Extended spectrum ß-lactamase (ESBL) mediated resistance to third generation cephalosporins in urinary tract infection isolates of *Klebseilla pneumonia.*

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الخلاصة

الغرض من الدراسة للتحري عن انتشار بكتريا *Klebsiella pneumoniae* المنتجة لأنزيمات ألبيتا لاكتاميز واسعة الطيف المعزولة من المرضى الذين لديهم نسبة ملحوظة من البكتريا في الإدرار.

تم دراسة المقاومة لمضادات ألبيتا لاكتام واسعة الطيف والمقاومة المتعددة للمضادات الحيوية ل 25 عزلة *K. pneumoniae*.

وجد أن جميع العزلات كانت مقاومة على الأقل لخمس أنواع من المضادات الحيوية المستخدمة في هذه الدراسة بينما أظهرت جميعها حساسية إلى الامبينيم. بينت النتائج إن 5 (22.7%) من العزلات منتجة لأنزيمات ألبيتا لاكتاميز واسعة الطيف. إن العزلات المنتجة لأنزيمات واسعة الطيف كانت أكثر مقاومة للمضادات الحيوية من العزلات الغير منتجة. كما إن صفة إنتاج أنزيمات ألبيتا لاكتاميز واسعة الطيف تم نقلها عمليا إلى السلالة المستلمة *Escherichia coli* MM294.

بينت الدراسة الحالية ظهور عزلات من *K. pneumoniae* منتجة لأنزيمات ألبيتا لاكتاميز ذات الطيف الواسع في مستشفيات النجف. إن استخدام الفحوصات الخاصة بالكشف عن هذه العزلات يجب أن تجرى في جميع المختبرات التشخيصية الروتينية وكذلك تجنب استخدام كافة مضادات الجيل الثالث من السيفالوسبورين لعلاج البكتريا المعزولة والتي تظهر مقاومة لأي مضاد منها.

Abstract

Objective: **To examine the dissemination of extended spectrum ß-lactamase (ESBL) producing isolates of *Klebseilla pneumoniae* obtained from patients with significant bacteriuria.**

Methods: **ESBL and multiantibiotic resistance were studied in a total of 25 isolates of *K. pneumoniae*.**

Results: **all isolates found resistance to at least five of the antibiotics used in this study. All the isolates were found sensitive to the antibiotic imipenem. 5 (22.7%) of these isolates were ESBL producing. The ESBL producing isolates were more resistant to antibiotics than non-ESBL isolates.**

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The ESBL activity could be experimentally transferred to recipient *Escherichia coli* MM294.

Conclusions: **present study has found the occurrence of ESBL producing *K. pneumoniae* isolates in Najaf hospitals. Test for the detection of ESBL producing bacterial isolates should be carried out in all diagnostic laboratories routinely and the therapeutic use of all the third generation cephalosporins should be avoided against bacterial isolates that appear resistant to any third generation antibiotic.**

Introduction

Bacteria of the *Klebseilla* genus constitute physiological flora of the human nasopharynx and gastrointestinal tract (1). However, they become with increasing frequency the pathogens causing hospital infections.

***Klebseilla* spp. are generally susceptible to cephalosporins but moderately resistant to penicillins by virtue of synthesis of chromosomally-mediated penicillinases. Some *Klebsiella* spp. also show reduce susceptibility to first and second generation cephalosporins by production of plasmid-mediated, TEM-like ß-lactamases (2). Since 1980s, the emergence of resistance to third generation cephalosporins has been reported in strains of *K. pneumoniae* (3).**

Resistance of *K. pneumoniae* to broad-spectrum cephalosporins such as cefotaxime, ceftriaxone and ceftazidime and other ß-lactam antibiotics poses a serious therapeutic problem in many parts of the world (4,5). Such resistance has often been associated with transferable plasmid encoded extended spectrum ß-lactamases (ESBLs). The resistance was caused by mutations in the common TEM-1, TEM-2 and SHV-1 ß-lactamases. ESBLs are more prevalent in *K. pneumoniae* than in any other enterobacterial species. In many parts of the world almost 10-40% reports have highlighted the emergence of ESBL producing strains endowed with an extremely wide spectrum of antibiotic resistance, including resistance to gentamicin, streptomycin, amikacin, and trimethoprim (6,7).

Although strains that produce ESBLs are characteristically resistant to newer cephalosporins and/or aztreonam, many strains producing these enzymes appear susceptible or intermediate to some or all of these agents in vitro, while expressing clinically significant resistance in infected patients (8,9). Such strains are often not recognized as producer of ESBLs, placing infected patients at risk of

receiving inappropriate therapy. Therefore, reliable detection of ESBLs is an important problem facing clinical laboratories.

The present study was conducted with an objective to examine the occurrence of ESBL producing isolates and multidrug resistant isolates of *K. pneumoniae* obtained from patients with significant bacteriuria. Transmissibility of antibiotics resistance and ESBL production was also studied.

Materials and Methods

Clinical isolates:

Twenty five isolates of *K. pneumoniae* were studied. They were recovered from patients with urinary tract infections in Al-Sadr Teaching and Al-Zahra Maternity and Children Hospitals in Najaf, from December 2006 to March 2007. *Klebseilla* isolates that were obtained as a pure and predominant growth (>10^5 colony forming units (CFU)/ml) from the urine specimens were only considered as significant bacteriuria. The organisms were identified based on colony morphology, biochemical reactions (10), and confirmed using the API 20E identification system (BioMerieux, France).

Beta-lactam resistance testing:

Ampicillin and amoxicillin were added separately to the Muller-Hinton agar (Biolife, Italy) at final concentration of 100 and 50 µg /ml, respectively. Preliminary screening of *K. pneumoniae* isolates resistance to above antibiotics was carried out using pick and patch method on above plates (11).

Production of beta-lactamase:

Rapid iodometric method for beta-lactam antibiotics resistance isolates was performed as described previously (12).

Antibiotic susceptibility testing:

Susceptibility to antibiotics was studied by a disc diffusion method on Mueller-Hinton agar medium. The antibiotics tested were amoxicillin (25 µg), ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), aztreonam (30 µg), carbenicillin (100 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (30 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantion (300 µg), and tobromycin (10 µg) manufactured by Himedia, India. The

cultures were incubated at 37°C for 18h under aerobic conditions, and then bacterial growth inhibition zones around the discs were measured and compared with NCCLS guidelines (11). *E. coli* ATCC 25922 was used as the reference strain for antibiotic susceptibility testing.

PCR amplification

DNA was extracted from bacterial cells by using the Wizard Minipreps DNA kit (Promiga, USA). The DNA was then used as a template in specific PCR for the detection of bla_{TEM}, and bla_{SHV}. PCR amplification was performed by using the following primers (Promiga): TEM/F (5'-CGCCGGGTTATTCTTATTTGTCGC-3') and TEM / R (5' -TCTTTCCGATGCCGCCGCCAGTCA- 3'); SHV / F (5' -ATGAGTATTCAACATTTCCG-3') and SHV/R(5'-CCAATGCTTAATCAGTGAGG-3'). Cycling conditions were as follows: initial denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 1 minutes (TEM) and 30 second (SHV), annealing at 55°C for 1 minute (TEM) and 68°C for 30 second (SHV), and elongation 72°C at 1 minute (TEM) and 50 seconds (SHV). The final elongation step was extended to10 minutes at 72°C. The PCR products were separated on 1.5% agarose gels. Bands were visualized with UV-transilluminater, after being stained with ethidium bromide.

Screen for ESBL:

ESBLs were detected by the double disc synergy test (DDST). Synergy was determined between a disc of amoxicillin-clavulanate (20/10 µg) and a 30 µg disc of each ceftazidime, cefotaxime, and ceftriaxone placed at a distance of 30 mm apart on a lawn culture of the isolate under test on Mueller-Hinton agar. The test organism was considered to produce ESBL, if the zone size around the test antibiotic disc increased towards the amoxicillin-clavulanate disc (11).

Determination of minimum inhibitory concentrations (MICs)

MICs of cefotaxime, ceftazidime ceftriaxone, cephalothin pipracillin, amoxicillin, and ampicillin (Panpharm, France) were determined by the agar dilution method with Muller-Hinton agar containing graded concentrations of antibiotics. A 5- µl portion of an inoculums (approximately 10^6 CFU/ml) cultured to the logarithmic

phase of growth in Muller-Hinton broth (Biolife, Italy) was applied. Plates were incubated overnight at 37°C (13).

Transfer of resistance:

Mating experiments were performed as previously described (1), with *E. coli* MM294 (rifampicin resistant, obtained from Institute for Genetic Engineering and Biotechnology for Post Graduate Studies/Baghdad University) as recipient. Exponential cultures of clinical isolates as donor (1 vol) and recipient (2 vol) were inoculated as a spot on Brain Heart Infusion Agar (BHIA)(Biolife, Italy). After overnight incubation at 37°C, the bacteria were resuspended, diluted and plated onto BHIA containing relevant selective agents at the following concentrations: ceftazidime (2 µg/ml) and rifampicin (256 µg/ml). Transconjugants growing in the selection plates were subjected to antibiotics susceptibility, DDST, MIC, and PCR analysis.

Statistical analysis:

The χ^2 test was used for statistical analysis. $P<0.05$ was considered to be statistically significant.

Results

Among 25 isolates of *K. pneumoniae* obtained from patients with significant bacteria, 22 (88%) isolates were β-lactam resistance. Of which 13 (59.1%) were able to produce β-lactamases.

The activities of 17 antibiotics against 22 β-lactam resistance isolates are shown in Table (1). All isolates were found to be resistant to a minimum of 5 antibiotics tested. Hence they were considered to be multidrug resistant. However, the highest resistance rates were found for ampicillin, amoxicillin with 100% each, carbenicillin (90.9%), cephalothin (77.3%), and tobramycin (63.6%). Intermediate resistance rates were obtained for gentamycin, kanamycin, nalidixic acid, ciprofloxacin, trimethprim, nitrofurantoin with 59.1% each, and ceftriaxone (50%). The lowest resistance rates were observed for cefotaxime, chloramphenicol with 40.9% each, and ceftazidime (36.4%).On the other hand, 100% of isolates were susceptible to imipenem, which was the most effective drug.

All 13 β-lactamase producing isolates were tested by PCR using primers specific to *bla*$_{TEM}$ and *bla*$_{SHV}$. Two (15.4%) isolates were positive for *bla*$_{TEM}$ genes, 7(53.8%) isolates were positive for *bla*$_{SHV}$

genes, 2(15.4%) isolates had both *bla*$_{TEM}$ and *bla*$_{SHV}$ genes, and 2(15.4%) isolates had negative results (Table 2).

The 22 β-lactam resistant isolates were screened for ESBL production by DDST. Of this 5(22.7%) isolates were confirmed as ESBL producers. The isolates were able to enhance the inhibition zones of the cefotaxime, ceftazidime, ceftriaxone, and azetreonam on the site facing the amoxicillin-clavulanate disc, excluding one isolate was found to be resistant to the effect of cefotaxime (Figure 1) and (Table 3).

Among the ESBL-producing isolates 3 were positive by PCR for *bla*$_{SHV}$, 1 isolate was able to produce both TEM and SHV enzymes, and 1 isolate was negative with PCR primers for *bla*$_{TEM}$ and *bla*$_{SHV}$ genes (Table 3).

MICs of ESBL-producing isolates against seven β-lactam antibiotics were determined by a two-fold agar dilution method (Table 4).

Table (1) Antibiotic resistance pattern of β-lactam resistant *K. pneumoniae* (n=22).

Type of antibiotic	No. (%) of resistant isolates
Ampicillin	22 (100%)
Amoxicillin	22 (100%)
Ciprofloxacin	13 (59.1%)
Tobramycin	14 (63.6%)
Carbenicillin	20 (90.9%)
Gentamycin	13 (59.1%)
Nitrofurantoin	13 (59.1%)
Kanamycin	13 (59.1%)
Nalidaxic acid	13 (59.1%)
Trimethoprim	13 (59.1%)
Chloramphenicol	9 (40.9%)
Cephalothin	17 (77.3%)
Cefotaxime	9 (40.9%)
Ceftazidime	8 (36.4%)
Ceftriaxone	11 (50%)
Imipenem	0

Table (2) Frequency of TEM and SHV among 13 β-lactamase-producing *K. pneumoniae* isolates

Type of β-lactamase	No. (%) PCR positive test
TEM	2 (15.4)
SHV	7 (53.8)
TEM and SHV	2 (15.4)
Negative result	2 (15.4)

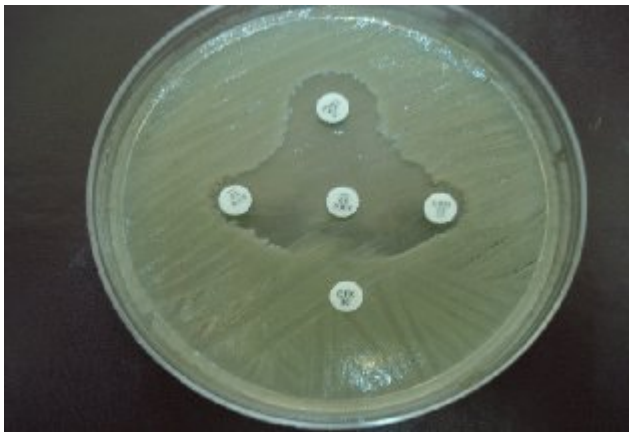

Figure (1) Response of ESBL-producing isolate of *K. pneumoniae* 26

with discs, 30 µg of cefotaxime (CTX, dawn), ceftazidime (CAZ, up), ceftriaxone (CRO, right), azetreonam (ATM, left) and amoxicillin-clavulanate (CAM, center).

Table (3) Phenotypic double disc synergy test for ESBL-producing *K. pneumoniae*

Type of isolate	CTX	CAZ	CRO	β-Lactamase production	Type of β-lactamase	
					bla_{TEM}	bla_{SHV}
K. pneumoniae 19	R	R	R	+	+	+
K. pneumoniae 41	R	R	R	+	-	+
K. pneumoniae 26	R	R	R	+	-	+
K. pneumoniae 39	S	R	R	+	-	+
K. pneumoniae 40	R	S	R	+	-	-

R, resistance; S, sensitive

Results show that all of the isolates displayed high level resistance to ampicillin, amoxicillin, pipracillin, and cephalothin with concentrations reached beyond the break point values. The MICs values of ampicillin, amoxicillin, and pipracillin for all ESBL producing isolates were >128 µg/ml, while the MIC values of cephalothin ranged from 64 µg/ml to >128 µg/ml. Three isolates were able to grow in concentration of cefotaxime equal to break point (64 µg/ml) and 2 isolates were able to grow in less than break point value (32µg/ml). The result also showed that MIC values of ceftazidime were ranged 16-64 µg/ml. For ceftriaxone, 100% of the isolates were resistant.

Table (4) MICs of selected β-lactam antibiotics for ESBL-producing *K. pneumoniae* isolates

Isolate	AMP≥32 µg/ml	MX≥32 µg/ml	PIP≥128 µg/ml	CEF≥32 µg/ml	CTX≥64 µg/ml	CAZ≥32 µg/ml	CRO≥64 µg/ml
K. pneumoniae 19	>128	>128	>128	>128	64	64	64
K. pneumoniae 39	>128	>128	>128	>128	32	64	64
K. pneumoniae 26	>128	>128	≥128	>64	32	64	64
K. pneumoniae 40	>128	>128	>128	>128	64	16	64
K. pneumoniae 41	>128	>128	>128	≥128	64	32	64

* Number between backets refer to break points recommended by NCCLS (2003b).
AMP, ampcillin;AMX, amoxicillin; PIP, piperacillin; CEF, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; CRO, cfetriaxon

Antibiotic resistance between ESBL-positive and ESBL-negative of 22 beta-lactam resistant isolates is compared in Table (5). Among the ESBL-producing isolates a significant proportion were found to be resistant to antibiotics. One ESBL-producing isolate were resistant to all antibiotic tested. However, ESBL-positive isolates were resistant to more antibiotics than non-ESBL-producing isolates. The resistance rates in ESBL-positive isolates to most antibiotics were significantly (P>0.05) higher than those of ESBL-negative isolates.

Table (6) shows antibiotics resistance pattern of original and transconjugation strains by revealing that the transferring of resistance to ampicillin, amoxicillin, cephalothin, trimethoprim, cefotaxime, ceftazidime, ceftriaxone, whereas the resistance to gentamycin was not transferred in only one isolate, while ciprofloxacillin was not transferred in all isolates.

The MIC to β-lactam antibiotics of transconjugants were detected. Table (7) revealed that these transconjugants were able to

grow in concentration ≤128 µg/ml of ampicillin and amoxicillin. Regarding pipracillin, all the transconjugants were able to grow in concentration less than break point (128 µg/ml). The MIC value of extanded spectrum β-lactam antibiotics reached to 64 µg/ml for cefotaxim, ceftriaxion and (32 µg/ml) for ceftazidime indicating that these isolates are still resistant to these antibiotics as their original isolates.

On the basis of PCR analysis of DNA, results indicate that *bla*$_{TEM}$ and *bla*$_{SHV}$ genes in the test isolates were encoded on the plasmid, which was shown transfer in conjugal mating experiments with recipient cells.

Table (5) The antibiotic resistance patterns of ESBL-producing and non-producing *K. pneumoniae* isolates to 16 antibiotics

Type of antibiotic	No. (%) of resistant isolates	
	ESBL(+) n=5	ESBL(-) n=17
Ampicillin	5 (100%)	17 (100%)
Amoxicillin	5(100%)	17 (100%)
Ciprofloxacin	4 (80%)	9 (52.9%)
Tobramycin	4 (80%)	10 (58.8%)
Carbencillin	5 (100%)	15 (88.2%)
Gentamycin	4 (80%)	9 (52.9%)
Nitrofurantoin	3 (60%)	9 (52.9%)
Kanamycin	4 (80%)	9 (52.9%)
Nalidaxic acid	4 (80%)	9 (52.9%)
Trimethoprim	4 (80%)	11(64.7%)
Chloramphenicol	2 (40%)	6 (35.3%)
Cephalothin	5 (100%)	12(70.6%)
Cefotaxime	4 (80%)	5 (29.4%)
Ceftazidime	4 (80%)	4 (23.5%)
Ceftriaxone	5 (100%)	7 (41.2%)
Imipenem	0	0

Table (6) Antibiotics disk resistant for transconjugants resulted from conjugation between ESBL-producing *K. pneumoniae* and standard strain *E. coli* MM294

Isolate	AMP	AMX	CIP	CEF	TE	CTX	CAZ	CRO	CAB	GN
Standard strain	S	S	S	S	S	S	S	S	S	S
K. pneumoniae. 19	R	R	R	R	R	R	R	R	R	R
K.pneumoniae.19 T	R	R	S	R	R	R	R	R	R	R
K. pneumoniae. 41	R	R	S	R	R	R	R	R	R	S
K. pneumoniae 41T	R	R	S	R	R	R	R	R	R	S
K. pneumoniae. 26	R	R	R	R	R	R	R	R	R	R
K.pneumoniae.26 T	R	R	S	R	R	R	R	R	R	R

AMP, ampcillin; AMX, amoxicillin;; CEF, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; CRO, cfetriaxone; GN, gentamicin; CAB, carbencillin; TE; tetracyclin

Table (7) MICs of β-lactam antibiotics for transconjugants resulted from conjugation between ESBL-producing *Klebsiella pneumoniae* and standard strain *E. coli* MM294

Isolate	AMP	AMX	PIP	CEF	CTX	CAZ	CRO
K. pneumoniae 41	>128	>128	>128	≥128	64	32	64
K. pneumoniae 41T	>128	>128	64	64	≥32	32	32
K. pneumoniae 19	>128	>128	>128	>128	64	64	64
K. pneumoniae 19 T	≥128	≥128	64	≥64	32	32	64
K. pneumoniae 26	>128	>128	≥128	>64	32	64	64
K. pneumoniae 26 T	≥128	≥128	32	64	32	32	32

AMP, ampcillin;AMX, amoxicillin; PIP, piperacillin; CEF, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; CRO, cfetriaxon.

Discussion

The emergence and dissemination of antibiotics resistance in bacteria has been well documented as a serious problem worldwide (14). Our results showed high rates of resistance in *K. pneumoniae* to β-lactam antibiotics (ampicillin and amoxicillin) (88%). This rate is higher than those given in Hilla, Iraq (73.8%) (10) and in Nigeria (66.7%) (15), while they are much lower than those reported in Tanzania (100%) (16). The mechanism of resistance to β-lactam antibiotics was mainly the production of β-lactamases, which was detected in 59.1% of resistant isolates, whereas 40.9% were failed to produce these enzymes. Although the β-lactamases undoubtedly play

a major role in the resistance to β-lactam antibiotics, the high ratio of resistance was not only attributable to the production of β-lactamases. The other mechanisms conferring resistance to these compounds is caused by reducing of the activity of β-lactam antibiotics in a resistant cell due to many factors such as; the sensitivity of the antibiotic to β-lactameses, the penetration through the outer membrane, the affinity for the target (PBPs), and the amount of β-lactamase (17).

In this investigation, 15.4% of β-lactamases producing *K. pneumoniae* gave PCR products with TEM-specific primers. The TEM β-lactamases spread worldwide and it is known to be found in many Enterobacteriaceae. However, *Klebsiella* spp. shows reduced susceptibility to first and second generation cephalosporins by the production of plasmid-mediated, TEM β-lactamase. Since 1980s, the emergence of resistance to third generation cephalosporins has been reported in strains of *K. pneumoniae* (18). The first ESBLs observed at the teaching hospitals of Clermont-Ferrand, France, in July 1984, the cefotaximase TEM-3/CTX-1 was produced by *K. pneumoniae* (19). In the United States, the enzymes which occur commonly in outbreak caused by *K. pneumoniae* are TEM-10, TEM-12, and TEM-26 (20).

The results in this study also showed that 69.2% out of the 13 β-lactamase-producing *K. pneumoniae* isolates yield amplification products with SHV-PCR specific primers. Most *K. pneumoniae* isolates have chromosomally or plasmid-mediated SHV-1 β-lactamase, which is a narrow-spectrum β-lactamase with activity against penicillins. More than 50 variants of SHV which are important worldwide and currently recognized on the basis of unique combination of amino acid replacement (21). SHV-2 and SHV-5 (plasmid-mediated β-lactamases) enzymes have been recorded in at least five countries, with the latter type widespread in Greece (22). In one study, Tasli and Bahar (23) found that 42 (46%) of the 91 *K. pneumoniae* strains were isolated from clinical samples in Turkey gave SHV-PCR- positive.

Among the 13 *K. pneumoniae* isolates produced β-lactamase, 15.4% isolates were unable to yield amplification products with TEM and SHV-PCR specific primers. Although the PCR results did not suggest that the TEM and SHV enzymes were present, it is possible that other enzymes such as AmpC, CTX-M, and OXA might be present in these isolates. In a recent study in the United States, it is estimated that in the 3-4% of clinical *K. pneumoniae* isolates carry plasmid-mediated AmpC enzymes (24).

The occurrence of ESBL-producing *K. pneumoniae* is a rapidly increasing problem in the world. These multiresistant bacteria pose a real therapeutic problem, with carbapenems the only intravenous antibiotics remaining consistently active for treatment in severe cases. The prevalence of ESBL-producing isolates varies from country to country and from species to species and also according to clinical samples (25, 26). The main goal of this study was to assess the impact of risk factors on the ESBL-producing *K. pneumoniae* isolates obtained from patient with significant bacteriuria. To our knowledge, the current study is the first report that focusing on the distribution of ESBL-producing *K. pneumoniae* in Najaf . The overall prevalence of ESBL-producing isolates (22.7%) was higher in the Najaf than in Hilla (10.5%) (27), United States (7.6%), Canada (4.9%) (28), and Sweden (3%), but lower than in Russia (nearly 50%) and Poland (nearly 40%) (29).

During the study period, 3 ESBL-producing isolates gave SHV-PCR-positive test. The first ESBL SHV enzyme was described in 1983 in clinical isolate of *K. pneumoniae* and because of its similarity to SHV-1, the new enzyme was named SHV-2. More than 50 varieties of SHV are recognized on the basis of unique combinations of amino acid replacement (30). In this study, one isolate of was able to produce both TEM and SHV enzymes. *Klebseilla* strains that produce 3 to 6 β-lactamases are commonplace in several countries (31). However, 1 isolate showed inhibition by clavulanate and β-lactamases active against cefotaxime, ceftazidime, ceftriaxone, and aztreonam but were negative with PCR primers for TEM and SHV genes. These are presumably other than TEM and SHV β-lactamases.

In this investigation, the MIC values of ceftriaxone were higher compared to those of cefotaxime and ceftazidime; this may be depending on the type of β-lactamase production. In a recent study, Tofteland *et al.* (32) reported that some of the minor *bla* genotypes showed clinically significant difference in MIC levels for different antibiotics, and that the *E. coli* TEM-128 had higher mean MIC for cefotaxime (9 µg/ml) than for ceftazidime (0.6 µg/ml) and aztreonam (1 µg/ml), while SHV-5 β-lactamase of *K. pneumonia* isolates produces higher ceftazidime MIC (256 µg/ml) than cefotaxime (64 µg/ml) and ceftriaxone (32 µg/ml).

In our study, the test isolates were able to enhance the inhibition zones of the cefotaxime, ceftazidime, ceftriaxone, and azetreonam on the site facing the amoxicillin-clavulanate disk, excluding one isolate was found to be resistant to the effect of

cefotaxime even in the presence of clavulanate. According to this result, the isolate may produce cefotaximases (CTX-M β-lactamases). These enzymes are similar to ESBL-hydrolysis broad spectrum cephalosporins and azetreonam (33). In contrast with TEM and SHV-type ESBLs, the CTX-M type enzymes display an enhanced activity against cefotaxime and ceftriaxone, but their activity against ceftazidime is significantly lower (34, 35). The presence of CTX-M-type ESBLs were described in various species of the family Enterobacteriaceae, most of them being plasmid encoded (33).

Organisms that express an ESBL are frequently resistant to other antibiotics, as many of these additional resistance genes are encoded on the ESBL-associated plasmid (35).

In this study, ESBL producing isolates were significantly more resistant to all antibiotics tested as compared to non-ESBLproducing isolates. other studies have reported on cross-resistance to aminoglycosides, fluoroquinoloes, and trimethoprim in ESBL producing organisms (36, 37). Mechanisms of coresistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antibiotics within the same conjugative plasmids (38).

From the present study, it appears that imipenem are the drug of choice for serious infections with ESBL-producing *K. pneumoniae* as has been recommended earlier (8). However, these should not be administered as empirical therapy for *Klebsiella* infections that are not life threatening because their overuse can pose a significant problem (9). Alternatively, ciprofloxacin, some aminoglycosides and nitrofurantoin may be used if they show in vitro activity against the isolates, leaving far behind the β-lactam- β-lactamase inhibitor combination. The situation may vary from region to region, so institutional local patterns of susceptibility should be used to determine the choice of antibiotics.

***Klebsiella* spp. have been an important source of tranferable antibiotic resistance (35). In present study, the resistance to ESBL antibiotics was transferred to the recipient strain along with resistance to gentamicin and other β-lactam antibiotics. ESBL-encoding genes are usually carried by large and transferable plasmids. Thus the plasmid localization of the genetic determinants facilitates their horizontal spread in bacterial populations, particularly by means of conjugation, accumulation of resistance genes results in strains that contain multiresistant plasmids(7). For**

this reason, such ESBL-producing multiresistant isolates pose a serious therapeutic problem in hospital setting.

Our study has shown the incidence of multidrug resistant and ESBL producing *K. pneumoniae* isolates among patients with significant bacteriuria. Hence it is suggested that, routine diagnosis of ESBL producing isolates should be done in hospitals in Najaf and more importantly, avoiding misuse and overuse of antibiotics may reverse the undesired effects of multidrug resistant and ESBL-producing bacteria.

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