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Evaluation of multi drug resistance among extended spectrum β-lactamase-producing Escherichia coli causing urinary tract infection in Erbil City

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Abstract

Background and objective: Bacterial resistant to broad spectrum β-lactams, which is mediated by the extended spectrum beta lactamase enzyme, has emerged recently as increasing problem. Extended spectrum beta lactamase producing strains can also displaying multi-drug resistance. Thus, increased number of infections due to these strains is a public health issue associated with high morbidity, mortality, high health-care costs and prolonged hospitalization. Therefore, this study aimed to evaluate multi-drug resistance among extended spectrum beta lactamase producing E. coli causing urinary tract infections.

Methods: A total of 400 mid-stream urine specimens were collected from patients with urinary tract infection. Disk diffusion agar method on Muller-Hinton agar plates was carried out. Double Disc Synergy Test was used for detection of extended spectrum beta lactamase producer. All the isolates that were screened out for extended spectrum beta lactamase production were also subjected to confirmation by using the Phenotypic Confirmatory Combination Disc Diffusion Test.

Results: The urinary tract infection cases were mainly due to Gram negative bacteria (87%). E. coli was isolated from 195 (48%) specimens. Sixty isolates of E. coli (31%) were found to be extended spectrum beta lactamase producers. The resistance to antibiotics tested was significantly higher ($P < 0.001$) among extended spectrum beta lactamase producing E. coli isolates compared with non-extended spectrum beta lactamase producing isolates.

Conclusion: The prevalence of multi-drug resistance to the antibiotics among extended spectrum beta lactamase producing E. coli isolates was established. Imipenems are recommended for the treatment of serious infections caused by these organisms.

Keywords: E. coli, Eextended spectrum β-lactamase, Multi-drug resistance, DDST, PCDDT, Urinary tract infection.

Introduction

Extended spectrum beta lactamases (ESBL) enzymes were first described in Germany in 1983 from Klebsiella pneumonia. ESBL enzymes are usually plasmid mediated and gain broad resistance to cephalosporins: (cefotaxime, ceftazidime, ceftriaxone), and monobactams (aztreonam).[1] A new class of ESBL, called CTX-M enzymes, has emerged during late 1990 and early 2000s, which was widely detected among Escherichia coli (E. coli) isolates. These ESBL-producing E. coli are able to resist penicillins, cephalosporins and are found mostly in urinary tract infections (UTI).[2,3] ESBL-producing strains can also display multi-drug resistance (MDR), including resistance to aminoglycosides and fluoroquinolones. Therefore, therapeutic options for these strains are limited.[4-6] Continuous exposure of such bacterial strains to ß-lactams could induce mutation and production of new ß-lactamases, expand their activity even against the fourth generation cephalosporins. Thus, these new ß-lactamases are called extended spectrum ß-lactamases.[7] ESBL-producing

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organisms have been found most commonly in uropathogens like K. pneumoniae and E. coli. Other enterobacteria and non-fermenting Gram negative rods also produce ESBLs but to a lesser extent.[8,9] In recent years, increasing ESBL- producers have been found in outpatient UTI, which aid the spreading isolates of MDR,[10] which is defined as resistance to at least three classes of antimicrobial agents.[11] Infections caused by MDR Gram negative bacteria have been associated with increased morbidity and mortality.[12] It has been found that morbidity and mortality have increased also among UTI patients, especially those treated by inadequate antibiotics against ESBLs-producing E. coli. Thus, rapid detection of these isolates is essential for effective treatment.[13] Various outbreaks of infection with ESBLs have been observed in many countries. These organisms are considered the main causative agents of nosocomial infections that lead to prolonged hospital stay.[3] Studies reported detection of ESBL-producing Enterobacteriaceae in low rates of 3-8% in Sweden, Japan and Singapore compared to much higher prevalence rates documented in studies from Portugal (34%), followed by Italy (37%), USA (44%), Latin American countries (30-60%) and Turkey (58%). While detection rates ranged from 8.5-38.5% have been found in the Kingdom of Saudi Arabia and (31.7%) in Kuwait; the highest level of 41% is from the United Arab Emirates.[14] This study aimed to evaluate the prevalence of MDR among ESBL-producing and non-ESBL producing E. coli isolated from urine cultures in patients attending Rezgari Teaching Hospital in Erbil City.

Methods

Specimen collection and bacterial isolates

This study was conducted at Rizgary Teaching Hospital in Erbil City, Iraqi Kurdistan Region, during the period from October 2012 to August 2013. A total of 400 mid-stream urine specimens were collected from patients suffering from UTI. Urine culture was done using standard wire loop 0.001 ml. It was streaked on 5% sheep blood agar and MacConkey agar plates. The inoculated plates were incubated aerobically at 37°C for 24-48 hrs.[15] Identification was done on the basis of colony morphology, Indole test, Simmon's citrate agar and standard biochemical tests. Identification of isolates was confirmed using API-20 E test (bioMerieux). Only significant bacterial growth (>10^5 cfu/ml) were included in the study.[5]

Antimicrobial Susceptibility Test (AST)

Disk diffusion agar method on Muller-Hinton agar (MHA) plate was achieved. The results were interpreted according to Clinical Laboratory and Standards Institute (CLSI) guidelines.[16] The following antimicrobials from (Rashmi Diagnostic Ltd., India) were tested: ampicillin (10µg), cefixime (30µg), gentamycin (10µg), cotrimoxazole (25µg), ciprofloxacin (10µg), amoxicillin/clavulanic acid (amoxiclav) (20/10 µg), aztreonam (30µg), ceftriaxone (30µg), ceftazidime (30µg), cefotaxim (30µg), nitrofurantion(10µg), piperacillin (25µg), and imipenem (10µg). All *E. coli* isolates that showed resistant to at least three different classes of antimicrobial agents were determined as MDR.[5]

Detection of ESBL isolate

National Committee of Clinical Laboratory Slandered (NCCLS) screening test

Isolates showing an inhibition zone size of ≤22 mm to ceftazidime (30µg), ≤25 mm to cefriaxone (30µg) and ≤27 mm with cefotaxime (30µg) were identified as potential ESBL-producing isolates and were short-listed for confirmation of ESBL- production.

Double Disk Synergy Test (DDST)

All isolates showing resistance to one or more of the third generation cephalosporins were tested for ESBL-production, using a double disk synergy test (DDST) as a standard disk-diffusion assay on MHA plates. The amoxicillin/ clavulanic acid (20/10µg) was placed in the

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center of the plate, and the following disks of β-lactam antibiotics were placed 15mm apart (edge to edge) from the center in order to observe the synergistic effect: cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg) and aztreonam (30µg). Following incubation, a clear extension of the edge of the inhibition zone of cephalosporin toward the augmenting disk was interpreted as positive for ESBL production.[17,18]

Phenotypic Confirmatory Combination Disk Diffusion Test (PCDDT)

All the isolates that were screened out for ESBL-production were also subjected to confirmation by using the PCDDT.[1,17] The disks of cefotaxime (30µg), cefotaxime/clavulanic acid (20/10µg), ceftazidime (30µg) and ceftazidime / clavulanic acid (20/10µg) were placed on the MHA plates. Following incubation, a ≥ 5mm increase in diameter of the inhibition zone of the cephalosporin/ clavulanic disk when compared to the cephalosporin disc alone were interpreted as phenotypic evidence of ESBL-production.[19]

Statistical analysis

The mean ±SD of antibiotic resistance were estimated and the paired sample t-test was used to compare these means. P-value <0.05 was considered statistically

Results

Clinical isolates

A total of 400 urine specimens were included in the study. Majority (87%) of UTI were due to Gram negative bacteria, while remaining 13% were due to Gram positive bacteria. E. coli was present in 195 (48%) of specimens, representing the most common isolates.

Detection of ESBLs

Of 195 clinical isolates of E. coli, only 60 (31%) showed to be ESBL-producers, while 135 (69%) were non ESBL-producers. DDST could detect only 55 ESBL-producers, whereas a total of 60 ESBL were detected by PCDDT. The sensitivity and specificity of DDST were 91.66% and 96.26%, respectively as shown in Table 1.

Table 1: Sensitivity and specificity of DDST in comparison with PCDDT for detection of ESBL-producing E. coli.

Detection of ESBL-Producing-E.coli (n=195)	PCDDT positive		PCDDT negative		Total
	n=60	(%)	n=135	(%)	
DDST positive	55	(91.66%)	5	(3.73%)	60
DDST negative	5	(8.33%)	130	(96.26%)	135
Total	60	(100%)	135	(100%)	195

Sensitivity: 91.66% & Specificity: 96.26%

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Antibiotic susceptibility

All ESBL-producing E. coli were 100% resistant to ampicillin, while (98%) and (96%) showed resistant to ceftazidime and cefotaxime, respectively. All isolates were sensitive (100%) to imipenem. Non ESBL-producing E. coli exhibited high resistance to ampicillin (85%), followed by amoxiclave (81%), and piperacillin (50%), while all were sensitive to imipenem (Figure 1). ESBL-producing *E. coli* could display significant resistance (P <0.001) to 13 types of antibiotics compared with Non-ESBL-producers as shown in Table 2.

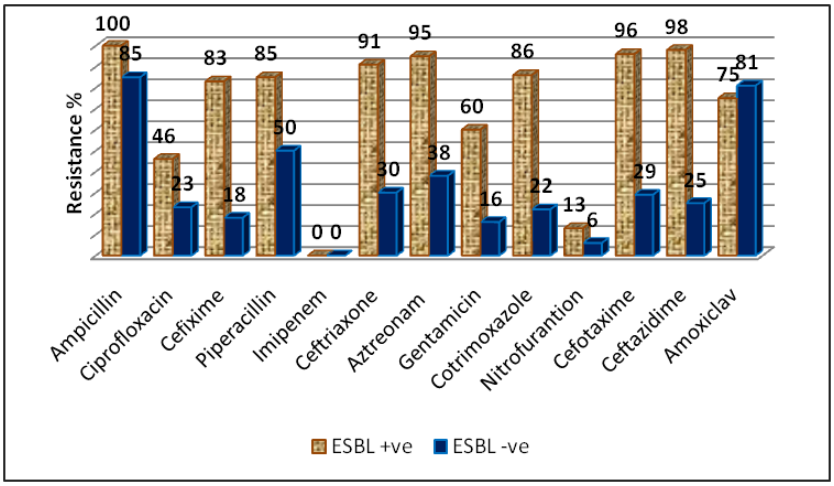

Figure 1: The percentage of resistance among ESBL-producers and non ESBL-producers E. coli isolates.

Table 2: Number and percentage of antimicrobial resistant of both ESBL-producers and non ESBL-producing E. coli isolates.

Antibiotics	ESBL-Producer n=60	Mean±SD	Non-ESBL-Producer n=135	Mean±SD
Ampicillin	60(100%)	99.73±0.64*	115(85%)	85.07±0.80*
Ciprofloxacin	28(46%)	45.38±0.63*	32(23%)	23.30±0.62*
Cefixime	50(83%)	83.48±1.02*	25(18%)	18.115±0.33*
Piperacillin	51(85%)	85.22±1.02*	68(50%)	50.27±0.067*
Imipenem	0	0	0	0
Ceftriaxone	55(91%)	91.48±0.89*	41(30%)	31.0±1.0*
Aztreonam	57(95%)	95.26±1.08*	52(38%)	38.16±0.9*
Gentamicin	30(60%)	60.34±1.41*	21(16%)	16.32±0.58*
Cotrimoxazole	52(86%)	86.4±1.32*	30(22%)	22.21±1.2*
Nitrofurantion	8(13%)	13.13±1.15*	9(6%)	6.17±1.04*
Cefotaxime	58(96%)	96.18±1.2**	40(29%)	29.18±1.07**
Ceftazidime	59(98%)	98.19±1.18*	35(25%)	25.44±1.45*
Amoxiclav	45(75%)	75.33±1.18**	110(81%)	81.10±0.97**

All data were expressed as mean ± SD; the mean difference is significant at (**) p < 0.01 and (*) at p < 0.001

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Multi-drug resistance was found to be greater among ESBL-producing isolates, showing resistance against 12 types of antibiotics as summarized in Figure 2.

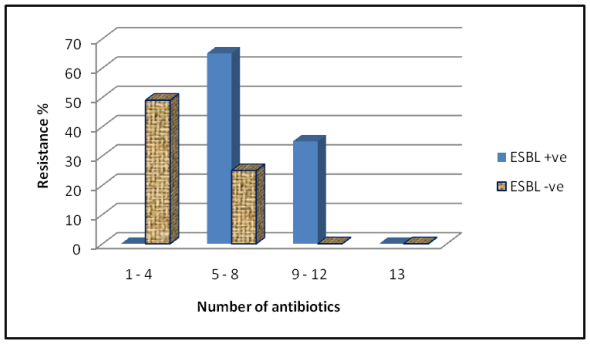

Figure 2: The percentage of MDR pattern (%) among clinical isolates of E. coli with ESBL and non ESBL-producers.

Discussion

This study showed the bacterial isolates involved in UTIs were mainly Gram negative bacteria (87%); the significant growth for E. coli was present in 48% of urine specimen. It is believed that uropathogenic E. coli reside in the colon, and are later introduced through the urethra to the bladder.[20] This work was designed to examine all E. coli strains isolated from urine specimens to study the prevalence of ESBL-producing isolates, since these pathogens are now recognized worldwide as most important causative agents of nosocomial and community-acquired infections.[14] ESBLs-producing E. coli represented 31%. Similar rates were reported from Kufa City, the southern Iraq (38%) and Iran (35%).[21] A study from Turkey also reported a rate of 25% of infection with ESBL-producing Gram negative bacteria.[22] The antimicrobial resistance displayed various results. All ESBL-producing E. coli were significantly resistant to 3rd and 4th generations cephalosporins: ceftazidime (98%), cefotaxime (96%), ceftriaxone (91%), and cefixime (83%). These results are consistent with the related studies in the same field.[7,13,23] Furthermore, ESBL-producing E. coli was highly resistant to amoxiclav (75%). These finding might be due to long term uses as empirical therapy to UTIs. Multi-drug resistant isolates showed to be greater among ESBL-producing E. coli than non ESBL-producers. Similar findings were reported in a number of recent studies.[7,13,24] In fact, ESBL-producing organisms of the family Enterobacteriaceae were primarily considered MDR initiated in hospitals. In recent years, an increase of such ESBL-producers has been observed in outpatient settings, especially related to UTIs, reducing the treatment options to a limited number of antibiotics.[10] Treatment of the infections caused by ESBL-producing organisms is not easy. This is because of the resistance to the extended spectrum cephalosporins themselves, and also associated with resistance to other antimicrobial agents coded by plasmids.[25] Therefore, an increase in the number of infections due to MDR organisms is a public health issue associated with high morbidity, mortality, high health-care cost and prolonged hospitalization.[19] In view of the fact that ESBL-producing organisms are MDR, therapeutic selections for these infections are limited. In recent years, imipenem has been recommended for treatment of infections caused by ESBL-producing organisms. Because of increasing incidence and pathogenic significance of ESBL-producing E. coli in community and nosocomial infections, gathering additional data on clinical risk factors for these isolates are recommended.[26]

Conclusion

Prevalence of MDR to the antibiotics among ESBL-producing E. coli isolates was established. The study suggests regular screening and surveillance for ESBL-producing E. coli. Imipenems are recommended for the treatment of serious infections caused by these organisms.

Conflicts of interest

The authors report no conflicts of interest.

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