Dissemination of Klebsiella pneumonia and Klebsiella oxytoca Harboring bla TEM genes isolated from different clinical samples in Erbil City

Fattma A Ali (PhD) [1] **and Rozhan Mohammed Ismael** (Msc)[2]

Abstract

Background: *Klebsiella spp*. is an opportunistic nosocomial pathogen causing a variety of infections including urinary tract infections, pneumonia, septicemia, wound infections, distributed between patient results from producing ESBL enzymes lead to multiresistance against antibiotic encoded by some genes like blaTEM.

Objective: To identify *Klebsiella pneumonia* and Klebsiella *oxytoca* isolates harboring gene encoding for ESBL enzyme and multiresistance antibiotics such as blaTEM.

Patients and Methods: Three hundred samples were collected from (urine, wound, sputum), Klebsiella spp. isolated and identified by using microscopical, morphological, biochemical tests and Vitek 2 compact system. Antibiotic susceptibility testing was screening according to the CLSI guideline and Vitek 2 compact system. Phenotypic screening of ESBLs was undertaken using (Double disk diffusion and Standard disk diffusion) Methods, also PCR technique was used for genotypic detection of ESBL genes (blaTEM) according to the standard protocol.

Results: We obtained in this study 88 (29.33%) total positive results of Klebsiella spp.84 isolates for *Klebsiella* pneumonia and 4 isolates for Klebsiella oxytoca isolated from 300 different clinical specimens (urine, wound and sputum) ,from patient attending public hospitals in Erbil province (Rizgary, Teaching hospital, Laboratory center, Raparin, Nanakaly hospitals at a period from September 2014 to March 2015.Susceptibility profilehas been done for all *Klebsiella spp* isolated by using 13 antimicrobial agent,our multifinding pointed out that highest resistance ,most of *Klebsiella spp* isolates were resistance to more than three antibiotics belonging to different classes used and these were considered to be multidrug resistant (MDR) isolates. The incidence rate of ESBL-producing Klebsiella spp was 51 (57.95%) by Standard disk diffusion Method,48 (54.85%) by Double disk diffusion . Remarkably,dissemination of blaTEM 13 (11.09%) genes among ESBLs-positive isolates and the length of amplified genes (840) bp for blaTEM genes.

Conclusion: It can be said that the incidence rate of *Klebsiell asp* carring genes encoding for ESBL enzyme representing their commonness in our institute and multi resistance to many classes of antibiotic, resulting in limited treatment options.

Key words: *Klebsiella spp*, antimicrobial resistance extend spectrum β-lactamase, Bla TEM gene, PCR.

Corresponding Author: fattmaabeer@yahoo.com.

Received: 31th June 2017

Accepted: 12th March 2017

[1] College of Health Sciences-Hawler medical university- Erbil -Iraq.
[2]Ministry of Health-Erbil -Iraq.

Introduction

The extensive use of the third generation cephalosporins like cefotaxime, ceftriaxone and ceftazidime has led to the evolution of newer β-lactamases such as the Extended Spectrum β-Lactamases (ESBLs). ESBL are Plasmid-mediated enzymes that hydrolyze the oxyimino β-lactams and the monobactams (Aztreonam) but have no effect on the cephamycins (cefoxitin, cefotitan) and the carbapenems (Imipenem). Being plasmid mediated, they can be easily transferred from one organism to another [1].

One of the most important nosocomial pathogen produced ESBLs is *Klebsiella pneumoniae*, frequently causing pneumonia, urinary tract, wound and blood infection resulting in significant morbidity and mortality due to had plasmid encoded ESBLs lead to failure treatment or treatment option limited. The predominant types of ESBLs enzymes in *K. pneumoniae* are SHV followed TEM and CTX-M that belonged to class A B- lactamases include ESBLs enzymes [2].

Klebsiella oxytoca is an opportunistic pathogen that causes primarily hospital-acquired infections, most often involving immunocompromised patients or those requiring intensive care. Reported outbreaks have most frequently involved environmental sources. *K. oxytoca*, like other Enterobacteriaceae, may acquire ESBL and carbapenemases outbreaks of multidrug-resistant *K. oxytoca* infection pose an increasing risk to hospitalized patients [3]. Plasmids may harbour ESBL-encoding genes. Along with these, the plasmids may also carry genes that confer resistance to other antibiotics, making treatment options even narrower. This is called co- resistance, and these genes are often carried in conjunction with the ESBL-gene. Bacteria that harbour genes that confer resistance to three or more different types of antibiotics are called multidrug resistant [4].

There are three mechanisms that can cause antibiotic resistance: prevention of interaction of drug with target organisms, decreased uptake due to either an increased efflux or a decreased influx of the antimicrobial agent and enzymatic modification or destruction of the compound The resistance of *Klebsiella spp*. was mediated by several mechanisms, the important one of which is the production of enzymes encoded by several genes that are carried on some bacterial plasmids, β-lactamase and extended spectrum β-lactamase. Extended spectrum β- lactamases are mostly plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactam antibiotics, including different types of penicillins and cephalosporines[3]. overexpress beta-lactamases or produce both SHV-1 and TEM-1 resistant to Piperacillin or first generation Cephalosporins. Clavulanic acid are active against the SHV-1 and TEM-1 β-lactamases of K. pneumonia as ß-lactamase inhibitors. However, clinical isolates have been described that are resistant to beta-lactamase inhibitor combinations[6].

Materials and Methods

This study was conducted in the Rizgary, Teaching hospital, Laboratory Center, Raparin, Nanakaly Hospitals in Erbil province at a period from September 2014 till March 2015.

A total of 300 samples were collected from different clinical specimens (sputum, urine, wound) from both sex (males and females) attending public hospitals with age group up to70 years.

Isolation of microorganism

The specimen was inoculated on Blood culture and MacConkey agar plates were incubated aerobically at 37°C for (24-48) hours, were identified using API system, Vitek 2 system [6].

Antimicrobial susceptibility test by Vitek2 system

With its ability to provide accurate "fingerprint" recognition of bacterial resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology. TheVitek 2 card contains 64 microwells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms [7]. The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods.

Phenotypic detection of ESBL enzyme

All bacterial species were screened for ESBL enzyme production by the following methods:

Screening test for ESBL (Standard disk diffusion method). ESBL detection was carried out by standard disk diffusion methods for all Gram negative isolates according to the Standard Institute of Antimicrobial Susceptibility Testing recommendation [8] by using various antimicrobials. ESBL positive meant the organism shows comparatively high level co-resistance to third generation cephalosporin such as; ceftazidime zone ≤ 22 mm, Aztreonam zone ≤ 27 mm, Cefotaxime zone ≤ 27 mm or Ceftriaxone zone ≤ 25 mm. A laboratory strain of *Klebsiella pnemoniae* ATCC (13883) was used as a control.

Double disk synergy test (confirmatory test)

Disk of Amoxicillin - Clavulanic acid 30 µg (20 µg of Amoxicillin +10 µg of Clavulanic acid) and one of the antibiotic disks of the third generation cephalosporin antibiotics such as (Ceftazidime disk 30 µg) used together. The disks were placed at a distance of 30 mm apart from the inhibitor disk on Muller Hinton agar, if the zone size around the tested antimicrobial increased towards the Amoxiclav disk this meaning the test was positive [9].

Isolation of plasmid from bacterial cell

The method that used for isolation of plasmid from *Klebsiella spp*. was performed by using Prime PrepTM plasmid DNA isolation kit.

All bacterial plasmids were run on 1% agaros gel for detection of their pattern. Fifteen µl of extracted plasmid was mixed with 3 µl of loading dye (6×) and the mixture was loaded in to prepared agaros gel and the gel ran for 3 min at a voltage of 1 to 5 volts/cm2 of the gel size. The results were read using ultra violet light in gel documentation system.

Polymerase Chain Reaction (PCR), PCR reaction mixtures were prepared in duplicates; negative controls were included in each run to validate the reaction. Each reaction mixture was prepared to a volume of 50 µl in a sterile PCR tube.

lyophilized primers provided by Cinnage® were processed in order to product a stock concentration of 100 µM by mixing the concentrated lyophilized primer with specific volumes (458.01, 528.40, 502.93, 568.43) of nuclease free de-ionized water according to the primer manufactures and concentration table (1). From this stock concentration a working primer concentration of 10 µM was prepared.

All isolated *Klebsiella spp.* were selected for detecting multi resistant gene namely (blaTEM).

In standard PCR, the reaction was prepared for two sets of primer separately worked cause of difference in annealing temperature was prepared by mixing the reverse with the forward of primer and addition to other component of the PCR reaction. A PCR amplification condition was modified according to the annealing temperature of the primer; while for denaturation and extension steps table (1).

Table (1): Primers used in the study.

Primer Name	Primer Sequence	Annealing Temp.	Target gene	Amplicon in	Reference
TEMF	ATGAGTATTCAACATTTCCGTG	55°C	*TEM*	840-bp	Sidjabat *et al.*, 2010
TEMR	TTACCAATGCTTAATCAGTGAG				

Agaros gel preparation and agaros gel electrophoresis

The agaros gel was prepared according to what was performed by Ozer *et al.*[11].

-To prepare 1% agarose gel, 1 g of agarose powder was mixed with 1× TBE (Tris base-boric acid-EDTA) buffer to reach a final volume of 100 ml, the mixture was thoroughly mixed by swirling the flask.

The mixture was melted in a microwave oven for about 2 minute until the mixture become clear; and after the mixture had been cooled to a temperature of appox55°C. 3 µl of 10 mg/ml of the intercalating agent Ethidium Bromide was added and the suspension mixed thoroughly by gentle swirling.

The agaros mixture was poured into the assembled gel support (10 × 20 cm); a comb was inserted and gel was allowed to set completely and solidify at the room temperature.

The gel cast was placed on its support into the electrophoresis running apparatus and the tank was filled sufficient electrophoresis running buffer (1× TBE) to cover the gel completely, then the comb was removed.

The DNA (PCR product) was mixed with 3 µl of loading dye and the samples were loaded in separate wells including a DNA size marker in one of the lane.

The lid of the tank was closed, electrophoresis separation was started by running the electric current at a voltage of 1 to 5 volts/cm2; bubbles arising from the anode pole and dye migration indicate the gel is in separation.

After complete migration of markers to the other end, the gel was removed and visualized under UV light transilluminator, the gel was photographed and documented.

Result

In present study a total of 300 samples collected from different hospitals in Erbil. Only 88 (29.33%) obtained and isolated were identified as *Klebsiella spp*. The result of this study indicate that the high percentage of *Klebsiella spp*. was 84 (29.33) distribution between 64 (71.73%) urine, 16 (17.73%) wound, 8 (9.09%) sputum as in table (1).

The results of present study were produce two types of culture (single and mixed), the most frequency was 78 (88.64%) of *Klebsella spp*. isolated from single culture of them 74 (88.09%) were *Klebsiella pnemoniae* and 4 isolated of *Klebsiella oxytoca*, only 10 (11.36%) of *Klebsiella* pnemoniae isolated from mixed culture. Regarding mixed bacteria in positive culture *Klebsiella spp* with *Escherichia coli* in 4 urine sample, *Klebsiella spp* with Pseudomonas aeruginosa in 3 wound sample and *Klebsiella* spp with *Staphylococcus aureus* in 3 sputum sample as in table(2).

Table (2): Distribution of *Klebsiella pnemoniae* and *Klebsiella oxytoca* in different clinical specimens.

Isolated Bacteria	*Male*			*Total No. %*	*Female*			Total female No %	Total No . (%)
	Urine No (%)	Sputum No (%)	Wound No (%)		Urine No (%)	Sputum No (%)	Wound No (%)		
Klebsiella pneumoniae	10 (11.36%)	5 (5.68%)	5 (5.68%)	20 (22.73%)	50 (56.82%)	3 (3.41%)	11 (12.5%)	64 (72.72%)	84 95.45 %)
Klebsiella oxytoca	4 (4.55%)	0 (0%)	0 (0%)	4 (4.55%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 4.55%)
Total No. (%)	14 (15.91%)	5 (5.68%)	5 (5.68%)	24 (27.28%)	50 (56.82%)	3 (3.41%)	11 (12.5%)	64 (72.72%)	88 100%)

Table (3): The incidence of *Klebsiella spp*. isolated in single and mixed positive culture.

Isolated pathogens	Positive culture result		Total No of isolated and %
	Mixed bacteria No. (%)	Single bacteria No. (%)	
Klebsiella pnemoniae	10(11.91)	74 (88.09)	84(95.45)
Klebsiella oxytoca	0(0)	4 (100%)	4 (4.55)
Total No and %	10(11.36)	78 (88.64)	88(100)

Antimicrobial susceptibility testing for *Klebsiella spp*

In present study as shown in table (4) for *Klebsiella pnemoniae* the most sensitive antibiotics were Imipenem 79 (94.04%) followed by Amikacin 69 (82.14%) and Ciprofloxacin 62 (73.81%).

While *Klebsiella oxytoca* the most effect antibiotics were Ciprofloxacin 4 (4.55%) and both are the same effect Imipenem 3 (3.41%) and Amikacin 3 (3.41%).

Table (4): Antimicrobial susceptibility tests for *Klebsiella spp*.

Antibiotics			*Klebisella pneumonia*			*Klebsiella oxytoca*			Total No %
			R	I	S	R	I	S	
Ciprofloxacin	CIP 10	No	19	3	62	0	0	4	88
		%	22.69	3.57	73.81	0	0	100	100
Amikacin	AK 10	No	7	8	69	1	0	3	88
		%	8.33	9.53	82.14	25	0	75	100
Doxycycline	DO 10	No	36	2	46	2	0	2	88
		%	42.86	2.38	54.76	50	0	50	100
Ceftriaxone	CRO 10	No	41	4	39	3	0	1	88
		%	48.81	4.78	46.43	75	0	25	100
Cefotaxime	CTX 30	No	42	3	39	4	0	0	88
		%	50	3.57	46.43	100	0	0	100
Imipenem	IPM 10	No	4	1	79	1	0	3	88
		%	4.76	1.19	94.04	25	0	75	100
Ceftazidime	CAZ 10	No	82	1	1	3	1	0	88
		%	97.62	1.19	1.19	75	25	0	100
Gentamicin	CN 10	No	23	0	61	2	0	2	88
		%	27.38	0	72.62	50	0	50	100
Piperacillin	PRL 30	No	69	8	7	3	1	0	88
		%	82.14	9.53	8.33	75	25	0	100
Amoxicillin	AX 25	No	84	0	0	4	0	0	88
		%	100	0	0	100	0	0	100
Cefazolin	CFZ10	No	82	0	2	4	0	0	88
		%	97.62	0	2.38	100	0	0	100
Cefoxitin	FOX30	No	81	0	3	4	0	0	88
		%	96.43	0	3.57	100	0	0	100
Cefuroxime	CXM10	No	80	0	4	4	0	0	88
		%	95.24	0	4.76	100	0	0	100

Current study a total of 88 isolates of Klebsiella spp. were screened for Extended Spectrum β-Lactamases (ESBL) enzyme production by using two method standard disk diffusion methed as in figure(1) and double disk diffusion method as in figure (2) and the result showed that 51 (57.95%) ESBL producer, while 37 (42.05%) total non ESBL produce of Klebsiella spp. while by Double disk diffusion method 48 (54.55%).

ESBL producers by Klebsiella spp., 40 (45.45%) non ESBL producer of Klebsiella spp.As in table 5 . In our study there was difference in the detection of ESBL between screening test and confirmatory method in the aspect that lower prevalence rate of ESBL (54.55%) was recorded by double disk synergy test compared to standard disk diffusion test (57.95%) as in table (4).

Table (5): Phenotypic detection of ESBL producing by *Klebsiella spp.* isolated from different clinical specimens.

	Double screening ESBL				Total No and (%)
	Standard disk diffusion (Screen test)		Double disk diffusion (DDST)		
	Positive No.(%)	Negative No.(%)	Positive No.(%)	Negative No.(%)	
Klebsiella pnemoniae	48 (57.14%)	36 (42,85%)	46 (54.76%)	38 (45.24%)	84(95.45)
Klebsiell aoxyoca	3 (75%)	1 (25%)	2 (50%)	2 (50%)	4 (4.55)
Total (%)	51 (57.95%)	37(42.05%)	48 (54.55%)	40 (45.45%)	88(100)

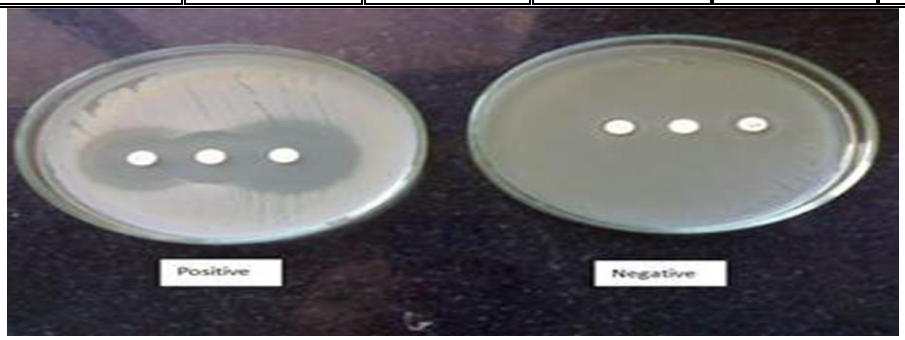

Figure (1): Stander disk diffusion method for ESBL production *by Klebsiella spp.* (A)ESBL positive, (B) ESBL negative. From left to the right (Ceftazidime, Aztreonam, Cefotaxime)

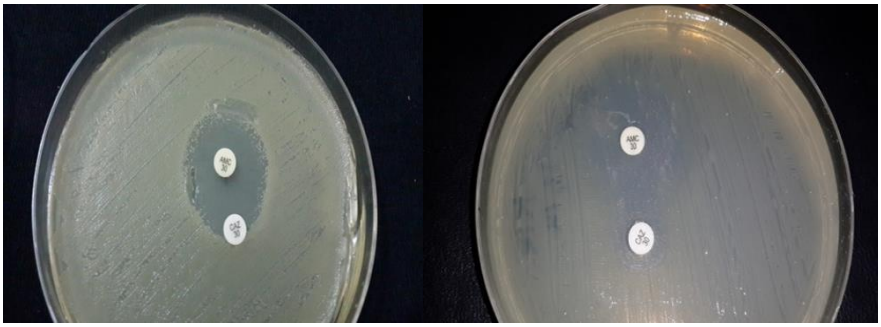

Figure (2): Double-disk diffusion method for ESBL production by *Klebsiella spp*. ESBL positive, (B) ESBL negative. (Amoxicillin- Clavulanic acid, Ceftazidime).

Molecular Detection of ESBL enzyme in *Klebsiella spp.*

Detection of blaTEM gene, PCR reactions were performed for all studies

bacteria, PCR detection of two ESBL genes yielded many positive results. Obtaining the expected size of amplicon was considered as in indicator for the gene presence. These amplicon sizes were consistent and the same results were obtained when PCR reported on the same sample. In our study 88 *Klebsiella spp.* plasmid were run on gel electrophoresis for detecting plasmid pattern PCR reaction for detecting blaTEM gene. Out of 88 *Klebsiella spp.*, 13 (14.77%) samples were positive result and 75 (85.23%) samples were negative result in table (5). blaTEM genes was recorded in 13 (14.77%) among all *Klebsiella spp* isolates isolated from different clinical samples with lengths of amplified gene was (840) bp as in figure (3).

In the current study, the prevalence of ESBL genes by using PCR was different according to the species of Klebsiella spp. our result revealed that most of blaTEM gene present in Klebsiella pnemoniae12 (30%), On the other hands, 1 stain of *Klebsiella oxytoca* carried blaTEM gene.

Table (6): Molecular detection of ESBL genes by PCR.

Isolated Bacteria	PCR result		Total No and %
	Positive No.(%)	Negative No.(%)	
Klebsiellapnemoniae	12 (14.29%)	72 (85.71%)	84 (95.45%)
Klebsiellaoxyoca	1 (25%)	3 (75%)	4 (4.55%)
Total No (%)	13 (14.77%)	75 (85.23%)	88(100%)

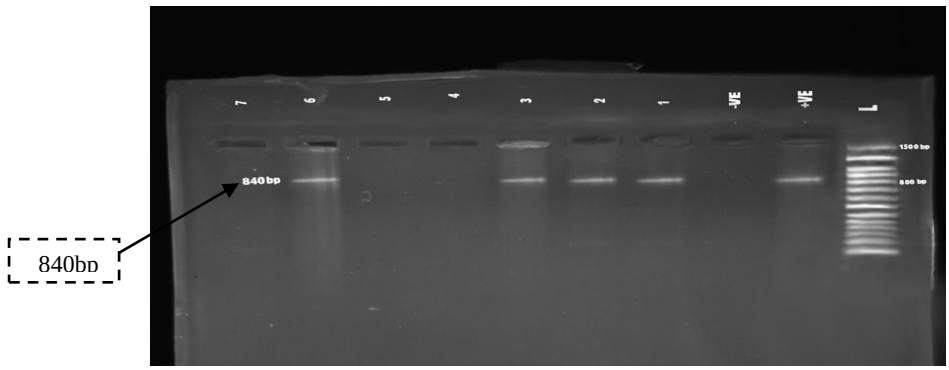

Figure (3): PCR product for *bla*TEM gene (840) bp resolved using 1% agarose gel electrophoresis

L: Ladder (1500bp), +ve: positive control (*Klebsiella pnemoniae*, ATCC (13883), -ve: negative control, **Lane1,2,3:** amplified PCR product of *bla*TEM gene (840) bp for *Klebsiella pnemoniae* and **Lane 6**: for *Klebsiella oxyoca* with *bla*TEM, **Lane 4,5,7:** *Klebsiella pnemoniae* negative for *bla*TEM gene.

Discussion

The result of this study indicate that the high percentage of *Klebsiella spp.* was 84 (29.33) distribution between 64 (71.73%) urine, 16 (17.73%) wound, 8 (9.09%) sputum. These results were in agreement with finding of Idomir *et al*., from Romania who found were (63.6%) isolated from urine, (10.4%) from wound and (10%) from sputum[10].

The result of present study agree with Al-Charrakh *et al*., from Hilla (Iraq) who collected 88 *Klebsiella spp* strains were isolated from different environmental and clinical [11].

The presence of this bacteria in large present in UTI might be attributed to the fact that these bacteria are often part of the resident flora and different virulence factors contributing to their pathogenicity and the difference in the result with others might be attributed to the number of taken sample size and the difference in the time of the study.

The results in this study showed that highest percentage of *Klebsiella spp*. was caused by single microorganism infection, the culture result doesn't always show single bacterial growth, frequently there are two or more growths of bacterial pathogens on normal flora found as combination and these result relatively similar with the founding of Anwar, from our country from Sulaimanyia in which reported that mixed infection in wound swab was observed mostly between *Klebsiella pnemoniae* and *Acinobacter baumanni* in 7 samples[12].

The most sensitive antibiotics were Imipenem followed by Amikacin and Ciprofloxacin which was similar to the founding of Gupta *et al*., [13].

This result showed in table (4) high rates of resistance in *K. pneumoniae* to ß-lactam antibiotics while it equal to those reported in Tenzania [14]. For ampicillin and amoxicillin 100%. While was higher than those obtained in Hilla-Iraq which found that (73.08%) of *Klebsiella* isolates obtained from clinical and environmental samples resistance to Amoxicillin Orrett, [15]. And higher than these obtained in Najaf/ Iraq the rate of resistance were 88% [16].

In our country does not have any systematic program for studying the antibiotic resistance pattern. Proper use of antibiotics is ensured by formulating an antibiotic policy. ESBL screening as a routine test has not yet been practiced in Iraq. ESBL occurs at an alarming rate among Enterobactericeae isolates among the hospitalized patients which can result in an outbreak in the community that may be difficult to treat. Bangladesh does not have any systematic program for studying the antibiotic resistance pattern. Proper use of antibiotics is ensured by formulating an antibiotic policy. ESBL screening as a routine test has not yet been practiced in in our country and developing country. ESBL occurs at an alarming rate among Enterobactericeae isolates among the hospitalized patients which can result in an outbreak in the community that may be difficult to treat.

Phenotypic detection of ESBL producing by *Klebsiella spp*. isolated from different clinical specimens. Extended-spectrum beta-lactamase (ESBL) producing strains of *K. pneumonia* have caused major therapeutic problems worldwide since the majority are resistant to various antibiotics [16]. These enzymes catalyze the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs have been reported worldwide in many different genera of Enterobactericeae and *Pseudomonas aeroginosa* [17]. However, these are most common in *Klebsiella pneumonia* and *E. coli* [18].

Now days, *K. pneumoniae* has become resistant to most of the β-lactams antibiotic

including carbapenem drugs because of ESBLs and MBLs production such as imepenem, leading to therapeutic failure. The overall prevalence of ESBL producers found to vary greatly in different geographical areas such as varies from 54.7% to 59.2% in Iran [19], 41% in United Arab Emirates [20]. The result showed that 51 (57.95%) ESBL producer, while 37 (42.05%) total non ESBL produce of *Klebsiella spp*. while by Double disk diffusion method 48 (54.55%) ESBL producers by *Klebsiella spp*., 40 (45.45%) non ESBL producer of *Klebsiella spp.* As in table 5 it similar to that recorded in Sulaimani (57%) by Mazin, [21], higher than recorded in Erbil (42%) by Hussen [22].

Cephalosporins and Aztreonam, have been increasingly reported and are due to the acquisition of plasmids encoding ESBLs [23]. In addition, *K. oxytoca* isolates that overproduce the chromosomally-encoded β-lactamase have been found to be resistant to broad-spectrum Cephalosporins (e.g. Cefotaxime and Ceftriaxone) and Monobactams [24]. A strain of *K. oxytoca* that produces a chromosomally-encoded β-lactamases conferring resistance to Ceftazidime was recently reported [25].

An interested finding in this part of the study there was an association of positive culture of *Klebsiella spp.* with number of antibiotic resistant and with ESBL production. Therefor in current study found that most isolates multi resistance to more than > 7 antibiotic (55.68%) and most of isolates ESBL producers. The majority of ESBL positive isolates showed high resistance to most of the tested antibiotics with highest rate of resistance to Amoxicillin and also Cefoxitin, Cefuroxime and Ceftazidime. In contrast with Al-Charrakh *et al*., [11] from Hilla (Iraq) who found All the ESBL β- lactamase-producing *Klebsiella* strains showed multiple-drug resistance to least 8 antibiotics.

Klebsiella oxytoca like other Enterobacteriaceae, may acquire ESBL and carbapenemases; out breaks of multi-drug resistant *K. oxytoca* infection pose an increasing risk to hospitalized patients [26].

In a study MDR Enterobacteriaceae and randomly selected non-MDR counterparts isolate from patients, healthcare workers and environmental surface in a newly opened hospital in Iraq were investigated to characterize plasmid found in these isolates and determine their contribution to antibiotic resistance. The study found that MDR for *Klebsiella pnemoniae* mostly demonstrating 2 plasmids compared to their non-MDR counterparts [27].

In the current study, the prevalence of ESBL genes by using PCR was different according to the species of *Klebsiella spp*. our result revealed that most of blaTEM gene present in *Klebsiella pnemoniae* 12 (30%), On the other hands, *Klebsiella oxytoca* carried blaTEM gene. In this content, it was clear that the gene is present especially in urine sample. In similar study that done by Izad *et al*. [28] In Iran out of 58 ESBL producing *Klebsiella pnemoniae* isolates 15 cases (78.56%) had TEM genes. This result indicates that this gene was present in Kurdistan Iraq and is regarded as a source of international disseminations and may be this MDR gene is transmitted to our locality from tourists of other neighboring countries such as Iran, Turkey and south and middle of Iraq and transmission mostly occurs by plasmid caring genes encoding for ESBL enzyme.

References

[1] Quale JM, Landman D, Bradford PA, Visalli M, Ravishankar J, Flores C, *et al.* Molecular epidemiology of a citywide outbreak of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae*

infection. Clin Infect Dis. 2002; 35(7):834-41.

[2] Eftekhar F, Rastegar M, Golalipoor M, Mansoursamaei N. Detection of Extended Spectrum B-Lactamases in Urinary Isolates of *Klebsiella pneumoniae* in Relation to blaSHV, blaTEM and blaCTX-M gene car¬riage. Iran J Public Health. 2012; 41(3):127–32.

[3] Chiangjong, W. Study of extended-spectrum beta-lactamase (ESBLs) producing Klebsiella pneumoniae: phenotypic and genotypic characteristics. M.Sc. Thesis. Mahidol University.2006.

[4] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin.Microbiol. Infect. 2012: 268-281.

[5] Girlich D, Naas T, Nordmann P. Biochemical Characterization of the Naturally Occuring Oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antiicrobial, Agents and Chemotherapy. Journal of Antimicrobial and Chemotheraph. 2004; 48(6): 2043-2048.

[6] Nagaraja P. Antibiotic resistance of Gardnerella vaginalis in recurrent bacterial vaginosis Indian Journal of Medical Microbiology.2008; 26 (2): 155- 157.

[7] Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of Extended-Spectrum β-Lactamases among Enterobacteriaceae by use of semi-automated microbiology systems and manual detection procedures. Journal of Clinical Microbiology. 2007; 45(8): 1167-1174.

[8] CLSI, (Clinical and Laboratory Standards Institute). Performance standard for antimicrobial susceptibility testing, Twenty-First informational supplement. 2011; 31(1):M100-S21

[9] Rajesh KR, Mathavi SK, Indra P. Research Detection of extended spectrum beta-lactamase producing gram negative bacilli in urinary isolate. International Journal of Biological and Medical. 2010; 1(4): 130-132.

[10] Ozer1 B, Duran N, Onlen Y and Savas L. Efflux pump genes and antimicrobial resistance of Pseudomonas aeruginosa strains isolated from lower respiratory tract infections acquired in an intensive care unit. The Journal of Antibiotics. 2012; 65 (8): 9-13.

[11] Al-Charrakh AH, Yousif SY and Al-Janabi HS. Antimicrobial spectrum of the action of bacteriocins from Klebsiella isolates from Hilla/Iraq. Iranian J Microbiol. 2011; 2(5): 76-78.

[12] Anwar K. Detection of metalo beta lactamase in Gram negative bacteria isolated from burn patient in Sulaimanyia. PhD Thesis. College of Mediciene, Sulaimanyia University.2013.

[13] Gupta V, Kumarasamy K, Gulati N, Garg R, Krishnan P, Chander J. AmpC β-lactamases in nosocomial isolates of *Klebsiella pneumoniae* from India. Indian Journal of Medical Research. 2012; 136(2): 237-241.

[14]Boomberg B, Manii KP, Ureassa WK, Tamil BS, Mwakagile DS, Jurgen R, *et al.*antimicrobial resistance predicts death in Tanzania children with blood stream. BMC infect. 2007:1-14.

[15] Orrett FA. Clinicalsources and prevalence of resistance to antimicrobials of Klebsiella pneumoniae strains in Trinidad. Saudi Med J. 2005; 26(11): 1766-1770.

[16] Almohana A, Ammran A, Al-Thahab. Occurrence and Detection of Carbapenemase-Producing *Klebsiella pneumoniae* Clinical Isolates in Najaf Hospitals Zuhair Sadiq Al Sehlawi College of Dentistry- Kufa University. Magazin of

Al-Kufa University for Biology.2013:2073-8854.
[17] Feizabadi MM, Etemadi G, Yadegarinia D, Rahmati M, Shabanpoor S, Bokaei S. Antibiotic-resistance patterns and frequency of extended-spectrum beta-lactamase-producing isolates of Klebsiella pneumoniae in Tehran. Med Sci Monit. 2006:362–365.
[18] Friedman NP, Miyake A, Young SE, Defries JC, Corley RP, Hewitt JK. Individual differences in executive functions are almost entirely genetic in origin. J Exp Psychol Gen.2008; 137(2):201-25.
[19] Bell JM, Chitsaz M, Turnidge JD, Barton M, Walters LJ, Jones RN .Prevalence and Significance of a Negative Extended-Spectrum β-Lactamase (ESBL) Confirmation Test Result after a Positive ESBL Screening Test Result for Isolates of Escherichia coli and *Klebsiella pneumoniae*. J Clin Microbiol. 2007; 45(5): 1478-1482.
[20] Al-Zarouni M, Senok A, Rashid F, Al-Jesmi SM, Panigrahi D. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med Princ Pract. 2008; 17(1):32–36.
[21] Mazin F. Detection of malti drug resistance in some gram negative bacteria production extend spectrum beta lactamase in interval care unit in Sulaimanyia city, diploma of medical since, Sulaimanyia, Diploma of Medical Science.2011.
[22] Hussen SQ. Microbiological study of burn wound infection, antibiotics susceptibility pattern and beta lactamase in Erbil city master theses hawler medical university.2010.
[23] Bradford PA. Extended-spectrum-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001; 14:933–51.
[24] Fournier B, Lu CY, Lagrange PH, Krishna MR, Philippon A. Point mutation in the pribnow box, the molecular basis of beta-lactamase overproduction in Klebsiellaoxytoca. Antimicrob Agents Chemother. 1995; 39(6):1365-1368.
[25] Mammeri H, Poirel L, Nordmann P. In vivo selection of chromosomally encoded β-lactamase variant conferring ceftazidime resistance in Klebsiella oxytoca .J. Antimicrob. Agents Chemother. 2003; 47 (12):3739-3742.
[26] Lin WH, Wang MC, Tseng CC, Ko WC, Wu AB, Zheng PX, *et al.* Clinical and microbiological characteristics of Klebsiella pneumoniae isolates causing community-acquired urinary tract infections. 2010; 38(6):459-64.
[27] Huang J, Roberts A J, Leschziner A E, Reck-Peterson SL, Huang J. Lis1 Acts as a "Clutch" between the ATPase and Microtubule-Binding Domains of the Dynein Motor. Cell. 2012; 150(5):975-86.
[28] Izadi N, Nasab MN, Mood HE, Meshkat Z. Prevalence of TEM and SHV genes in clinical isolates of *Klebsiella pnemoniae* from Mashhad, North-East Iran. Iranian Journal of Pathology. 2014; 9(3):199-205.