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Risk factors for extended-spectrum β-lactamases-producing *Escherichia coli* urinary tract infections in a tertiary hospital

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Abstract

Objective. To assess the risks factors for urinary tract infections (UTIs) caused by Extended-Spectrum Beta-Lactamases (ESBLs)-producing *E. coli* and the molecular characterization of ESBLs. **Materials and methods**. A case-control study was performed to identify risk factors in consecutively recruited patients with UTIs caused by ESBLs or non-ESBLs-producing *E. coli* in a tertiary hospital in Mexico. **Results**. ESBLs-producing *E. coli* were isolated from 22/70 (31%) patients with *E. coli* UTIs over a three month period. All isolates were resistant to cephalosporins and quinolones but susceptible to carbapenems, amikacin and nitrofurantoin. Prior antibiotic treatment with more than two antibiotic families (OR=6.86; 95%CI 1.06-157.70; *p*=0.028), recurrent symptomatic UTIs (OR=5.60; 95%CI 1.88-17.87; *p*=0.001) and previous hospitalization (OR=5.06; 95%CI 1.64-17.69; *p*=0.002) were significant risk factors. Sixteen isolates harbored the beta-lactamase $(bla)_{\text{CTX-M-15}}$ gene and five the $bla_{\text{TEM-1}}$ gene. **Conclusions**. One of every three patients presented UTIs with ESBLs-producing beta-lactams and fluoroquinolone resistant *E. coli*. Risk factors and resistance patterns must be taken into account for developing antibiotic use policies in these settings.

Key words: *Escherichia coli*; beta-lactamases; urinary tract infections; risk factors; Mexico

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Resumen

Objetivo. Evaluar los factores de riesgo en infecciones de vías urinarias (IVUs) causadas por *E. coli* productora de Beta-Lactamasas de espectro extendido (BLEEs) y caracterizar las BLEEs. **Material y métodos**. Estudio de casos y controles en pacientes consecutivos con IVUs causadas por *E. coli* productoras o no de BLEEs en un hospital de referencia. **Resultados**. *E. coli* productora de BLEEs se aisló en 22/70 (31%) pacientes con IVUs por *E. coli* durante un periodo de tres meses. Todos los aislamientos fueron resistentes a cefalosporinas y quinolonas, pero susceptibles a carbapenemes, amikacina y nitrofurantoina. Factores de riesgo significativos incluyeron tratamiento previo con más de dos familias de antibióticos (OR=6.86; IC95% 1.06-157.70; *p*=0.028), IVUs sintomáticas recurrentes (OR=5.60; IC95% 1.88-17.87; *p*=0.001) y hospitalizaciones previas (OR=5.06; IC95% 1.64-17.69; *p*=0.002). Dieciséis aislamientos presentaron el gen betalactamasas $(bla)_{\text{CTX-M-15}}$ y cinco el gen $bla_{\text{TEM-1}}$. **Conclusiones**. Uno de cada tres pacientes presentó IVU con *E. coli* resistente a beta-lactámicos, fluoroquinolonas y productora de BLEEs. En estos casos, los factores de riesgo y patrones de resistencia deberían tomarse en cuenta para recomendar tratamiento.

Palabras clave: *Escherichia coli*; beta-lactamasas; infecciones de vías urinarias; factores de riesgo; México

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Urinary tract infections (UTIs) are an important cause of morbidity in the general population, being *Escherichia coli* the principal etiologic agent.[1] At present the production of extended spectrum beta-lactamases (ESBLs) by this uropathogen complicates treatment because their presence not only implies resistance to beta-lactam antibiotics but is also associated with resistance to other families of antibiotics.[2]

ESBLs production is one of the main contributors to the problem of antimicrobial resistance. ESBLs are enzymes produced by gram-negative bacilli, most commonly derived from TEM or SHV parents, but the prevalence of CTX-M types has increased dramatically since 1998 in most parts of the world.[3] Typically, the isolation of ESBLs-producing *E. coli* has occurred in the hospital setting, but this organism has begun to disseminate in the community.[4]

Numerous studies have investigated the risk factors for UTIs caused by ESBLs-producing *E. coli* in the world, however, they are limited in Mexico.[5-7] The identification of predisposing factors is relevant to the prevention of this common infectious disease diagnosed in outpatients. The increasing prevalence of ESBLs-producing *E. coli* prompted our interest to investigate risk factors for ESBLs-producing *E. coli* in patients with community-onset UTIs. The aims of this study were to identify potential risk factors for hospital or community acquired UTIs by ESBLs-producing *E. coli*, determine the prevalence of antimicrobial resistance, identification and molecular characterization of genes encoding ESBLs and to determine the clonal relationship between clinical isolates.

Materials and methods

Study design and population

A case-control study was conducted to identify risk factors in consecutively recruited patients who presented with UTI caused by ESBLs-producing *E. coli* to Hospital General Naval de Alta Especialidad, Secretaría de Marina Armada de México (Hosgenaes), Mexico´s Naval Referral Hospital, a 150 bed tertiary care teaching hospital, which provides medical services to the naval branch of Mexico´s armed forces. The study was approved by the hospital´s research and ethics commission. The diagnosis of UTIs was defined by the presence of symptom related to the urinary tract, pyuria (≥10 leucocytes per high-power field) and positive clean catched urine culture [$\geq 10^5$ colony-forming units (CFU) / mL] of *E. coli*.[8] Only the first isolate from each patient was included in the analysis. Adult patients with UTIs due to ESBLs-producing *E. coli* (cases) and non-ESBLs-producing *E. coli* (controls) were prospectively identified through records from the hospital´s clinical microbiology laboratory over a three month period, August to October, 2011. Controls were matched in a 2:1 ratio to case patients and potential risk factors were recorded by abstracting medical records. A case was identified as an inpatient or outpatient with symptoms related to UTIs and a positive urine culture for ESBLs-producing *E. coli*. Controls were patients with UTIs due to non-ESBLs-producing *E. coli*. Clinical information included age, gender, previous hospitalization (within the past year), recent antibiotic treatment within 60 days, number of antibiotic treatments, antimicrobial regimen for UTIs, recurrent symptomatic urinary tract infections, underlying diseases (urological abnormalities, diabetes mellitus), and use of indwelling urinary catheter.

Bacteria, antibiotic susceptibility, and ESBLs production

Identification, susceptibility pattern, and ESBLs production were initially detected by the Phoenix system (Becton Dickinson, USA). Susceptibility profile of the isolates against to 22 antibiotics was determined using a disk diffusion method as the CLSI guidelines[9] and determining minimum inhibitory concentration (MIC) by BIOMIC System (Giles Scientific, USA). The ESBLs-producer *E. coli* was confirmed for ceftazidime and cefotaxime with or without clavulanate by double disk synergism method.[9] *Klebsiella pneumoniae* ATCC 700603 was used as control in all assays. An increase of >5 mm in a zone diameter for either antimicrobial agent tested in combination with clavulanate and their respective zone diameter of the agents when tested alone confirmed the presence of ESBLs-producing isolates.[9]

Detection and characterization of *bla* genes

PCR was used to detect the $bla_{CTXM,}$ $bla_{SHV,}$ and bla_{TEM} genes using previously described primers.[10-12] Amplified products were subjected to nucleotide sequencing at the Instituto de Biotecnología, Universidad Nacional Autónoma de México and analyzed using Clone Manager suite 7.0 and Basic Local Alignment Search Tool (BLAST).

Pulsed Field Gel Electrophoresis

A possible clonal relationship among the isolates was determined. Genotyping of ESBLs-producing *E. coli* was performed by Pulsed Field Gel Electrophoresis (PFGE), as described previously in a standard protocol established by the Centers for Disease Control and Prevention (PulseNet; Centers for Disease Control and

Prevention 2008), using genomic DNA and enzymatic restriction analysis with *Xba*I. The DNA band patterns were analyzed using Tenover criteria.[13] The percentage of similar profiles was calculated using the Dice coefficient,[14] isolates with a Dice coefficient correlation >85% were considered to belong to the same pulsotype (clone).

Statistical analysis

Qualitative variables were compared using chi square test. Logistic regression models were used to calculate odds ratios and 95% confidence intervals (CI). For all comparison, a *p* value of <0.05 was considered statistically significant.

Results

A total of 70 patients with UTIs caused by *E. coli* were recruited during the study period. Confirmatory double disc synergy diffusion test showed that 22 (31%) isolates were phenotypic positive for ESBLs-production (one in three patients with UTIs), 19 of these isolates came from the outpatient services and the other three isolates from inpatient (Surgery, Internal Medicine and Intensive Care Unit).

Univariate and multivariate analysis were performed with patient characteristics, epidemiological data and variables associated with development of UTIs caused by ESBLs-producing *E. coli* (table I). Gender and

Table I
Risk factors for acquisition of UTIs caused by ESBLs-producing *Escherichia coli* isolates

	Cases		*Controls*				
Variable	*n=22*	*%*	*n=48*	*%*	*OR**	*CI95%*	*p-value*
Age, years Mean (SD)	54.4	(16.12)	59.0	(14.5)			0.45‡
Gender							
Female	21	95.5	44	91.7	1.89	0.22-49.36	0.466#
Male	1	4.5	4	8.3			
Diabetes mellitus type2							
Positive	10	45.4	23	47.9	0.91	0.33-2.49	0.936
Negative	12	55.6	25	52.1			
Urologic abnormalities							
Positive	13	59.1	13	27.1	3.88	1.31-11.47	0.005
Negative	9	40.9	35	72.9			
Previous hospital days							
Positive	17	72.3	19	39.6	5.06	1.64-17.69	0.002
Negative	5	22.7	29	60.4			
Foley catheter							
Positive	8	36.4	6	12.5	3.90	1.13-14.08	0.008
Negative	14	63.6	42	87.5			
Previous antibiotic							
Positive	21	94.5	36	75.0	6.86	1.06-157.70	0.028
Negative	1	5.5	12	25.0			
Number of antibiotic groups							
3->	12	54.5	4	8.3	5.14	1.39-18.89	0.000&
2	6	27.3	10	20.8			
1	3	13.6	26	54.2			
0	1	4.6	8	16.7			
Antibiotic for UTI							
Positive	15	68.2	24	50.0	2.11	0.74-6.47	0.198
Negative	7	31.8	24	50.0			
Urinary symptoms							
Positive	15	68.2	13	27.1	5.60	1.88-17.87	0.001
Negative	7	31.8	35	72.9			

* The rest of the odds ratio values were estimated by Woolf method and the confidence interval by Cornfield method at 95% level of significance
‡ Mean was evaluated by t test at 95% level of significance
# Gender was evaluated by chi-square test at 95% level of significance
& The odds ratio for number of antibiotics groups was stratified by Mantel-Haenszel method and the *p* value was calculated by chi-square for trend

mean age were not found to be statistically significant risk factors between the case and control groups. Previous exposure to antibiotics (OR=6.86; 95%CI 1.06-157.70; p=0.028), recurrent symptomatic UTIs (OR=5.60; 95%CI 1.88-17.87; p=0.001), use of >2 of different families of antibiotics (OR, 5.14; 95%CI 1.31-11.47; p=0.0), previous hospitalization (OR=5.06; 95%CI 1.64-17.69; p=0.002), urological abnormalities (OR=3.88; 95%CI 1.31-11.47; p=0.005), and urinary catheterization (OR=3.90; 95%CI 1.13-14.08; p=0.008), were significantly associated with development of ESBL-producing *E. coli* UTIs.

Susceptibility phenotype of ESBLs-producing *E. coli* isolates are summarized in table II. As expected, multidrug-resistant comprised 100% of these isolates, they were resistant to three or more different families of antibiotics; aminopenicillins, third-generation cephalosporins and fluoroquinolones. Nevertheless, all isolates remained fully susceptible to carbapenems and highly susceptible to amikacin and nitrofurantoin.

PCR amplification of DNA from 22 ESBL-producing *E. coli* isolates showed the presence of two ESBLs families; CTX-M in 16/23 (70%) isolates and TEM in 5/23 (22%) isolates (four isolates from outpatients and one from inpatient), SHV was not detected. Sequencing of CTX PCR products demonstrated 100% homology to the sequence CTX-M-15, GenBank accession no. AY044435, and TEM PCR products demonstrated 100% homology to the sequence TEM-1 GenBank accession no. J01749. One isolate from the Surgery ward and one isolate from an outpatient simultaneously harbored $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ genes. Four isolates with ESBLs production were negative for *bla* genes studied.

PFGE analysis of 22 ESBLs-producing *E. coli* isolates yielded 14 different clones and one isolate was not

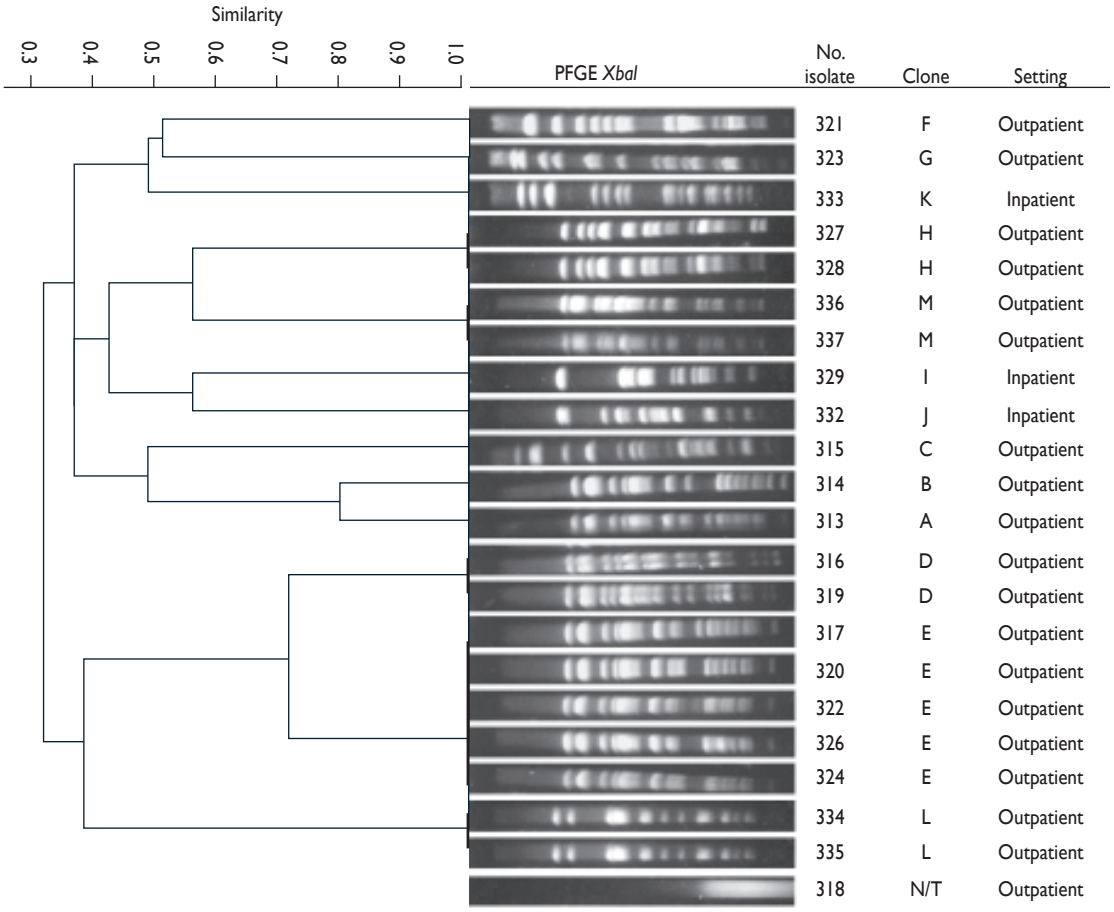
Table II
ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF 22 ESBLs-PRODUCING *ESCHERICHIA COLI*

		E. coli (n=22)							
Antibiotic family	*Antibiotic*	*Breakpoints CLSI/2014 (µg/mL)*		*MIC50 BIOMIC (µg/mL)*	*MIC90 BIOMIC (µg/mL)*	*MIC Range BIOMIC (µg/mL)*	*Resistant (%)*	*Intermediate (%)*	*Susceptible (%)*
Beta-lactam	Ampicillin	S≤8	R≥32	>58	>58	>58->58	100	0	0
	Cefoxitin	S≤8	R≥32	19	>69	4.6->69	31.8	31.8	36.4
	Cefuroxime	S≤8	R≥32	>75	>75	>75->75	95.4	0	4.5
	Ceftazidime	S≤4	R≥16	12	>126	1.1->126	72.8	9	18.2
	Cefotaxime	S≤1	R≥4	>138	>138	51->138	100	0	0
	Ceftriaxone	S≤1	R≥4	> 51	>51	>51->51	100	0	0
	Cefepime	S≤2	R≥16	23	>79	8->79	54.6	36.4	9
	Imipenem	S≤1	R≥4	≤ 0.56	0.68	≤0.56-0.83	0	0	100
	Meropenem	S≤1	R≥4	0.59	0.84	0.3-0.84	0	0	100
	Aztreonam	S≤4	R≥16	> 58	> 58	12->58	81.8	18.2	0
Beta-lactam/Beta-lactamases	Amoxicillin-Clavulanate	S≤8/4	R≥32/16	44	>64	11->64	86.3	13.7	0
	Ticarcillin-Clavulanate	S≤16/2	R≥128/2	>232	>232	32-> 232	95.4	4.6	0
	Piperacillin-Tazobactam	S≤16/4	R≥128/4	20	369	3.6-512	13.6	50	36.4
Aminoglycosides	Gentamicin	S≤4	R>16	24	>41	0.66->41	59	0	41
	Streptomycin[a]	S> 15	R≤11				36.4	13.6	50
	Tobramycin	S≤4	R>16	> 42	>42	4.4->42	86.4	4.6	9
	Ciprofloxacin	S≤1	R>4	> 19	>19	>19->19	100	0	0
Fluoroquinolones	Ofloxacin	S≤2	R>8	> 16	>16	>16->16	100	0	0
	Levofloxacin	S≤2	R>8	> 16	>16	5.1->16	100	0	0
Folate pathway inhibitors	Trimethoprim-Sulfamethoxazole	S≤2/38	R≥4/76	2.6	>10	≤ 0.21->10	45.5	9	45.5
Nitrofurans	Nitrofurantoin	S≤32	R>128	8.5	64	0.85->152	9	4.6	86.4

determined. These results indicate a high clonal variability (figure 1), and close genetic relatedness among five of the isolates belonging to clone E. These five isolates were from outpatients, three of them with a history of previous hospitalization. Additionally, these five isolates presented identical resistance patterns to 16/21 antimicrobials tested (included Beta–lactams, Beta-lactams-Beta-lactamase inhibitors combinations, aminoglycosides, and fluoroquinolones) and carried only the $bla_{\text{CTX-M-15}}$.

Discussion

E. coli remains as one of the most important ESBLs-producing bacteria in human infections worldwide. Several studies have addressed the impact of risk factors associated with community-onset UTIs caused by ESBLs-producing *E. coli*.[15-17] The most common and significant risks factors associated with these infections included recent hospitalization,[15-17] prior use of cephalosporins,[16,17] catheterization,[15,16,18] and diabetes.[19,20] However, studies on risk factors for infections due to ESBLs-producing *E. coli* in Mexico are limited and have been related to bloodstream and surgical wound infections.[5-7,21] Our results are similar to reports in other parts of the world and show that the previous use and the number of different families of antibiotics used, mainly cephalosporins, along with prior hospital stay, are major risk factors for acquisition of the UTIs caused by ESBLs-producing *E. coli*.[15-20,22]



N/T: non-typeable

Figure 1. Dendrogram constructed from PFGE patterns of twenty two ESBLs-producing *Escherichia coli* isolates. Clone E contained most of the isolates

In this prospective case-control study, ESBLs-producing *E. coli* was isolated in 33% of patients with UTI (78% of these cases were outpatients), a prevalence comparable to that found in a previous report from Latin American countries.[23] These isolates were 100% resistant to aminopenicillins, cephalosporins and fluoroquinolones, thereby the management of related UTIs could be more difficult. However, results in our study showed that amikacin, carbapenem and nitrofurantoin have in vitro efficacy. Because of the risk of nephrotoxicity and ototoxicity in the elderly,[24] amikacin should be used in short term therapy[24] and for that reason nitrofurantoin and carbapenems may be the antibiotics of choice in the treatment of these infections.

The types of ESBLs present in isolates in this hospital were CTX-M-15 and TEM-1 in 70% and 22% of the isolates, respectively. These results correlate with ESBLs families most commonly associated with ESBL-producing *E. coli* causing community-onset UTIs in most areas of the world.[5,7,17,25-30] Because four strains were phenotypically negative to the $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} genes, these results suggest the involvement of other ESBLs genes that were not tested for in this study. For example, bla_{VEB} gen has been reported within the genes identified from ESBL-producing *E. coli* isolated from community-onset UTIs in Taiwan[31] and $bla_{\text{TLA-1}}$ gen has been identified in 11% of the ESBL-producing *Enterobacteriaceae* causing nosocomial infections in Mexico.[32]

Although clonal variation was observed in the isolates studied, clone E was the most frequent, including five isolates from the community, three of them from patients with a recent history of previous hospitalization, which lead us to hypothesize that these isolates were acquired previously in the hospital.

The molecular analysis of the isolates in this study, which revealed the presence of CTX-M-15 and TEM-1 in different clones, suggests a likely horizontal dissemination of plasmids between ESBLs-producing *E. coli* and the association with multidrug-resistance observed. This is the first report to document the presence of CTX-M-15 and TEM-1-producing multi-drug-resistant *E. coli* causing UTIs in Mexico.

What are the clinical implications of these finding? When ESBLs-producing isolates are resistant to second- and third-generation cephalosporins, the treatment options are limited to carbapenems, aminoglycosides and nitrofurantoin.[16] Our findings strongly suggest that in this hospital setting, carbapenems and nitrofurantoin could be selected as empirical treatment options for out- and inpatients with ESBLs-producing *E. coli* UTIs and as these patients are at risk of developing sepsis, carbapenems but not nitrofurantoin could also be considered in the empirical treatment of bloodstream infections in patients with underlying[33] or recent history of *E. coli* urinary tract infection.

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Declaration of conflict of interest. The authors declare that they have no conflict of interests.

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