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RESEARCH ARTICLE

Detection Of ESBL And AmpC β-Lactamases In Gram Negative Isolates From Some Iraqi Medical Centers In Baghdad

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Abstract

The aims of this study were to determine the prevalence of (ESBL) and (AMPC) β-lactamase production and their effect on antimicrobial susceptibility pattern by different phenotypic detection tests in gram negative clinical isolates from the patients admitted to some Iraqi medical centers in Baghdad. A total of 34 clinical isolates isolated from different clinical specimens from patients at different medical centers of Baghdad ,Iraq .The maximum number [12,35.29%]of isolates were of *Pseudomonas aeruginosa*, followed by those of [10,29.41%] *Escherichia coli* ,[7,20.58%] *Klebsiella pneumoniae* and[5,14.7%] *Acinetobacter baumannii*. And were tested for ESBL and AMPC production by screening test .15(44.1%),7(20.50%) were found to be ESBL and AMPC producers respectively.Amongst of these[7(70%) , 1(10%) *E.coli*] followed by [5(71.4%), 3(42.8%), *Klebsiella pneumonia*] and[3(60%) 3(60%) *Acinetobacter baumannii*] respectively. While the number of potential ESBL production were observed in 7(20.5%)isolates only by double disk synergy test (DDST).The highest positivity was found to be in 6(60%) , *E.coli* followed by 1(14.2%) *K.pneumoniae* . And increased the numbers to10(29.4%)isolates were positive for ESBL by Combined disk method amongst these 6(60%) of *E.coli* and 4(57.1%) *Klebsiella pneumonia*. (CDT was found to be better than DDST in the detection of ESBL).But lower the numbers to 3(8.82%) isolates were positive for AMPC by Combined disk method amongst these of 1(10%) *E.coli* and 2(28.5%) *Klebsiella pneumoniae*. Organism wise distribution of 7 pure ESBL isolates showed 5 *E.coli*(71.4%) and *2 Klebsiella pneumoniae* (28.5%) while isolates show3 mixed ESBL and AMPC producers *E.coli* 1 (33.3%), *Klebsiella pneumonia* 2(66.6%) . Detection of ESBL in the presence of AMPC β-lactamases were determined ,the co-existence phenotype of both ESBL and AMPC in3(8.82%) of which 1(10%) and 2(28.5%) isolates were *E.coli* and *K.pneumoniae* respectively. and 24(70.5%) isolates didn't show any evidence of the production of β-lactamases. 3(8.82%) isolates were positive for both ESBLs and AmpC by Combined disk method. A higher rate of ESBL and ESBL andAMPC production were seen among isolates from urine as compared to other sources .And they were significantly multidrug resistant (MDR) with uniform sensitivity to imipenem.

Introduction

The emergence and spread of antibiotic-resistant bacteria causing infection is of great concern to clinicians ,spreading of resistance to commonly used antibiotics in both human and animal populations has posed adverse impact on morbidity and mortality due to diseases caused by resistant bacteria(Bonomo and Szabo,2006). Gram negative bacteria are the common pathogens causing wide spread infections, both nosocomial and community acquired. In the Gram negative bacteria, one of the important mechanisms of resistance is production of β-lactamases (Kolar *et al*.,2010).There are more than 340 different beta lactamases so far identified and the growth spurt shows no signs of slowing (Sharma *et al*., 2012).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 Beta 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 (Quale *et al*.,2002).

The detection of extended-spectrum β -lactamase-producing (ESBL) bacteria is of importance for infection control and epidemiological surveillance (Garrec *et al*.,2011) . Since ESBL positive isolates show false susceptibility to expanded spectrum cephalosporins in standard disk diffusion tests, it is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Specific detection methods such as double disk potentiation methods recommended by (National Committee for Clinical Laboratory Standards) NCCLS have to be adopted. ESBL are inhibited by β- lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBL (Lytsy *et al*., 2008).Phenotypic methods can be divided into screening tests and confirmatory tests .The confirmatory step is based on the synergy between cephalosporins and clavulanic acid(Patrso and Bonomo2005) . *Ambler classC* (AmpC) β-lactamase production is one of the mechanisms of resistance to β-lactam antibiotics in Gram negative bacteria conferring resistance to a wide variety of β-lactam antibiotics including 7- α-methoxy cephalosporins (cefoxitin or cefotetan), oxyimino cephalosporins (cefotaxime, ceftazidime, ceftriaxone), monobactam (aztreonam) and are not inhibited by clavulanic acid(Parveen *et al*.,2012). Distinguishing between the AmpC and the ESBL producing organisms has epidemiological significance and it may have a therapeutic importance as well , the AmpC producing organisms can act as hidden reservoirs for ESBL. *Enterobacteriaceae* which produce both AmpC and ESBL have been increasingly reported worldwide. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBL (Pitout *et al*.,2003). The aims of this study were to determine the prevalence of (ESBL) and (AMPC) β-lactamase production and their effect on antimicrobial susceptibility pattern by different phenotypic detection tests in gram negative clinical isolates from the patients admitted to some Iraqi medical centers in Baghdad.

Material and Methods

Clinical isolates: Different clinical specimens such as urine(n= 18), wound swabs (n=9), ear(n=2), blood(n=4), sputum(n=1) ,were obtained from patients admitted to Some Iraqi medical centers in Baghdad over a period of four months (January to April, 2012). The samples were processed and isolates were identified by standard biochemical methods (Winn *et al*.,2006).

Antibiotic susceptibility testing : The isolates were subjected to antimicrobial susceptibility testing using Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines (12) , using commercially available 6mm disks (Bioanalyse(Ankara/Turkey) The susceptibility of the isolates was determined against 19 antibacterial agents by disk diffusion method, They include: cefoxitin(FOX) (30µg), cefoxitin/boronic acid (FOX/BA)(30/400ug) ,cefotaxime (CTX)(10µg), ceftriaxone (CRO) (30µg), ceftazidime (CAZ) (30µg), cefixime (CFM) (5ug), ceftazi-dime/clavulanic acid (CZC) (30ug/10), cefipime (FEP)(30µg), imipenem (IPM) (10µg),carbenicillin (PY) (25ug), aztreonam (ATM) (30µg),amoxicillin (AX) (25ug), amoxycillin/ clavulanic acid (AMC) (20/10µg), pipercillin (PRL) (30µg), ciprofloxacin(CIP) (5µg), netilmicin (NET) (30ug), nalidixic acid (NA) (30ug), gentamicin (CN) (10ug), rifampin(RA)(5ug),On Mueller Hinton agar Plate (Lab M Limited Topley House, United Kingdom),using overnight culture at a 0.5 McFarland standard followsed by incubation at 35 ^{o}c for 16 to 18 h. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes.

Screening for ESBL and AmpC β-lactamases: According to CLSI guidelines (12), those with a zone inhibition of ≤18mm with cefotaxime, ≤18mm with ceftriaxone, ≤16 mm with ceftazidime, ≤18mm with aztreonam were considered potential ESBL producers and further proceeded for confirmation.

Isolates showing resistance or reduced sensitivity to cefoxitin, cefotaxime, ceftriaxone, ceftazidime, aztreonam and sensitive to cefepime were considered as a screen positive AmpC producer and subjected to combined disk diffusion test .

Detection of ESBL and AmpC β-lactamases:

Tests for ESBL production

1. Double disk synergy test (DDST):

Isolates were screened for ESBL production by the double-disk potentiation method (Jarlicr *et al*.,1980). A 0.5 McFarland of test isolate was swabbed on Mueller-Hinton agar plate and disks containing the standard ceftazidime, cefotaxime, and ceftriaxone 30 µg each were placed at a distance of 15 mm (edge to edge) from a centrally placed an amoxicillin clavulanic acid disk (20 and 10 ug, respectively) (Coudron *et al*.,1997). And incubated at 35°C for

18-24 hours, an enhanced zone of inhibition between any one of the β -lactam disks and the clavulanic acid disk was interpreted as presumptive evidence for the presence of an ESBL. The organisms which were screened and found positive for ESBL production were subjected to confirmatory test.

2. Combined disk test CDT (Phenotypic confirmatory test):
A disk of ceftazidime (30µg) alone and a disk of ceftazidime + clavulanic acid (30µg/10µg) were placed independently, 30mm apart, on a lawn culture of 0.5 Mc-Farland opacity of the test isolate on Mueller Hinton Agar (MHA) plate and incubated for 18-24 hours at 35°C. An increase of ≥5mm zone of inhibition diameter around the ceftazidime/clavulanic acid in comparison to ceftazidime confirmed ESBL production (Coudron *et al*.,1997; Bhattac,2006).

3. Combined disk diffusion test: The differences in inhibition zones for cefoxitin (30 µg) disk alone and in combination with (400 µg) of phenyl boronic acid was determined. An increase of >5 mm in zone diameter in the presence of phenyl boronic acid compared with cefoxitin tested alone was considered to be positive for the presence of an AmpC β-lactamase production(Perez-Perez and Hanson, 2002).

Statistical Analysis: Significance between the resistance level of various drugs in ESBL and AmpC and non ESBLs & AmpC isolates was performed using the Proportion test (Z). and the *P value* is < 0.05.(Mahaja, 1999).

Results

Of the various clinical samples tested during the study period, a total of 34 isolates were obtained. Out of 34 isolates, 18(52.9%) were from urine, 9 (26.4%)from wound swabs, 1(2.9%) from sputum, 2(5.8%) from ear and 4(11.7%) were from blood. Among 34 isolates, 12(35.29%) were *Pseudomonas aeruginosa*, 10(29.41%), were *Escherichia coli*,7(20.58%)were *Klebsiella pneumoniae*,5(14.7%) were *Acinetobacter baumannii* [Table1].

Table 1: clinical isolates isolated from various clinical specimens during this study

					Frequency	**Percentage%**
Bacterial species	Urine	Wound swabs	Sputum	Blood	Number(34)	
Pseudomonas aeruginos	1	4	1	4	12	35.29
Escherichia coli	10	-	-	-	10	29.41
Klebsiella pneumoniae	7	-	-	-	7	20.58
Acinetobacter baumann	-	5	-	-	5	14.7
Totale=34	18 (52.9%)	9 (26.4%)	1 (2.9%)	4 (11.7%)	34	(100%)

The resistance patterns for the ESBLs and AMPC producer isolates and non producer isolates are shown in [Table 2]. All ESBLs producer isolates were resistant 100% to amoxicillin, amoxycillin/ clavulanic acid, pipercillin, carbenicillin, ceftazidime, cefotaxime, ceftriaxone, cefixime , aztreonam and rifampin. Other resistance rates were: 80% cefipime, 60% gentamicin ,50% nalidixic acid&ciprofloxacin,40% netilmicin,30% cefoxitin but they were susceptible 100 % to cefoxitin/boronic acid, ceftazidime/clavulanic acid and Imipeneme. On the other hand, All ESBLs and AMPC producer isolates 100% were resistant to both β-lactam and non β-lactam (gentamicin ,ciprofloxacin and rifampin).Other resistance rates were: 66.6% nalidixic acid, 33.3% netilmicin, while the minimum resistance were seen with cefoxitin/boronic acid, ceftazidime/clavulanic acid and Imipeneme (0%).

The (Non-ESBL and AMPC) producer isolates were tested for susceptibility to antibiotics and showed a higher degree resistance to rifampin 87%, nalidixic acid 58% followed by amoxicillin, amoxycillin/ clavulanic acid, carbenicillin and cefixime (29.16% each).

In the present study, reflected that multidrug resistance was significantly (P < 0.05) higher in ESBL β-lactamase producers than non-ESBL and non- AmpC producers. Resistance to cefoxitin(30%) , and aztreonam (100 %) were observed in most of the ESBL producers. Interestingly, ESBL and ESBL and AmpC producers also showed concurrent resistant to netilmicin (40%,33.3%), gentamicin(60%,100%) nalidixic acid (50,66.6%), ciprofloxacin (50%,100%) and rifampin (100%each) respectively.

Table 2: Antimicrobial resistance of ESBL and AMPC producers and non producers of clinical isolates.

	ESBL isolates(n=10) **CDT***	ESBL and AMPC isolates (n=3) **CDT***	Non-ESBL and AMPC isolates (n=24)	***P value***
Antibiotic	Resistant %	Resistant %	Resistant %	**< 0.05**
AX	(10)100%	(3)100%	(7)29.16%	**<0.05**
AMC	(10)100%	(3)100%	(7)29.16%	**<0.05**
PRL	(10)100%	(3)100%	(6)25.00%	**<0.05**
PY	(10)100%	(3)100%	(7)29.16%	**<0.05**
FOX	(3)30%	(3)100%	(4)16.60%	**<0.05**
FOX/BA	(0)0.00%	(0)0.00%	(4) 16.60%	**
CAZ	(10)100%	(3)100%	(5)20.80%	**<0.05**
CZC	(0)0.00%	(0)0.0%	(5)20.80%	**
CTX	(10)100%	(3)100%	(4)16.60%	**<0.05**
CRO	(10)100%	(3)100%	(4)16.60%	**<0.05**
CFM	(10)100%	(3)100%	(7)29.16%	**<0.05**
FEP	(8)80%	(3)100%	(5)20.80%	**<0.05**
ATM	(10)100%	(3)100%	(4)16.60%	**<0.05**
IPM	**(0)0.00%**	**(0) 0.00%**	**(0)0.00%**	**
NET	(4)40%	(1)33.3%	(1)4.16%	**<0.05**
CN	(6)60%	(3)100%	(4)16.60%	**<0.05**
NA	(5)50%	(2)66.6%	(14)58.33%	**<0.05**
CIP	(5)50%	(3)100%	(4)16.60%	**<0.05**
RA	(10)100%	(3)100%	(21)87.50%	**<0.05**

CDT* Combined disk test

**** Comparison could not be made**

AX =amoxicillin, AMC =amoxycillin/ clavulanic acid, PRL =pipercillin, PY =carbenicillin, FOX= cefoxitin, FOX/BA=cefoxitin/boronic acid, CAZ =ceftazidime, CZC ceftazidime/clavulanic acid ,CTX =cefotaxime , CRO =ceftriaxone, CFM cefixime, FEP cefipime, ATM= aztreonam, IPM= imipenem , NET =netilmicin, CN =gentamicin, NA =nalidixic acid, CIP =ciprofloxacin, RA =rifampin .

The number of potential ESBLs and AMPC β-lactamase producers detected by screening test were 15(44.1%)and 7(20.50%) respectively.

The double disk synergy test (DDST) method was used to detect the production of ESBL. Results showed that ESBL production was observed in 7(20.5%)isolates by (DDST) amongst these 6(60%) and 1(14.2%) isolates were *E.coli* and *K.pneumoniae* respectively [figure1] and reduction test from 15(44.1%) screening positive isolates .

The remaining 8 isolates (1-*E.coli*, 4- *K.pneumoniae* and 3- *A.baumannii*) exhibited a high degree of resistance to third-generation cephalosporins but did not produce ESBL or could be co- producers of AmpC hence giving false positive results.

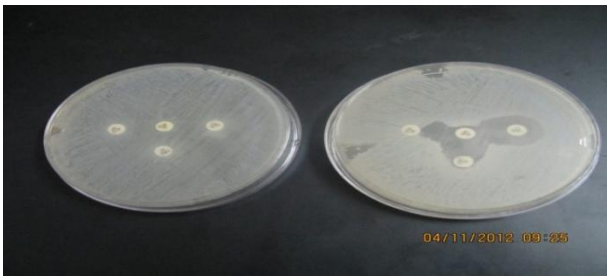

Figure 1. Positive double-disk synergy tests DDST between a disk containing clavulanic acid (AMC) (20/10µg) and a disk containing an extended-spectrum cephalosporin (3G),an enhanced zone of inhibition between any one of the β -lactam disks and the clavulanic acid disk was interpreted as presumptive evidence for the presence of an ESBL in *Klebsiella pneumoniae*

And results were confirmed by the presence of a zone of inhibition around a disk containing a combination of the cephalosporin (ceftazidime) and clavulanic acid when compared with the zone around a disk containing the cephalosporin (ceftazidime) alone[figure2]. Only 10 (29.4%) were found to be true ESBL producers . Out of 10, 6 isolates(60%) were *E.coli* followed by 4(57.1%) were *K.pneumoniae* . A higher rate of ESBL 10(55.5%) production was seen among isolates from urine. maximum numbers of ESBL production 6(33.3%) *E.coli* followed by 4(22.2%) *K. pneumoniae.*The remaining 24 isolates (4 *E.coli*, 3 *Klebsiella pneumoniae* , 5 *Acinetobacter baumannii*, 12 *Pseudomonas aeruginosa*)which were negative by phenotypic confirmatory method. [Table3].

Table 3: ESBLs production by clinical isolates included in this study.

Bacterial species (number)	Number (%) of positive isolates screening(CLSI)	Number (%) of positive isolates DDST*	Number (%) of positive isolates CDT**	Final + result	Final- result
E.coli (n=10)	(7) 70.00%	(6)60.00%	(6)60.00%	(6) 60.00%	(4) 40.00%
K.pneumoniae (n=7)	(5) 71.40%	(1)14.20%	(4)57.10%	(4) 57.10%	(3)42.80%
A.baumannii (n=5)	(3) 60.00%	(0) 0.00%	(0) 0.00%	(0) 0.00%	(5)100%
P.aeruginosa (n=12)	(0) 0.00%	(0) 0.00%	(0) 0.00%	(0)0.00 %	(12)100%
Total=34	(15)44.1%	(7)20.5%	(10)29.4%	(10) 29.4%	(24)70.5%

DDST* Double Disk Synergy Test
CDT** Combined Disk Test

Figure 2: Positive Phenotypic confirmatory test (combination disk method) for detection of ESBL , Muller – Hinton agar plate showing an isolate *Klebsiella pneumoniae* resistant to ceftazidime (CAZ) (30µg) and along with an increase zone of inhibition around ceftazidime clavulanic acid (CZC) (30ug/10) .

Seven(20.50%) isolates were found to be resistanse to cefoxitin by screening test**,** Only 3 (42.8%) isolates were found to be true AMPC β-lactamase producers by a phenyl boronic acid . Out of 3, 2 isolates (28.5%) were *K.pneumoniae* followed by 1(10%) were *E.coli* [figure3].Remaining 4(57.1%) were non AMPC β-lactamase producers. A higher rate of AMPC β-lactamase 3(16.6%) production was seen among isolates from urine. maximum numbers AMPC production 2(11.11%) *K. pneumoniae* followed by 1(5.55%) *E.coli* .The remaining 27(79.4%)isolates (9 *E.coli*, 4 *Klebsiella pneumoniae* , 2 *Acinetobacter baumannii*, 12 *Pseudomonas aeruginosa*) which were negative. [Table4].

Figure 3: Positive Phenotypic confirmatory test (combination disk method) for detection of AMPC, Muller – Hinton agar plate showing an isolate *Klebsiella pneumoniae* resistant to cefoxitin(FOX) (30µg) and along with an increase zone of inhibition around cefoxitin/boronic acid(FOX/BA)(30/400ug).

Table 4: AMPC β-lactamases production by clinical isolates included in the study

	Number (%) of positive isolates Screening(CLSI)	Number (%) of positive isolates CDT*	Number (%) of negative isolates CDT*
Bacterial species(number)		AMPC final+ve	AMPC final-ve
E.coli(n=10)	1(10%)	1(10%)	9(90%)
K.pneumoniae(n=7)	3(42.80%)	2(28.5%)	5(71.4%)
A.baumannii(n=5)	3(60%)	0(0%)	5(100%)
P.aeruginosa(n=12)	0(0%)	0(0%)	12(100%)
Total(n=34)	7(20.5%)	3(8.82%)	31(91.17%)

CDT * Combined Disk Test

Detection of ESBL in the presence of AMPC β-lactamases were determined ,the co-existence phenotype of both ESBL and AMPC in3(8.28%) of which 1(10%) and 2(28.5%) isolates were *E.coli* and *K.pneumoniae* respectively [Table 5] .

Table 5: ESBL and AMPC β-lactamases production by clinical isolates included in the study

	Number (%) of positive isolates **CDT***
Bacterial species(number)	ESBL&AMPC β-lactamases
E.coli(n=10)	1(10%)
K.pneumoniae(n=7)	2(28.5%)
A.baumannii(n=5)	0(0%)
P.aeruginosa(n=12)	0(0%)
Total(n=34)	3(8.82%)

CDT * Combined Disk Test

DISCUSSION:

Urinary tract infections (UTIs) is one of the most common infection is an important cause of morbidity and mortality in human(Crain and Gerschel 1990).The most common bacteria responsible for of UTIs in this study were found to be *E. coli* 10(55.5%)and *Klebsiella pneumoniae* 7(38%) followed by *Pseudomonas aeruginosa* 1(5.55%)**.** The result of this study showed Enterobacteriaceae isolates were the dominant bacterial species isolated from urine cultures .The findings were supported by another study (Kadri *et al*.,2002 ; Zafar *et al*.,2006)**.**

The prevalence of ESBL-producing enteobacterial isolates evaluated in the present study 10(55.5%) were seen among isolates from urine. maximum numbers of ESBL production 6(33.3%) *E.coli* followed by 4(22.2%) *K. pneumoniae* compared to other bacterial species tested . Based on the results of the current study, the prevelance of ESBL producing isolates of *E.coli* and *K. pneumoniae* are higher in our country in comparison with USA and Canada (Altayar *et al*.,2012 ; Lowe *et al*.,2012).But was lower than that have studied from India, nearly 40% urinary isolates of *E. coli* and *K. pneumoniae* were ESBL positive(Babypa and Appalaraju 2004).

Ejaz *et al.* (2011) reported ESBL producing 57% *E. coli* and 71.7% *K. pneumoniae* from the patients suffering from urinary tract infections. The findings of our study are nearly to other studies in case of ESBL producing *E. coli*(57.4%) while the number of ESBL producing *K. pneumonia* was lower in our study as compared to others.

Antimicrobial resistant bacteria and their resistance determinants are considered hazards in antimicrobial resistance risk assessment. Therefore, the information obtained could be used as part of risk analysis of the distribution and development of antimicrobial resistance. However, data regarding the situations of antimicrobial resistance and mechanism basis of resistance is still limited especially in most developing countries (Chuanc and Padungtod ,2009).

All ESBL producer isolates*(E. coli* and *K. pneumoniae*) showed higher resistance were seen against amoxicillin, amoxycillin/ clavulanic acid, pipercillin, carbenicillin, ceftazidime, cefotaxime, ceftriaxone, cefixime , aztreonam and rifampin (100% each) but resistance rate were 80% cefipime. The resistant rate to 3rd and 4th generation cephalosporins and monobactam ranged from (80%

to 100%) ; however no resistance was detected in the combination of 3rd generation cephalosporins and clavulanic acid. This result indicated that the resistance to 3rd generation cephalosporins was due to the production of extended-spectrum β- lactamases (ESBL).And patients infected with these strains cannot be treated with β -lactam antimicrobial agents and monobactams.

These results are similar from those of a study conducted in Pakistan , where higher numbers of ESBL producing *E. coli* and *K. pneumonia* showed higher resistance to ceftazidime, cefotaxime, cefixime (Ejazh *et al*.,2011).

These results are different from those of a study conducted in Pakistan, where higher numbers of ESBL producing *E. coli* and *K. pneumonia* showed higher resistance to nalidixic acid&ciprofloxacin and a lower numbers of ESBL producing *E. coli* and *K. pneumoniae* showed lower resistance to amoxycillin/ clavulanic acid, gentamicin. However no resistance was detected in the combination of 3rd generation cephalosporins and clavulanic acid.

This result indicated that the resistance to 3rd generation cephalosporins was due to the production of extended-spectrum β- lactamases (ESBL).Similar finding was reported by AL-gamy *et al*.(2012).

The result of this study showed Carbapenems (imipenem) and beta-lactam/beta-lactamase inhibitor Combinations are the most effective drugs against uropathogenic *E. coli*. *K. pneumoniae* from the patients suffering from urinary tract infections. The findings were supported by another study (Jaitly *et al*.,2012)**.**

But the sensitivity of ESBL producers against carbapenems should not lead to excessive use of such drugs in clinical practice(Bush and Medeiros 1995).ESBL producers are associated with increased morbidity and mortality. The majority of ESBL producing *E. coli* and *K. pneumoniae* were resistant to the common antibiotics used in the treatment of urinary tract infections. The early detection and reporting of suitable antibiotics can reduce the treatment failure in ESBL UTI.(Ejazh *et al*.,2011).

ESBL production by bacteria is an important threat to clinical therapeutics. These organisms elaborate plasmid-encoded β-lactamases, a variety of which have been described among members of the family Enterobacteriaceae. First described in 1983, ESBL producers have contributed to the dramatic increase in recent years to resistance among gram-negative bacteria to β-lactam agents. Plasmid-borne genes code for enzymes that hydrolyze penicillins, cephalosporins, and aztreonam, and are inhibited by clavulanic acid (Altayar *et al*.,2012).

The presence of a high percentage of isolates producing ESBLs which are resistant to different antibacterial agents may result in treatment failure in infected patients. Careful detection of antimicrobial resistant strains is needed in order to avoid underestimation or misidentification of ESBLs. An effective hospital infection control policy is also necessary in order to prevent further resistance to antimicrobials in the region(Mansou *et al*.,2004)

These results are similar from those of a study conducted in Iran , where 80%*Acintobacter* spp.exhibited high resistance to third- generation cephalosporins but did not produce ESBL. These results suggest the intrinsic resistance to antibiotics in non-ESBL producing isolates may be due to mutation(s) in the genome involved in antibiotic susceptibility (Shakbai, 2012).

Based on the results of the current study, the 15 (44.1%)of ESBL producing isolates showed resistance to cefotaxime, ceftriaxone, ceftazidime and aztreonam(100%each).The double disk synergy test (DDST) allowed for the detection of 7(20.5%) of the ESBL producing isolates, while the combined disk test(CDT) was able to detect 10(29.4%)of the ESBLisolates. In the present study,CDT was found to be better than DDST in the detection of ESBL .Other workers have reported (DDST)showed 100% concordance with (CDT)for ESBLdetection(Singh *et al*.,2012).

The phenotypic expression of plasmid mediated ESBL producing strains is resistant to 3th generation cephalosporines(3GC). β-lactamase inhibitors such as clavulanic acid can restore susceptibility to inactive cephalosporin(Lal *et al*.,2007).

cefoxitin resistance was seen in 7/34(20.5%) isolates .Only 3/7(42.8%)showed ≥5mm zone enhancement with addition of phenylboronic acid to cefoxitin .4/7(57.1%)showed resistance to cefoxitin but, did not show enhancement of zone with addition of phenylboronic acid .In all such isolates, cefoxitin resistance may be due to mechanism other than AMPC production. Rudresh *etal.* (Rudres and Nagarathnamma, 2011)reported that sensitivity of FOX-PB method was found to be 85% for AMPC detection. But the present study showed a sensitivity of 42.8% for AMPC detection using FOX- PB disk potentiation method.

The inhibitor based confirmatory method, used in this study , proved helpful to detect(AMPC) β-lactamase enzymes,the most common mechanism of resistance in gram negative bacilli after the ESBL production.When both enzymes are present together

can mask the phenotype of each other. So the detection becomes very difficult. The inhibitor based method detects when both enzymes are present simultaneously and it is easy to perform.The results are helpful to formulate an empirical therapy in different clinical situations(Patel *et al*.,2010).

The co-existence of ESBL and AmpC found in 10% *E.coli* and 28.5% of *Klebsiella pneumonea*. could be due to dissemination of plasmid encoding both AmpC and ESBL enzymes among *Enterobacteriaceae* and thus might give false negative tests for the detection of ESBL. In such situations, it is desirable to develop an ESBL detection test that includes a substrate displaying a higher degree of resistance to AmpC enzymes like cefepime. Singhal *et al* (2005) observed the co-existence phenomenon in two (1%) isolates (one each of *Klebsiella* spp. and *E. coli*) .

Regular monitoring of the incidence of the β-lactamase production by the organisms is necessary. Along with the detection of ESBL, it is necessary to detect AmpC β-lactamases, as they can act as hidden reservoirs for the ESBLs *Enterobacteriaceae* which produce both AmpC and ESBLs have been increasingly reported worldwide. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs. To prevent the spread of the β-lactamase producing strains, hospitals must have functional hospital infection control committee with an appropriate hospital antibiotic policy, with regular updates(Jaitly *et al*.,2012).

(CDT) should be performed routinely to clinical microbiological laboratories to detect (ESBL) and (AMPC) β-lactamase producers so that suitable antimicrobial therapy can be instituted. some of the (ESBL) producers also produced (AMPC) β-lactamase ,the inhibitor based test was useful in identifying cefoxitin susceptible (AMPC) producers and could also effectively differentiate (ESBL) and(AMPC) producing isolates. ESBL and AMPC β-lactamase producing bacterial pathogens may cause a major therapeutic failure if not detected.

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