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Extended-spectrum β-lactamase producing *E. coli* in urinary tract infections: A two-center, cross-sectional study of prevalence, genotypes and risk factors in Amman, Jordan

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ABSTRACT

Background: To determine the prevalence, phenotypes, and genotypes of extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) among patients with urinary tract infection along with identifying the associated risk factors.
Methods: A cross-sectional study was conducted at two tertiary hospitals in Amman, Jordan between June and October, 2016. One hundred twenty one *E. coli* isolates from hospitalized patients with urinary tract infection were phenotypically assessed for ESBL production using the double disc diffusion test. Positive isolates to ESBL production were further genotyped using multiplex PCR. A nested case-control study was used to determine the independent risk factors.
Results: ESBL-producing *E. coli* were found in 75/121 (62%) isolates. Molecular genotyping demonstrated that CTX-M group1 (42.7%) predominated followed by combination of SHV and CTX-M group1 (20%). In the regression model, previous hospitalization and use of urinary catheter were identified as independent risk factors for ESBL-producing *E. coli* infections.
Conclusion: We report a high prevalence of ESBL-producing *E. coli* which is in concordance with other studies from developing countries. Additionally, CTX-M group1 has emerged as the predominant ESBL produced by *E. coli*, which is consistent with reported results throughout the world. Independent risk factors to UTI infections due to ESBL-producing *E. coli* include previous hospitalization and use of urinary catheter.

Introduction

Urinary tract infection (UTI) is considered to be the most frequent bacterial infection; *Escherichia coli* (*E. coli*) being the commonest causative agent [1]. Recently, the antimicrobial resistance among *Enterobacteriacea* is becoming a rising health problem due to the production of extended-spectrum β-lactamases (ESBLs) [2]. ESBLs are groups of β-lactamases causing treatment failure in clinical practice [3]. Globally, ESBL infections vary in prevalence and are becoming increasingly frequent [2,4].

ESBLs are enzymes produced by Gram-negative bacilli, and most commonly are classified into three types: TEM, SHV, and CTX-M [4]. Identifying the molecular characterization of ESBL genes has an essential role in epidemiological studies. In addition, understanding risk factors associated with ESBL infection can identify patients who should receive empiric ESBL-targeted antimicrobial therapy [5].

In Jordan, little is known about the prevalence of ESBL-producing *E. coli* and the circulating ESBL gene types [6–8]. Moreover, there is no available data on the risk factors associated with ESBL-producing *E. coli* UTI infections.

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Table 1
16S rRNA targeted primers used for the detection of *E. coli* isolates.

Primers	Sequence (5′–3′)	Length bases	Amplicon size	Primers concentration (μmol/mL)
16 E1_for	GGGAGTAAAGTTAATACCTTTGCTC	25	584	1
16 E2_rev	TTCCCGAAGGCACATTCT	18		1
16 E3_rev	TTCCCGAAGGCACCAATC	18		1

Table 2
Primers used for identifying ESBL gene types.

Primers	Genes amplified	β-Lactamase (s) targeted	Sequence (5′–3′)	Length bases	Amplicon size	Primers concentration (pmol/mL)
TEM_for	bla_{TEM}	TEM variants including TEM-1 and TEM-2	CATTTCCGTGTCGCCCTTATTC	22	800	0.4
TEM_rev			CGTTCATCCATAGTTGCCTGAC	22		0.4
SHV_for	bla_{SHV}	SHV variants including SHV-1	AGCCGCTTGAGCAAATTAAAC	21	713	0.4
SHV_rev			ATCCCGCAGATAAATCACCAC	21		0.4
MultiCTX-MGp1_for	bla_{CTX-M} group 1	Variants of CTX-M group 1 including CTX-M-1, CTX-M-3 and CTX-M-15	TTAGGAARTGTGCCGCTGYA	20	688	0.4
MultiCTX-MGp1_rev			CGATATCGTTGGTGGTRCCAT	21		0.2
MultiCTX-MGp2_for	bla_{CTX-M} group 2	Variants of CTX-M group 2 including CTX-M-2	CGTTAACGGCACGATGAC	18	404	0.2
MultiCTX-MGp2_rev			CGATATCGTTGGTGGTRCCAT	21		0.2
MultiCTX-MGp9_for	bla_{CTX-M} group 9	Variants of CTX-M group 9 including CTX-M-9 and CTX-M-14	TCAAGCCTGCCGATCTGG	19	561	0.4
MultiCTX-MGp9_rev			TGATTCTCGCCGCTGAAG	18		0.4

Materials and methods

Study type and population

A cross-sectional study was conducted between June 19th and October 12th, 2016 at the Jordan University Hospital (JUH) and Islamic Hospital (IH) to determine the prevalence of ESBL-producing *E. coli* and their genotypes. JUH is a tertiary teaching hospital with a capacity of 550 beds and IH is one of the largest private hospitals in Jordan. This study was approved by the Institutional Review Board (IRB) at the two hospitals; JUH (Ref. 13807) and IH (Ref. 2393).

Patients admitted to the internal medicine, obstetrics and gynecology and ICU departments (>3 days after hospital admission) with confirmed UTI-associated *E. coli* regardless of their age and gender were included in this study. Recruited cases were patients with positive *E. coli* urine culture (ca. 1×10^5 colony forming units/ml) and physician diagnosed of UTI infection. At the School of Pharmacy—The University of Jordan, patients' culture samples were further confirmed to be *E. coli* using uniplex polymerase chain reaction (PCR) and were further analyzed for ESBL production and gene types and subtypes. Only one sample from each patient was included in the study.

Consented patients were interviewed and medical files and medication sheet were reviewed to collect the following data: history of hospitalization, history of clinic visit, previous urogenital surgery, current or previous use of urinary catheter, recurrent symptomatic UTI, antibiotic treatment in past 3 months, and insertion of permanent devices.

A nested case-control study was conducted to identify risk factors for UTI infection due to ESBL-producing *E. coli*. Cases were identified as hospitalized patients with symptoms related to UTIs and a positive urine culture for ESBL-producing *E. coli*, while controls were patients with UTIs due to none ESBL-producing *E. coli*. Cases of ESBL-producing *E. coli* were matched in a 1:1 ratio to control of none ESBL-producing *E. coli* according to age, gender, and settings.

ESBL double disc diffusion test

ESBL production among all genetically confirmed *E. coli* isolates was detected using the double diffusion disc (Bioanalyse™, Turkey) as recommended by CLSI [9].

E. coli strain ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as ESBL negative and positive quality control strains, respectively.

Genotypic characterization of Ambler class A resistant isolates

Uniplex PCR detection for the 16S ribosomal RNA (*16S rRNA*) gene and a multiplex PCR with 5 different sets of primers were performed to genotypically confirm *E. coli* species and to detect ESBL types, respectively. Two specific primers of *E. coli* were used to detect the *16S rRNA* sequences [10] in all *E. coli* received from the hospitals (Table 1). Five pairs of specific primers were used in all phenotypically confirmed ESBL for typing of ESBL genes; bla_{TEM}, bla_{SHV}, bla_{CTX-M} group 1, bla_{CTX-M} group 2 and bla_{CTX-M} group 9 [11] (Table 2). Those genes were selected because they are considered a paradigm in the evolution of a resistance mechanism and the most predominant types [11–13]. Each PCR reaction was carried out using 2 μl of bacterial DNA as template, 1X of both forward and reverse primers (μmol/mL or pmol/mL) of each targeted gene, 12.5 μl of Master Mix type Go Tag Green Master mix (Promega, USA), and the volume was made up to 25 μl using nuclease free water. Uniplex PCR and multiplex conditions and electrophoresis processes were used according to Tsen and Jian [10] and Dallenne

et al. [11]. DNA was visualized under a UV transilluminator (UVP, USA) provided with a gel documentation system using the Quantity One software (Biorad, USA). Fragment sizes of each PCR were determined by comparison with a 100 bp DNA ladder (Promega, USA).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS), version 20 (IBM Co., Armonk, NY, USA). Risk factors for ESBL-producing *E. coli* UTI were identified by univariate analysis. For categorical variable, either Chi square or Fisher's exact test was used. Significance was set at *P*-value <0.05. All variables that were associated with ESBL-producing *E. coli* UTI in the univariate analysis at the $P < 0.1$ level were included in the logistic regression using the backward conditional method. The final model included confounding variables significant at a two-tailed *P*-value of <0.05.Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association.

Results

Study population and ESBL types and prevalence

One hundred twenty one UTI patients were included: 41 (33.9%) males and 80 (66.1%) females. All isolates were genetically proven to be *E. coli*. Double disc diffusion test results showed that 75 (62%) of the tested *E. coli* (121) were ESBL-producers. The distribution of ESBL isolates in JUH and IH were 59 (65.6%) and 16 (51.6%) respectively.

All phenotypically ESBL-producing *E. coli* isolates were confirmed to have genes encoding ESBL. CTX-M group1 type (82.7%) was the most prevalent ESBL enzyme followed by SHV type (30.7%) and CTX-M group 9 type (28%). The prevalence of different types of ESBL encoding genes among *E. coli* isolates is provided in Tables 3 and 4.

Table 3
Total count and percentage of each ESBL genotypes.

ESBL genes	Frequency (%)
bla_{TEM}	2(2.7%)
bla_{SHV}	23(30.7%)
bla_{CTXM} group 1	62(82.7%)
bla_{CTXM} group 2	2(2.7%)
bla_{CTXM} group 9	21(28%)

Table 4
The distribution of genes encoding ESBL among 75 clinical isolates of ESBL producing *E. coli.*

ESBL genes	Isolates count (%)
bla_{SHV}	3 (4.0%)
bla_{CTXM} group 1	32 (42.7%)
bla_{CTXM} group 2	1 (1.3%)
bla_{CTXM} group 9	6 (8.0%)
bla_{CTXM} group 1 + bla_{CTXM}group 9	10 (13.3%)
bla_{CTXM} group 1+ bla_{CTXM} group 2+bla_{CTXM} group 9	1 (1.3%)
bla_{SHV} + bla_{CTXM} group 1	15 (20.0%)
bla_{SHV} + bla_{CTXM} group 9	3 (4.0%)
bla_{TEM} + bla_{CTXM} group 1	2 (2.7%)
bla_{SHV} + bla_{CTXM} group 1+ bla_{CTXM} group 9	2 (2.7%)

Risk factors associated with UTI in patients infected with ESBL-producing E. coli

Because the ESBL detection rate (62%) was unexpectedly high and exceeded half the sample size and in order to identify the associated risk factors, all remaining patients with none ESBL-producing *E. coli*, control, (46 patients, 38%) were matched in a 1:1 ratio to 46 cases infected with ESBL-producing *E. coli* (n = 75) according to age, gender and setting.

On univariate analysis, recent hospitalization, history of clinic visit, current use or previous use of urinary catheter in past 6 months, recurrent symptomatic urinary tract infections and benign prostate hypertrophy in men were identified as significant risk fac-

Table 5
Univariate analysis of risk factors associated with ESBL-producing *E. coli.*

Variables	Cases[a] count (%)	Controls[b] count (%)	*P*-Value (≤0.05%)	OR; (95% CI)
Hospitalization in past 6 months[c]	33 (71.7%)	20 (43.5%)	0.004	3.60; (1.51–8.61)
Marital status (Married)	34 (73.9%)	35 (76.1%)	0.81	1.12; (0.44–2.89)
History of out clinic visit in past 6 months	42 (91.3%)	35 (76.1%)	0.035	3.70; (1.09–12.53)
Central line used in past 6 months	7 (15.2%)	6 (13.0%)	0.76	1.19; (0.37–3.88)
Any permanent devices[d]	10 (21.7%)	7 (15.2%)	0.42	1.54; (0.53–4.49)
	0 (0.0%)	2 (6.9%)	0.99	NA
Previous urogenital surgery in past 6 months[e]	21 (45.7%)	13 (28.3%)	0.08	2.13; (0.89–5.06)
Current or previous use of urinary catheter in past 6 months	33 (71.1%)	21 (45.7%)	0.01	3.02; (1.27–7.18)
Recurrent symptomatic urinary tract infections[f]	34 (73.9%)	23 (50.0%)	0.02	2.83; (1.18–6.80)
Benign prostate hypertrophy (male)	14 (70.0%)	6 (35.3%)	0.04	4.27; (1.07–17.00)
Antibiotic treatment in past 3 months[g]	36 (78.3%)	26 (56.5%)	0.02	2.76; (1.11–6.89)
Return from travel in past 6 months	11 (23.9%)	10 (21.7%)	0.80	1.13; (0.43–2.99)
Steroid use in past 3 months	11 (23.9%)	14 (30.4%)	0.48	0.71; (0.28–1.80)
ICU admission	8 (17.4%)	10 (21.7%)	0.60	0.76; (0.27–2.13)
Diabetes mellitus[h]	20 (43.5%)	17 (37.0%)	0.52	1.31; (0.56–3.02)
Cancer[i]	5 (10.9%)	7 (15.2%)	0.54	0.68; (0.19–2.32)
Cardiovascular diseases[j]	19 (41.3%)	18 (39.1%)	0.83	1.09; (0.47–2.52)

NA: not available.
[a] Cases are ESBL- producing *E. coli* isolates.
[b] Controls are non ESBL- producing *E. coli*.
[c] Previous admission to a hospital for more than 1 day.
[d] Insertion any device such as urethral stent or arterial stent.
[e] Urogenital surgeries as diagnostic cystoscopy, biopsy or urethral or bladder dilatations.
[f] Three or more episodes of UTI in one year.
[g] Antibiotic use either IV injection or oral.
[h] Type I or II.
[i] Cancer at any stage.
[j] Hypertension, heart arrhythmia, myocardial infarction or heart failure.

Table 6
Multivariate analysis of risk factors associated with ESBL-producing *E. coli*.

Variables	*P*-Value (≤0.05%)	OR	95% CI
Hospitalization in past 6 months	0.029	2.79	1.11–7.03
Current or previous use of urinary catheter in past 6 months	0.031	2.74	1.09–6.85

tors associated with UTI due to ESBL-producing *E. coli*. Interestingly, the frequencies of major comorbidities such as diabetes or cancer did not show significant difference between cases and controls. The full list of factors with univariate ORs and 95% CIs are shown in Table 5. While on multivariate logistic regression analysis, recent hospitalization and current or previous use of urinary catheter in past 6 months were identified as independent risk factors in cases (Table 6).

Discussion

A high prevalence of ESBL-producing *E. coli* with a predominance of CTX-M group 1gene is reported in the current study. The independent risk factors associated with infection with ESBL-producing *E. coli* were recent hospitalization and urinary catheterization.

Globally, ESBL infections prevalence vary and are becoming increasingly frequent [2,4]. Prevalence results of the current study are in agreement with recent reports in MENA region, but higher than reports in other parts of the worlds [14,15]. Recent research studies in the neighboring MENA region, reported a high prevalence of ESBL rate among *E. coli* isolates ranging from 38.2% to 39.4% among community acquired UTI and from 50.5% to 70% among hospital acquired UTI patients [16–18]. Similarly, in Jordan, in the last few years (2012–2015) a high rate of ESBL-producing *E. coli* (43–54%) was reported among patients with community and hospital acquired UTI [7,8,19] which is significantly higher than that reported in 2009 (10.8%) [20].

The differences in the medical use pattern of antibiotic [21], self-prescription of antibiotics which are sold over the counter and absence of antibiotic stewardship programs (ASP) may account for the disparity in the incidence of ESBL infections between countries [22,23]. In Jordan, a high percent of the population believed that antibiotics treat common cold and cough and misused antibiotics as analgesics where one-half of all dispensed antibacterial drugs were without a prescription (46%), either via self-medication (23.2%) or pharmacist recommendation (23.1%) [24,25]. The current practice of using antibiotics without prescription, and insufficient knowledge of proper antibiotic use [25] might promote a high prevalence of resistance.

Identifying the molecular characterization of ESBL genes is essential in epidemiological studies and in dealing with outbreaks [26]. In this study, different types of ESBL were disclosed: CTX-M group 1, 2, 9, SHV and TEM. Molecular characterization of ESBL-positive isolates showed that the CTX-M group 1 type was the most predominant enzyme which is in concordance with global reports [8,27–29]. This is a remarkable change from the situation during the early 1990s when TEM ESBL was the most common gene worldwide [30]. A related point to consider is that two studies with high percentages of TEM and SHV genes were reported in northern Jordan in 2012–2015 [7,14].

According to Naseer and Sundsfjord [31] and Pitout et al. [32], CTX-M associated *E. coli* clones are causing systemic infection as well as repeated UTIs in patients worldwide which may explain the high rate of recurrent UTI among our patients [12,17,33].

Interestingly, in concordance to other studies worldwide [17,33], two or more β-lactamases gene co-existed in the same strain with predominance of $bla_{\text{CTX-M}}$ groups; $bla_{\text{CTX-M}}$ group 1, followed by $bla_{\text{CTX-M}}$ group 9.

Studies on risk factors for infection with ESBL-producing bacteria have been conducted in different parts of the world [34,35], but not in the Jordan. To our knowledge, our study is among the few to use a nested case-control design in the assessment of risk factors for ESBL-producing bacteria, and the first to do so within Jordanian health settings. In our regression model, recent hospitalization and use of current or previous use of urinary catheter in the past 6 months were identified as independent risk factors and were in concordance with most published work [36,37].

Notably, on multivariate regression analysis no statistically significant differences between the groups regarding history of recurrent symptomatic urinary tract infections, previous urogenital surgery, and recent antibiotic treatment in the past 3 months were found as independent risk factors although they were significantly different according to univariate analysis. These results were different from the worldwide findings that reported a strong association between ESBL-producing *E. coli* in UTI and recent antibiotic use [34,35,38], and recurrent UTI [35] on multivariate analysis. It was difficult to find reasonable explanations for the lack of correlations between these factors on multivariate but not on univariate. This could be explained by the small sample size that failed to notice the differences.

Although, using multivariate analysis, diabetes mellitus was reported as independent risk factor for ESBL-positive UTI [38,39], nevertheless, our study could not show that neither with multivariate nor with univariate analysis.

In conclusion, this study indicates that the prevalence of ESBL-producing *E. coli* in UTI is in concordance to other studies from developing countries and higher than those reported from developed ones. Also, CTX-M group1 is increasingly recognized in Jordan as well as throughout the world. Independent risk factors of UTIs caused by ESBL-producing *E. coli* were previous hospitalization and catheterization.

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Competing interests

None declared.

Ethical approval

This study was approved by the Institutional Review Board (IRB) at the two hospitals; JUH (Ref. 13807) and IH (Ref. 2393).

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