ANTIBIOTIC RESISTANCE PATTERNS OF MULTIDRUG RESISTANT AND EXTENDED-SPECTRUM B-LACTAMASE PRODUCING ESHCHERICHIA COLI URINARY ISOLATES AT QUEEN RANIA AL-ABDULLAH HOSPITALFOR CHILDREN, JORDAN

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ABSTRACT

To determine the prevalence and the antibiotic resistant patterns of the multi-drug resistant Extended-Spectrum B-Lactamase(ESBL) producing E. coli isolates from children urine samples, in Queen Rania Al-Abdullah Hospital for children.A total of 61 non-repetitive urine samples from various outpatient clinics and inpatient wards were collected retrospectively over a period of 5 months (May 2012 to September 2012). The resistant patterns, screening and confirmatory tests for phenotypic detection of ESBL-producers were studied using the VITEK 2 system against a set of antibiotics found on the antimicrobial susceptibility extend card AST-EXN8.Children were nearly equally infected by both types of E. coli isolates, ESBL-producers 31 (50.8%) and non ESBL-producers 30 (49.2%). ESBL-producing E. coli showed maximum rate resistance to Cefuroxime and Piperacillin (100%), Aztreonam, Cefixime, Ceftriaxone plus Levofloxacin (96.8%), Ampicillin/Sulbactam and Cefepime (93.5%), and Moxifloxacin (90.3%), while minimum resistance rate was seen with Tigecycline (12.9%), Colistin (3.2%) and meropenem (0%). ESBL-producing isolates were significantly more resistant than Non-ESBL-producers ($p < 0.05$) to the following antimicrobials (Ampicillin/Sulbactam, Aztreonam, Cefepime, Cefixime, Ceftriaxone, Levofloxacin, Moxifloxacin, Piperacillin and Tetracycline). Multi-drug resistance was found to be higher in ESBL-producing isolates, which were resistant to at least 9 antibiotics. To limit the spread of the multi-drug resistant ESBL-producers E. coli isolates, we should perform screening test for these isolates on daily basis, isolate the infected patients and choose the best therapeutic option.According to the resistant pattern and safety issue, Morepenem can be considered as first line treatment and colistin as last resort therapy.

Keywords: Resistant patterns, E. coli, MDR,ESBL, UTI, children, VITEK 2, AST-EXN8.

Key Message:
Due to rapid emerge of ESBL producing uropathogens over the last decade,we believe it's now a mandatory to perform screening and confirmatory tests for detection of those microorganisms in our daily routine work, to choose the best therapeutic option to limit or even prevent their spread within our community.

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamases enzymes belongs to group 2be produced by Gram negative Enterobacteriaceae (such as Klebsiellaspp and Escherichia coli).[1] Due to rapid emerge of ESBL producing uropathogens over the last decade the antimicrobial susceptibility profile have been changed dramatically. [2-5] ß-lactams antimicrobial agents are among the most widely used antibiotics to treat those community and hospital acquired infections. [6, 7] All ESBLs producers share the resistant to all generations of cephalosporins, penicillins, and aztreonam (except for cephamycins or carbapenems) and inhibited by clavulanic acid.[8-12]

Community acquired or nosocomial Urinary Tract Infections (UTI) are one of the common bacterial infections in childhood period. [13,14] ESBL-producers isolates can lead to UTI that range from uncomplicated to life threatening UTI in both developed and developing countries.[15,17] Morbidity and mortality usually increased when subjects with UTI were treated by antibiotics with inadequate in vitro activity against these ESBLs producing isolates, for that a rapid and accurate detection of these isolates is essential for effective treatment.[18,19] The increasing prevalence of UTI caused by ESBL-producing E. coli worldwide makes empirical treatment by conventional and newer antimicrobial agents is quite difficult[5, 19-21] The aims of this study were to determine the prevalence and antibiotic resistant patterns of ESBL-producing E. coli isolates from urine cultures, in Queen Rania Al-Abdullah Hospital (QRAH) for children, King Hussein Medical Center, Amman-Jordan, using the VITEK 2 system.

SUBJECTS AND METHODS

Bacterialisolates

In a retrospective study, A total of sixty one non-repetitive urine samples which were obtained from various outpatient clinics and inpatient wards of QRAH for children over a period of 5 months (May 2012 to September 2012). All samples which collected where send to the Department of Microbiology of Princess Iman Center for Research and Laboratory Sciences for

identification and characterization. Only one strain per patient was used and cultures with single strain were included in this study.This study was approved by the Ethical Committee of the Royal Medical Services in Jordan.

Antimicrobial Susceptibility Test

In united state, the FDA had approved four automated systems for rapid identification of the bacterial isolates and evaluation of their antimicrobial susceptibility, including screening and detection of ESBL-producers. These include The VITEK 2 System (bioMérieux, Marcy l´Etoile, France), the MicroScanWalkAway, The Sensititre ARIS 2X, and The BD Phoenix Automated Microbiology System.[22,23]VITEK 2 system with the advanced expert system (AES) has a high Sensitivity and specificity values (94-100%) that considered a rapid and reliable for routine laboratory work.[18, 24-33]

VITEK 2 system usually uses different Antimicrobial Susceptibility Test cards (AST-cards) according to the type of isolates we expect or studied, where the resistance of the isolates to various classes of antibiotics included was determined in accordance to the manufacture's recommendations. The following antibiotic were included in the AST-ENX8 card which we used in this study , Ampicillin/Sulbactam (SAM), Aztreonam (ATM), Cefepime (FEP), Cefixime (CFM), Cefrtiaxone (CRO), Cefuroxime (CXM), Chloramphenicol (C). Colistin (CS), levofloxacin (LEV), Meropenem (MEM), Minocycline (MNO), Moxifloxacin (MXF), piperacillin (PIP), Tetracycline (TE), Tigecycline (TGC), Trimethoprim (TMP), and ESBL test [3 paired sets of cephalosporin with and without clavulanic acid (CA)for ESBL detection; Cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ) (FEP/ FEP+CA ; CTX/ CTX+CA and CAZ/ CAZ+CA)]. (9). Quality control isolate strains (E. coli ATCC25922 andE. coli ATCC 35218) were included in each run.

Detection of ESBL

VITEK 2 system with the antimicrobial susceptibility extend card AST-EXN8card was designed to perform both screening and confirmatory tests for phenotypic detection of ESBL on the same plate. VITEK 2 system has two different ESBL detection procedures. The first one uses specific computer software called advanced expert system (AES), that performs analyzes and interpretation of minimal inhibitory concentration (MIC) of the antibiotics used.The use of several antimicrobial agents increases the sensitivity of ESBL detection[34] thus the second procedure was based on ESBL test on same AST-EXN8 card, where the antibiotic susceptibility of the isolates to cefepime, and 3[rd]generatin cephalosporin (cefotaxime and ceftazidime)with or without clavulanic acid were evaluated.[9, 35]

Statistics analysis

SPSS version17.0 was used for data analysis. Chi-square tests as well as two-tailed Fisher's exact test were used when appropriate to compare categorical variables.P-value of $< 0.05$was considered as statistically significant.

RESULTS

During the study period, we only include all the positive urine cultures of E. coli isolatesthat were tested against AST-EXN8card, sixty one cultures, while we exclude any positive urine cultures that were tested against other AST-cards, manually,or show mixed growth.

Children were nearly equally infected by both types of E. coli isolates, ESBL-producers 31 (50.8%) and non ESBL-producers30 (49.2%) see table 1.Never the less, There was significantly higher proportion of E. coli isolated from female (83.6%) than male (16.4%) childrenwith UTI in general and also according to the type of E. coli isolates see figure 1.

The frequency of antimicrobial resistance for the 16 antimicrobial agents included in AST-EXN8 card against E. coli isolates UTI pathogens (ESBL-producersand Non ESBL-producers) are summarized in Table 2. ESBL-producing E. coli showed maximum rate resistance to Cefuroxime as well asPiperacillin (100%), Aztreonam, Cefixime, Ceftriaxone plus Levofloxacin (96.8%), Ampicillin/Sulbactam and Cefepime (93.5%), Moxifloxacin (90.3%), while minimum resistance rate was seen with Tigecycline (12.9%),Colistin (3.2%) and meropenem (0%). The Non ESBL-producing E. coli showed maximum resistance rate toCefuroxime (93.3%), Piperacillin along with Chloramphenicol (80%) and Trimethoprim (66.7%), while minimum rate of resistance was seen with Aztreonam, Tigecycline, and Ceftriaxone (6.7%), while no resistance were seen with Cefepime,Colistin,as well as meropenem (0%). ESBL-producing isolates were significantly more resistant than Non-ESBL-producers ($p < 0.05$) to the following antimicrobials (Ampicillin/Sulbactam, Aztreonam, Cefepime, Cefixime, Ceftriaxone, Levofloxacin, Moxifloxacin, Piperacillin and Tetracycline). Multi-drug resistance (MDR) was higher among ESBL-producing E. coli isolates than non ESBL-producing E.coli isolates in general See figure 3.

Table 1.Prevalence of ESBL-producers and non ESBL-producersE.coliuropathogenes according to gender.

	MALE	FEMALE	Total	P-value
ESBL	6 (19.4%)	25 (80.6%)	31 (50.8%)	0.001
NON ESBL	4 (13.3%)	26 (86.7%)	30 (49.2%)	< 0.001
Total	10 (16.4%)	51 (83.6%)	61 (100%)	< 0.001

Figure 1.Prevalence of ESBL-producers and non ESBL-producer E. coli uropathogenes according to gender.
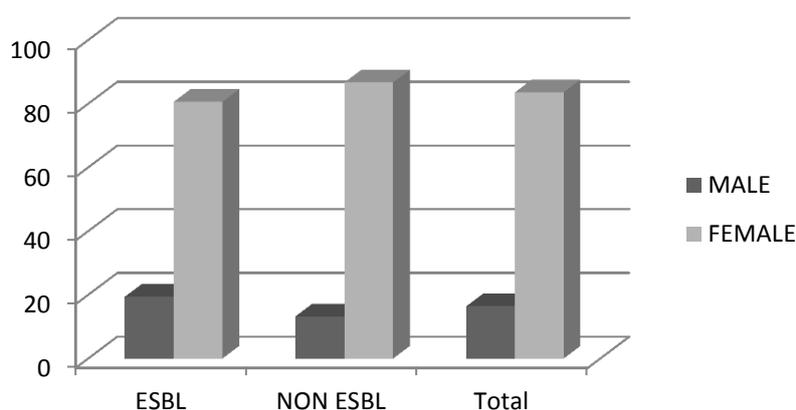
Table 2.number and percentage of antimicrobial resistant of both ESBL-producers and non ESBL-producersE.coli isolates.

Antibiotic	Resistance n (%)	ESBL n = 31	NON ESBL n = 30	P-value
Ampicillin/Sulbactam	48 (78.7%)	29 (93.5)	19 (63.3)	0.004
Aztreonam	32 (52.4%)	30 (96.8)	2 (6.7)	< 0.001
Cefepime	29 (47.5%)	29 (93.5)	0 (0.0)	< 0.001
Cefixime	40 (65.6%)	30 (96.8)	10 (33.3)	< 0.001
Ceftriaxone	32 (52.4%)	30 (96.8)	2 (6.7)	< 0.001
Cefuroxime	59 (96.7%)	31 (100)	28 (93.3)	0.238
Chloramphenicol	49 (80.0%)	25 (80.6)	24 (80)	0.601
Colistin	1 (0.02%)	1 (3.2)	0 (0.0)	0.508
Levofloxacin	47 (77%)	30 (96.8)	17 (56.7)	< 0.001
Meropenem	0	0	0	
Minocycline	38 (62.3%)	22 (71)	16 (53.3)	0.124
Moxifloxacin	45 (73.8%)	28 (90.3)	17 (56.7)	0.003
Piperacillin	55 (90.2%)	31 (100)	24 (80)	0.011
Tetracycline	41 (67.2%)	25 (86.6)	16 (53.3)	0.022
Tigecycline	6 (9.8%)	4 (12.9)	2 (6.7)	0.352
Trimethoprim	47 (77%)	27(87.1)	20 (66.7)	0.055

Table 3. DistributionMDR pattern of E.coli isolates.

Pattern	Resistant pattern	n (%)		
		ESBL	Non-ESBL	Total
Pattern 1	Resistant to 1 – 4 drugs	0	7 (23)	7 (11)
Pattern 2	Resistant to 5 – 8 drugs	0	16 (53)	16 (26)
Pattern 3	Resistant to 9 – 12 drugs	13 (42)	7 (23)	19 (31)
Pattern 4	Resistant to 13 - 16 drugs	17 (55)	0	17 (28)

We can use this figures instead of table 2 or 3

Figure 2. Antimicrobial resistant of both ESBL-producers and non ESBL- producersE.coli isolates.
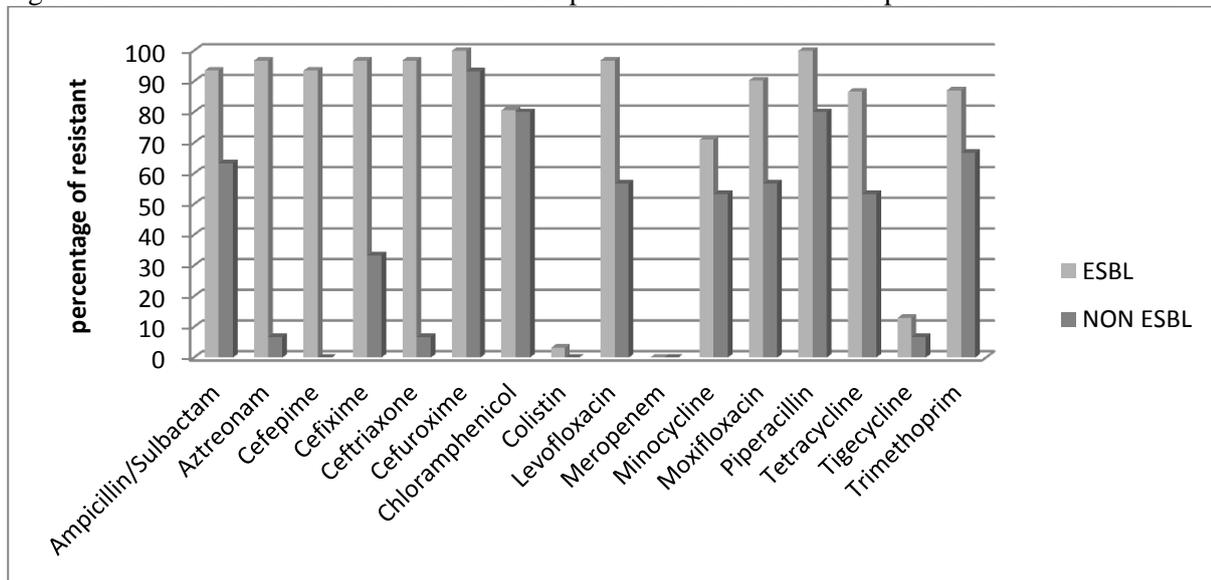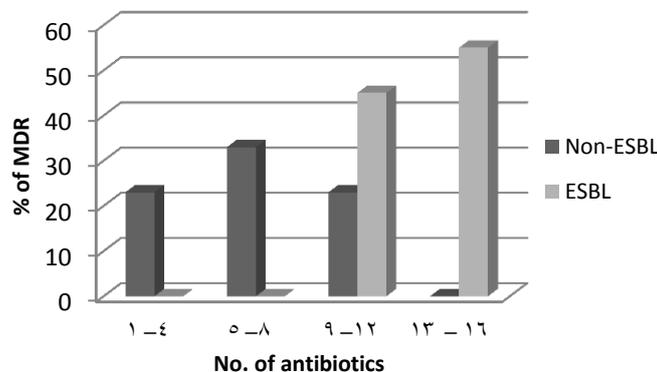

Figure 3.DistributionMDR pattern of E.coli isolates.

DISCUSSION

ESBL-producing *E. coli* isolates has emerged as serious uropathogens in both in hospital and community acquired UTIs in children and adults, leading to significantly higher treatment failure rate and mortality when compared with non ESBL-producers isolates.[36] The study was conducted to determine the prevalence and resistance profile of ESBL-producers of E.coli isolates in QRAH for children against a certain set of antibiotics using the VITEK 2 system. This study found that ESBL-producers isolates were as

high as 50.8%, which is comparable with other studies from Jordan 50.3%[37] or Pakistan (54% - 57.4%)[38-40], but higher than studies from India (40%)[17], Tanzania (39.1%)[41], Iran (21% and 35%)[42,43], Saudia Arabia (24.5% in children)[44], Lebanon 17.7% (23.5% from hospitalized children with UTIs and 14.1% from community UTIs), and from other studies in Jordan 10.8%.[45]

The prime factors for increasing resistant to 3rd generation cephalosporin or other broad spectrum antibiotics are; the over prescriptions of these antibiotics beside the lack of routine screening for ESBL-producer E.coli or isolation guidelines for the infected patients.[46] ESBL-producer isolates showed significant higher resistant rate to 3rd and 4th generation cephalosporin (Cefixime 93.5%, Ceftriaxone 96.8%, and Cefepime, 93.5) than non ESBL-producer isolates, while these results were comparable with what have been found in literature.[40, 47,48]

ESBL-producers Isolates were found to have significant higher resistant rates than non ESBL-producer isolates to Aztreonam (96.8%), and penicillin's (Ampicillin/Sulbactam 93.5% and Piperacillin 100%), sameresistant rates (Azactam, 90%-92%.[38, 49]Piperacillin100%[5, 49], and amoxicillin/clavulanic acid, 83.8%-85.6%.[38,40,41]) havebeen also found in different studies.

Meropenem showed the best in vitro activity (100%) against of both ESBL-producers and non ESBL-producer isolates; nearly same results (90-100%) were also found in most of studies for cabapenems (imipenem, meropenem, ertapenem). Since carbapenems are relatively safe in children they are still considered the drug of choice for UTIs caused by multi-drug resistant ESBL-producing *E. coli*.[44, 48-50]

The excellent in vitro activity for Colistin (3.2%) and Tigecycline (12.9%)against both ESBL-producers and non ESBL-producer isolates has been reported in this study, Colistin should be reserved as the last resort against the multi-drug resistant ESBL-producing *E. coli*[48] (48). Fluoroquinolone have high resistant rates according to the literature; Norfloxacin, 83%[40], Ofloxacin 70%[43],and Ciprofloxain (25-85%).[51-52] Levofloxacin, Moxifloxacinwhich usually not used to treat UTIs, had also showed a higher resistance rate for ESBL-producer *E. coli* (96.8%, 90.3%), so we should use this class with caution even when we use Ciprofloxacin which considered more safe in younger children than other Quinolones.[47]Chloramphenicol, Tetracycline, and Minocycline were associated with high resistant rate (80.6%, 86.6%, and 71% respectively) against of both ESBL-producers and

non ESBL-producer isolates. All are not preferred to be use in UTIs of children because of resistant and safety issues.[38, 47]Trimethoprim resistancewas considered high for both ESBL-producers (87.1%) and non ESBL-producer isolates (66.7%), this may due to long term use as empirical therapy to UTI in some countries,.[38,40,41,47,49]

MDR was found to be higher among ESBL-producing than non ESBL-producing E.coli isolates in this study and in literature.[36-37, 44]All of the ESBL-producing isolates were found to be resistant to at least (9) antibiotics; at the same time there were 17 ESBL-producing isolates were resistant up to (13–16) antibiotics, while none of non ESBL- producing isolates were found to be resistant to more than (12) antibiotics, and 23 of these isolates where found to be resistant to less than (9) antibiotics; See table 3.

The study has some limitations. First,the study was done retrospectively, for that we collect the patient information's and the sample data from the information's that have been provided to the VITEK 2 system, which arein most cases so limited to enable us to differentiate between community and nosocomial UTIs or even the source of the sample within the hospital if. Second, the limited number of samples tested on VITEK 2 system and the antibiotic classes that have been tested, so in future we may need to have a large multi-center studies to address the size of the problem, and to study the resistant patterns to other classes of antibiotics as aminoglycosides and other Quinoloneswhich are more specific and safer to be used in children UTI as ciprofloxacin, or to take in the account the comorbidity factors. But even under all of these limitations we still have a high percentage of ESBL-producers isolates, with high resistant rates; since all of these isolates were resistant to at least nine antibiotics from different classes, moreover these findings are generally consistent with what have been observed in our region or internationally.

In summary, the majority of therapy for UTI is empiric, where clinicians not always depend on laboratory guidance, beside the misuse and self-medication of relatively cheaper antibiotic without any prescription is common in our community. The findings of this study demonstrated an increase in the prevalence of multi-drug resistant ESBL-producers isolates, up to an alarming levels within our hospital region, which limit their treatment options, so we believe it's now a mandatory to perform screening and confirmatory tests for detection of those microorganisms in our

daily routine work, and to provide the clinicians with updated resistant pattern data to choose the best therapeutic option to limit or even prevent their spread within our community. Morepenem has a good activity against ESBL-producers isolates and relatively safe in children to be considered as drug of choice for these microorganisms, where Colistin may consider as the last resort of treatment.

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