		positive	negative	
bla ampc	Count	8	23	31
	Probability Count	5.3	25.7	31.0
	% among genes	25.8%	74.2%	100.0%
	% among status	50.0%	29.9%	33.3%
	% Of the whole	8.6%	24.7%	33.3%
bla CIT	Count	5	26	31
	Probability Count	5.3	25.7	31.0
	% among genes	16.1%	83.9%	100.0%
	% among status	31.2%	33.8%	33.3%
	% Of the whole	5.4%	28.0%	33.3%
bla FOX	Count	3	28	31
	Probability Count	5.3	25.7	31.0
	% among genes	9.7%	90.3%	100.0%
	% among status	18.8%	36.4%	33.3%
	% Of the whole	3.2%	30.1%	33.3%
Total	Count	16	77	93
	Probability Count	16.0	77.0	93.0
	% among genes	17.2%	82.8%	100.0%
	% among status	100.0%	100.0%	100.0%
	% Of the whole	17.2%	82.8%	100.0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.869[a]	2	.238

Likelihood Ratio	**2.887**	**2**	**.236**
Linear-by-Linear Association	**2.800**	**1**	**.094**
N of Valid Cases	**93**		



a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.33.

Figure (2): Multiplex PCR technique to investigate of AmpC β-lactamase genes among *K.pneumoniae* isolates positive for *bla*$_{\text{AmpC}}$ gene : Lane (L), 2000 bp DNA marker , Lanes (3, 8,11 and 15) *K.pneumoniae* showing (462 bp) *bla*$_{\text{CIT}}$ gene and Lanes (4, 6 and 11) *K.pneumoniae* showing (190 bp) *bla*$_{\text{FOX}}$ gene .

4-Discussion :

Klebsiella is a serious pathogen has the ability to cause essential nosocomial infections and high proportion of fatality , in the current study *Klebsiella pneumonia* was isolated from different samples at a higher rate of *Klebsiella oxytoca*, which was identical with other researchers as Feglo *et al*. [13]. Therefore, *Klebsiella pneumoniae* was described as a prominent bacteria responsible for acquired infections within the hospital particularly acquired pneumonia , inflammation of the urinary tract and blood infections [14] . This study revealed that *K. pneumoniae* subspecies *pneumoniae* was prevalent type (58.8%) . In a previous study confirmed that the presence of *K. pneumoniae* subsp. *pneumoniae* in clinical samples were the most common 87% [15]. However, in another study conducted by Al-Muhanna does not correspond with results of this study which found that subspecies *aerogenes* was the most prevalent among *Klebsiella spp*. member, it was present in 35 (81.4%) isolates[16], this ratio was higher than that we reached in this study, which found that *K.pneumoniae* subspecies *aerogenes* was (23.5%) . The proportion of (2%) was for each of the subsp. *ozanae* and subsp. *rhinoscleromatis* , another study

approach was performed by researcher Brisse *et. al.* recorded that low proportion (14.6%) of *K. pneumoniae* subsp. *ozaenae* whereas subsp. *rhinoscleromatis* not exist [14] .

The results described in (Fig.1) refers to the high rate of resistance (100%) for each ampicillin and amoxicillin , similar results were performed by other researcher Aminzadeh *et al*.[17] . As well as , this study also reported high resistance levels for each the carbenicillin and piperacillin (96.1%, 92.1% respectively) . This corresponds with the results of Al-Sehlawi who found *K. pneumoniae* isolates were resistant 100% for carbenicillin and 92.2% for piperacillin[18] . The reasons of the high resistance in this study may be attributed to repeated use or misuse of these antibiotics in hospitals .The resistance ratio for ceftriaxon was elevated and close to the results of Al-gerir [19], while the resistance ratio for cefotaxim and ceftazidim was (74.5% and 66.7% respectively) , higher resistance recorded by Gunseren *et al.* for cefotaxime (96.7%) and ceftazidime (85.5%) [20]. This results also revealed cefoxitin resistance was 60.8% , other researchers such as Al-Hilali recorded same results[21] , but this results incompatible with Nijssen *et al*. were found *K. pneumoniae* was 95.6% sensitive to cefoxitin [22] .

This resistance in *K. pneumoniae* attributes to production of AmpC β- lactamase or because reduction entry of antibiotic through the outer membrane [23]. Moreover, the proportion of the susceptibility to imipenem was (98%) and is the most effective antibiotics , this is compatible with Hadi who did not record any resistance against imipenem because the infected patient with molecular class C β- lactamases producing pathogens used imipenem therapy as last option [24, 25]. Aminoglycosides such as amikacin appeared effective toward *Klebsiella* spp. as showed in (Fig.1) although this result was observed compatible with results recorded by Ullah *et al.,* [26]. However, *K. pneumoniae* isolates relatively affected by gentamicin (51%) and tobramycin (49%). The aminoglycosides resistance caused by appearance various enzymes such as aminoglycosid altered enzymes **,** as well as the reasons of resistance altering the permeability of the outer membrane [27]. Also noted reduction of the effectiviness norfloxacin (43.1%) , ciprofloxacin (49%) and nalidixic acid (47.1%) and this is possible to attributed to the widespread use of these antibiotics in Al-Muthanna hospitals or because plasmid encoded resistance genes [28] .

The modified three-dimensional method can be used for primary screening of the molecular class C β- lactamases in laboratory , This study revealed (45.2%) out of 31 cefoxitin resistant isolates was productive for AmpC β- lactamase by this method , while the remaining 17(54.8%) cefoxitin resistant isolates were unproductive molecular class C β- lactamase because lack of penetration antibiotic across porins or these isolates have molecular class C genes but silent genes might not express in these isolates [29]. As well as another study carried out by Singhal *et al.*[30]and Al-Sehlawi [18]which have recorded that (33.3% and 42.5%, respectively) cefoxitin resistant isolates were confirmed to be AmpC producer by this way .The

production of molecular class C β-lactamase in *K. pneumoniae* due to excessive production of chromosomal encoded molecular class C β-lactamase or plasmid encoded molecular class C β-lactamase.

The other method AmpC disk test appeared that (35.5%) out of the 31 cefoxitin resistant isolates was productive for AmpC β- lactamase . Note that all of these isolates confirmed as a producer of AmpC β-lactamase by previous method (MTDT). Another study in India accomplished by Gupta *et al*. [31] recorded that 32(32%) out of the 48 cefoxitin resistant *K. pneumoniae* isolates gave positive results with AmpC disk method and considered producing for AmpC β- lactamase , this phenotypic methods has advantages such as speed, ease and accuracy in performance but does not distinguish between the causes of cefoxitin resistance whether by plasmid encoded enzymes or chromosomal encoded enzymes or reduced levels production of porins, as well as these phenotypic methods does not discriminate families plasmid encoded AmpC β- lactamase [32].

The polymerase chain reaction technique (PCR) is gold standard to determine AmpC gene in all the 31 cefoxitin resistant isolates . In addition to AmpC gene was found in 8(25.8%) cefoxitin resistance isolates by this technique and all of which were considered productive for the molecular class C β- lactamase via modified three dimensional and AmpC disk methods . This study in accordance with other results reported by other researchers Mohamudha *et al*. who found that (29.3%) of the *K. pneumoniae* carried plasmid mediated AmpC gene[33]. As well as, other study carried out by Shanthi *et al*.who found that (12/52) of the *K. pneumoniae* isolates carried plasmid mediated AmpC genes [34].Also this results showed the remaining 23(74.2%) cefoxitin resistance isolates were negative for plasmid mediated AmpC genes, therefore Cefoxitin resistance attributed to other means of resistance [35]. All eight *Klebsiella* isolates containing the AmpC gene were additionally screened to determine the presence each of the FOX and CIT genes by the traditional PCR technique . In the current study , blaCIT was essentially present in 5(62.5%) of *K. pneumoniae* isolates. In 2009, CMY-6(CIT type) was recorded from Uttar Pradesh [36]. In a similar study completed by Upadhyay *et al*. and Shanthi *et al*. found that bla_{CIT} gene was the most prevalent among *K. pneumoniae* and *E.coli* isolates [37] , [34].

The recent study carried out by Al-Sehlawi reported that CIT family was existed in (8/20) *K. pneumoniae* isolates which containing on plasmid mediated AmpC genes [18]. The FOX gene was first described in Argentina and Italy and believed that *Aeromonas caviae* may be source of this gene [38]. The current study showed that FOX gene were existed in 3/8(37.5%) *K. pneumoniae* isolates (Figure 2). In a previous study performed by Black *et al*. who reported that 28/44(63.6%) *K. pneumoniae* isolates carried plasmid mediated AmpC genes belonging to the FOX gene [39]. In other study revealed that 2/14 (14.3%)of the *K. pneumoniae* isolates carried *bla*FOX gene [40].

5-Conclusions:

The PCR technique is the best way to diagnose AmpC β- lactamase , which was awarded *Klebsiella Spp*. resistance to many of the β- lactam

antibiotics such as ampicillin and amoxicillin either imipenem is the optimal treatment for resistant bacteria . The phenotypic methods are less specificity as to discriminate between the bacterial resistance generated from production of AmpC β- lactamase or reduced levels production of porins and may be give false positive results as well as does not discriminate families plasmid encoded AmpC β- lactamase . The PCR technique determined CIT gene is the most prevalent among the Klebsiella isolates in Al-Hussein hospital , the identification of encoded genes for AmpC β- lactamases helps the doctor to describe the optimal treatment for the patient and control on the resistant bacteria and reduce its spread .

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