Comparison of Antibiotic Sensitivity between Extended Spectrum Beta Lactamase Producers and ESBL Negative Escherichia coli Clinical Isolates.

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Abstract:

Background: Recurrent urinary tract infection [UTI] and treatment failure were common challenges in the control of UTI in Iraqi community.
Objective: To determine the difference in antibiogram and multidrug resistance between ESBL positive and negative *E. coli* clinical isolates.
Materials and methods: prospective cross-sectional study was conducted during the period from 1st of June 2015 to the end of January 2016. The study population was 563 women, of them 425 [75.5%] were outpatients, and 138 [24.5%] were inpatients. Their age range was between 18 and 80 years, with a mean age of 33.59±15.29 years. Urine samples were immediately cultured on blood agar and MacConkey agar by spread plate technique. Bacterial colonies with different morphology were selected, purified and identified according to their biochemical characteristics using conventional standard methods.
Results: The rate of resistance was higher in ESBL positive as compared to ESBL negative producers *E. coli* isolates for all tested antibiotics. A high rate of resistance was demonstrated by most of the tested antibiotics. A low resistance rate in both ESBL positive and negative *E. coli* were demonstrated against amikacin, imipenem and nitrofurantoin**.** ESBL producer E. coli isolates were resistant to ≥5 of MDR in 98% of isolates [92/94], while the corresponding value was 71% [29/41], while MDR to ≥7 was 56% [53/94] in ESBL producer and 17% [7/41] in ESBL none producer *E. coli.*
Conclusion: ESBL producing was of significant influence on the emergence of resistance in E. coli clinical isolates.
Key words: ESBL, E. coli, Antibiotic resistance, UTI.

Introduction

Antibiotic resistance has significant health impact and contribute to treatment failure of bacterial infections including urinary tract infections [1-3]. Many factors may play a role in the emergence of antibiotic resistance which may include host and organism factors [4-7]. One of the risk factors related to the causative agents of UTI is the ability of the bacteria to produce extended spectrum beta lactamase [ESBL] [8-14]. The ESBL producing organisms are with global distribution, and they increased with time and varied geographically [15-20] . Additionally, study population is influenced aetiology of UTI and antibiotic susceptibility and response to treatment [21,22]. Thus this study was conducted to determine the association between ESBL *E. coli* producer clinical isolates and the frequency of antibiotic resistance in different population.

Materials and methods

Study design:

A prospective cross-sectional study is conducted during the period from 1st of June 2015 to the end of January 2016. The population included in the study was 563 women, of them 425, [75.5%], were outpatients, and 138, [24.5%], were inpatients. Their age range was between 18 and 80 years, with a mean age of 33.59±15.29 years. The study proposal was approved by the Ethical Committee of College of Science, Tikrit University and a verbal informed consent was taken from each woman before enrolment in the study.

Bacterial isolation:

Urine samples were centrifuged and the sediments were immediately cultured on blood agar and MacConkey agar by spread plate technique. Bacterial colonies with different morphology were selected, purified and identified according to their biochemical characteristics using conventional standard methods [23]. The antibiotic susceptibility test based on formation of zones of inhibition of bacterial growth in a Muller- Hinton agar medium as a result of diffusion of the antibiotic agent from discs holding specific quantities of them reflecting the degree of sensitivity of the bacterium under test. Beta Lactamase activity of the isolates was determined using rapid iodometric method [24].

Statistical analysis:

Statistical analysis was performed using SPSS [version20]. The data were presented as percentages, mean value and standard deviation. Chi square used to calculate significance of frequency, while t test was used to determine significance in mean difference. P value of < 0.05 is regarded significant.

Results

The rate of resistance was higher in ESBL positive as compared to ESBL negative producers *E. coli* isolates for all tested antibiotics. The differences were significant for Amoxicillin-Clav (X^2 =4.31, P=0.035), piperacillin (X^2 =4.53, P=0.027), ceftriaxone (X^2 =5.75, P=0.015), cefprozil (X^2 =9.62, P=0.003), ceftazidime (X^2 =4.28, P=0.039), amikacin (X^2 =3.71, P=0.05), tobramycin (X^2 =10.95, P=0.001), gentamicin (X^2 =5.10, P=0.021), tetracycline (X^2 =4.84, P=0.022), ciprofloxacin (X^2 =5.29, P=0.017), norfloxacin (X^2 =5.98, P=0.011), nalidixic acid (X^2 =5.10, P=0.021), and Aztreonam (X^2 =6.31, P=0.011). However, a low resistance rate in both ESBL positive and negative *E. coli* were demonstrated against amikacin, imipenem and nitrofurantoin, Table (1).

The mean value of multiple antibiotic resistant index [MARI] mean was higher in ESBL producer *E. coli,* [0.60±27], than in ESBL negative, [0.46±0.21] isolates. However, this difference was not significant, [t=1.91, P>0.05]. In addition, 36.4% [8/22] of MARI in ESBL positive isolates were with value of ≥0.75, while the corresponding value was 9.1% [2/22] in ESBL negative *E. coli* isolates. Furthermore, 17/22 [77.27%] were higher in ESBL positive than in ESBL negative isolates, Table (2).

In pregnant women, the MDR mean value was significantly (t=4.26, P=0.0001) higher in ESBL producer *E. coli* (6.38±1.13) than that in ESBL negative *E. coli* (5.09±1.19). In addition, in diabetic women, the MDR mean value was significantly (t=3.41, P=0.0013) higher in ESBL producer *E. coli* (6.92±0.98) than that in ESBL negative *E. coli* (5.75±1.22). Furthermore, in female student, the MDR mean value was significantly (t=2.26, P=0.0363) higher in ESBL producer *E. coli* (6.62±1.04) than that in ESBL negative *E. coli* ([5.43±1.27). Also, in the pool of the three groups , the MDR mean value was significantly (t=6.18, P<0.0001) higher in ESBL producer *E.coli* (6.64±1.08) than that in ESBL negative *Escherichia coli* ESBL (5.34±1.22), Table (4). ESBL producer E. coli isolates were resistant to ≥5 of MDR in 98% [92/94], of isolates, while the corresponding value was 71% [29/41],

while MDR to ≥7 was demonstrated in 56% [53/94] of the ESBL producer and 17% [7/41] in ESBL none producer *E. coli*, Table 4.

Discussion

Antimicrobial resistance is a global health problem with worldwide clinical 14substantial burden and their rate increased with time [25]. ESBL producers bacterial isolates demonstrated higher resistance rate to antibiotics as compared to ESBL negative isolates [26-31]. Although the rate of resistance was higher in ESBL positive as compared to ESBL negative producers *E. coli* isolates for all tested antibiotics, however, the differences were significant for Amoxicillin-Clav and pipercillin from penicillin group, while the differences in susceptibility did not reach significant level for ampicillin and carbencillin. These finding indicated that susceptibility of *E. coli* to amoxicillin-Clav and piperacillin are more influenced by ESBL activity of the isolates. ESBL producer *E. coli* isolates were with significantly higher resistance rate than ESBL negative to ceftriaxone, cefprozil, and ceftazidime from the cephalosporin group. However, ESBL producer *E. coli* isolates show higher resistance rate to cefotaxime and cefixime but the differences were not significant. While both ESBL positive and negative *E. coli* were with about the same resistance rate to cefaclor. The above findings indicated healthcare problem since both penicillin and carbencillin groups of antibiotics form the first and second line treatment approach for UTI. The present study findings were consistent with that reported by others which indicated a significant high resistance in ESBL positive *E. coli* as compared to ESBL negative isolates [32-34]. The significant reduction in sensitivity of ESBL producer *E. coli* as compared to ESBL negative isolates for ceftazidime and cefotaxime was consistent to that reported by Abdel-Moaty *et al*.[32], but did not agreed for amoxicillin-Clav. However, other study from Egypt [35], found a significant higher resistance rate to pipercillin in ESBL positive as compared to ESBL negative isolates, a finding consistent with the present study. Ejaz *et al*. [36], Pakistan, reported that ESBL positive *E. coli* demonstrated higher resistance against cefotaxime, cefuroxime and ceftazidime and lower resistance rate against pipercillin/ tazobactam.

The ESBL producer *E. coli* was implicated in community and hospital acquired infection and thus limited the treatment options of the infections induced by such bacteria [26]. The extent of resistance rate that was demonstrated in this study of ESBL *E. coli* isolates was consistent with previous reports [26-31].

In aminoglycosides, the resistance rate to amikacin, tobramycin and gentamycin was significantly higher in ESBL producer *E. coli* than in ESBL negative isolates. This did not agree with others in regard to gentamicin susceptibility between ESBL positive and negative isolates [32], while agreed with others in regard to amikacin [35], and tobramycin [37] susceptibility. Al-Otaibi *et al*. [38], found that ESBL producer *E. coli* were significantly highly resistant to ciprofloxacin and third generation cephalosporin. Another study from Bangladesh, reported high resistance rate of ESBL positive *E. coli* against amikacin and gentamicin [30].

ESBL positive *E. coli* was with significantly higher resistance rate to tetracycline and aziteonam than ESBL negative isolates. ESBL positive E. coli isolates were higher resistant to cotrimoxazole than ESBL negative isolates and this was in line of previous studies [26,30,31,32,35,36,38]. The resistance rate was significantly higher in ESBL producer *E. coli* than that of negative isolates to ciprofloxacin, norfloxacin and nalidixic acid of the quinolone antibiotics group. This finding was consistent to that reported by some [30,35,36,37] but not to others [32]. Both ESBL *E. coli* positive and negative isolates demonstrated the same resistance rate against gemifloxacin.

Low resistance rate in both ESBL positive and negative *E. coli* were demonstrated against amikacin, imipenem and nitrofurantoin. This finding was consistent with Ejaz et al

study [36] in regard to nitrofurantoin, but not for amikacin as they reported resistance rate of 46.5%, while in this study the corresponding value was 8%. In addition, Islam et al [30] reported high resistance rate of ESBL positive *E. coli* against amikacin, while other studies reported low rate of resistance [35,38]. Concerning imipenem, all the studies [30,31,32,37,38] indicated a low resistance rate in both ESBL positive and negative isolates suggesting it recommendation as empirical treatment for serious and non-responding UTI. Mekki *et al* .[39], reported that ESBL producing *E.coli* show resistance rate of 100% to nalidixic acid, nitrofurantoin, co-trimoxazole, and gentamycin, 97.96% to ciprofloxacin, 95.9% to cefuroxime and 69.39% to amikacin.

In a recent study that performed in Erbil, Iraq, ESBL producing *E. coli* shows maximum resistance to Ampicillin, Cefazolin, Cefepime, Cefotaxime, Tetracycline, Mezlocilin, Piperacillin, Cefuroxime (100% for each) and ceftazidime (93.7%) while minimum resistance was to Ertapenem (3.1%), Tigecycline (3.1%), Fosfomycin (3.1%), Imipenem (6.2%), Amikacin (9.6%), and Nitrofurantoin (9.6%) [8].

The MARI mean was higher in ESBL producer *E. coli than* that in ESBL negative indicating a reduction in ESBL positive *E. coli* susceptibility to antibiotics. With the exception of amikacin, imipenem, nitrofurantoin and gemifloxacin, MARI ranged from 0.47 to 0.89 in ESBL producer *E. coli*. The higher rank of MARI in ESBL positive *E. coli* was 0.76 for cephalosporins group, followed by 0.73 for penicillin group, 0.71 for azitreonam, 0.64 for trimethoprim, 0.59 for tetracycline, 0.55 for aminoglycosides, 0.51 for quinolone group, 0.02 for nitrofurantoin and imipenem.

In ESBL negative *E. coli,* the higher rank of MARI was 0.61 in both penicillin and cephalosporins groups, followed by 0.49 for azithromycin and trimethoprim; 0.39 for tetracycline, 0.37 for aminoglycosides, 0.34 for quinolones, and 0.15 for nitrofurantoin and imipenem. Thus in both ESBL positive and negative *E. coli* isolates nitrofurantoin, imipenem and amikacin from aminoglycosides group were with very low MARI, indicating their effectiveness in the treatment of UTI in women caused by *E. coli.*

The MDR was significantly more frequent in ESBL producer *E.coli* than in ESBL negative *E. coli* in pregnant, diabetic women and female student group and when the data of the 3 groups pooled together. The present study indicated that *E. coli* ESBL producing isolated show higher frequency of MDR as compared to ESBL negative isolates. However, ESBL negative isolates demonstrated MDR frequency of ≥5 antibiotic groups in 71% [29/41], while ESBL positive showed MDR to ≥5 antibiotic groups in 98% [92/94]. Additionally, the present study showed 9 patterns of MDR and thus MDR is an extensive problem in urinary tract infections in our study cohort. In a recent study in Erbil, Iraq [8], MDR was more predominant in ESBL positive *E. coli* than in ESBL negative isolates and 71.89% of ESBL positive isolates show MDR of ≥5 antibiotic groups, while the corresponding value in ESBL negative was 40%. Cruz *et al*. [29], 2014, Philippines, reported that MDR to 4 antibiotic groups, while Chakrawarti *et al.,[19]*, 2015, Nepal, reported nine MDR patterns for *E. coli* isolates. In addition, Aka and Haji, [40], 2015, Erbil, Iraq, reported that they found MDR more frequent in ESBL positive *E. coli* isolates; however, their data are multiple antibiotic resistance number and not MDR trend. Other studies [41-45] reported that MDR isolates were ESBL producers. In order to reduce the health impact of ESBL producing urinary isolates and to control the increased prevalence of antimicrobial agents' resistance and MDR, and extensive intervention required to develop guidelines for antibiotics prescription and improve prescription process to reduce resistance rate and support the improvement of UTI management.

In conclusion, ESBL producing was with significant influence on antibiotic resistance emergence in E. coli clinical isolates.

Table (1). Susceptibility of ESBL Positive and Negative *E. coli* Isolates.

Antibiotic	ESBL Positive (94) Number [%]	ESBL Negative (41) Number [%]	X^2	P value
Amoxicillin-Clav	79 [84.04]	28 [68.29]	4.31	0.035
Ampicillin	83 [88.30]	35 [85.37]	0.22	>0.05
Piperacillin	64 [68.09]	20 [48.78]	4.53	0.027
Carbencillin	50 [53.19]	17 [41.46]	1.57	>0.05
Ceftriaxone	72 [76.60]	23 [56.10]	5.75	0.015
Cefotaxime	54 [57.45]	18 [43.90]	2.10	>0.05
Cefixime	75 [79.79]	28 [68.29]	2.09	>0.05
Cefprozil	80 [85.10]	25 [60.97]	9.62	0.003
Cefaclor	61 [64.89]	26 [63.41]	0.03	>0.05
Ceftazidime	84 [89.36]	31 [75.61]	4.28	0.039
Amikacin	8 [08.51]	0 [00.00]	3.71	0.05
Tobramycin	78 [82.98]	23 [56.10]	10.95	0.001
Gentamycin	69 [73.40]	22 [53.66]	5.10	0.021
Tetracycline	56 [59.57]	16 [39.02]	4.84	0.022
Ciprofloxacin	50 [53.19]	13 [31.71]	5.29	0.017
Norfloxacin	44 [46.81]	10 [24.39]	5.98	0.011
Gemifloxacin	24 [25.53]	10 [24.39]	0.20	>0.05
Nalidixic acid	71 [75.53]	23 [56.10]	5.10	0.021
Aztreonam	67 [71.28]	20 [48.78]	6.31	0.011
Nitrofurantoin	17 [18.10]	6 [14.63]	0.24	>0.05
Imipenem	2 [02.13]	0 [00.00]	0.88	>0.05
Trimethoprim	60 [63.83]	20 [48.78]	2.68	>0.05

Table (2). Multiple Antibiotic Resistance Index [MAR Index] for *E. coli* Isolates.

Antibiotic		ESBL Positive MAR Index	ESBL Negative MAR Index
Group	Drug		
Penicillin	Amoxicillin	0.84	0.68
	Ampicillin	0.88	0.85
	Piperacillin	0.68	0.49
	Carbencillin	0.53	0.41
Cephalosporins	Ceftriaxone	0.77	0.56
	Cefotaxime	0.57	0.44
	Cefixime	0.80	0.68
	Cefprozil	0.85	0.61
	Cefaclor	0.65	0.63
	Ceftazidime	0.89	0.75
Aminoglycosides	Amikacin	0.08	0.00
	Tobramycin	0.83	0.56
	Gentamycin	0.73	0.54
Tetracycline	Tetracycline	0.59	0.39
Quinolones	Ciprofloxacin	0.53	0.32
	Norfloxacin	0.47	0.24
	Gemifloxacin	0.26	0.24
	Nalidixic acid	0.76	0.56
Monobactams	Aztreonam	0.71	0.49
Nitrofurantoin	Nitrofurantoin	0.02	0.15
Carbapenems	Imipenem	0.02	0.15
Trimethoprim	Trimethoprim	0.64	0.49
Mean ± SD		0.60± 0.27	0.46± 0.21
t Value	1.91		
P Value	>0.05		

Table (3). Multiple Antibiotic Resistance Index Frequency of *E. coli* Isolates

MAR Index	ESBL Positive Number [%]	ESBL Negative Number [%]	Total Number [%]
< 0.1	3 [13.64]	1 [04.55]	4 [09.10]
0.1- 0.19	0 [00.00]	2 [09.10]	2 [04.55]
0.2 – 0.29	1 [04.55]	2 [09.10]	3 [06.82]
0.3 – 0.39	0 [00.00]	2 [09.10]	2 [04.55]
0.4 - 0.49	1 [04.55]	5 [22.73]	6 [13.64]
0.5 – 0.59	4 [18.18]	4 [18.18]	8 [18.18]
0.6 – 0.69	3 [13.64]	4 [18.18]	7 [15.91]
0.7 – 0.79	4 [18.18]	1 [04.55]	5 [11.36]
0.8 – 0.89	6 [27.27]	1 [04.55]	7 [15.91]

$X^2 = 13.9$, P >0.05

Table (4). Multidrug Resistance Frequency in *E. coli* Isolates

No. Of Drug	ESBL Positive				ESBL Negative			
	Pregnant	Diabetic	Student	Total	Pregnant	Diabetic	Student	Total
3	0	0	0	0	1	0	1	2
4	2	0	0	2	7	3	0	10
5	7	3	2	12	6	1	2	9
6	13	10	4	27	6	4	3	13
7	14	14	4	32	1	4	1	6
8	5	11	3	19	1	0	0	1
9	1	1	0	2	0	0	0	0
Mean± SD	6.38 ±1.13	6.92 ±0.98	6.62 ±1.04	6.64 ±1.08	5.09 ±1.19	5.75 ±1.22	5.43 ±1.27	5.34 ±1.22
Total	42	39	13	94	22	12	7	41
t	4.26	3.41	2.26	6.18				
P	0.0001	0.0013	0.0363	<0.0001				

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