Multidrug resistance and risk factors associated with community-acquired urinary tract infections caused by *Escherichia coli* in Venezuela

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Original article

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Multirresistencia a medicamentos y factores de riesgo asociados con infecciones urinarias por *Escherichia coli* adquiridas en la comunidad, Venezuela

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Abstract

Introduction: The treatment of urinary tract infections has become more challenging due to the increasing frequency of multidrug-resistant *Escherichia coli* in human populations.

Objective: To characterize multidrug-resistant *E. coli* isolates causing community-acquired urinary tract infections in Cumaná, Venezuela, and associate possible risk factors for infection by extended-spectrum beta-lactamases (ESBL)-producing isolates.

Materials and methods: We included all the patients with urinary tract infections attending the urology outpatient consultation and emergency unit in the *Hospital de Cuman*á, Estado Sucre, Venezuela, from January through June, 2014. *bla*TEM, *bla*SHV and *bla*CTX-M genes detection was carried out by PCR.

Results: We found a high prevalence of multidrug-resistant *E. coli* (25.2%) with 20.4% of the isolates producing ESBL. The ESBL-producing isolates showed a high frequency (66.7%) of simultaneous resistance to trimethoprim-sulphamethoxazole, fluoroquinolones and aminoglycosides compared to non-producing isolates (2.4%). Of the resistant isolates, 65.4% carried the *bla*TEM gene, 34.6% the *bla*CTX-M and 23.1% the *bla*SHV. The *bla*CTX-M genes detected belonged to the CTX-M-1 and CTX-M-2 groups. Plasmid transfer was demonstrated by *in vitro* conjugation in 17 of the 26 ESBL-producing isolates. All three genes detected were transferred to the transconjugants. Age over 60 years, complicated urinary tract infections and previous use of a catheter predisposed patients to infection by ESBL-producing *E. coli*.

Conclusions: The high frequency of multidrug-resistant ESBL-producing isolates should alert the regional health authorities to take measures to reduce the risk of outbreaks caused by these types of bacteria in the community.

Keywords: Escherichia coli++ urologic diseases++ beta-lactamases++ rug resistance, bacterial++ community-acquired infections.

Resumen

Introducción: El tratamiento de las infecciones urinarias constituye un reto creciente por el aumento de *Escherichia coli* proveniente de la comunidad multirresistente a los medicamentos.

Objetivo: Caracterizar aislamientos de *E. coli* multirresistente causantes de infecciones urinarias adquiridas en la comunidad en Cumaná, Venezuela, y detectar los posibles riesgos de infección por aislamientos productores de betalactamasas de espectro extendido (BLEE).

Materiales y métodos: Se incluyeron todos los pacientes atendidos en la consulta externa de urología y en urgencias del Hospital de Cumaná entre enero y junio de 2014 y que evidenciaban infecciones urinarias. La detección de los genes *bla*TEM, *bla*SHV y *bla*CTX-M se hizo mediante la reacción en cadena de la polimerasa (PCR).

Resultados: Se encontró una alta prevalencia de *E. coli* multirresistente a los medicamentos (25,2 %), con 20,4 % de aislamientos productores de BLEE y una gran frecuencia de resistencia simultánea a trimetoprim-sulfametoxazol, fluoroquinolonas y aminoglucósidos (66,7 %) comparados con los no productores (2,4 %). En el 65,4 % de los aislamientos resistentes, se encontró el gen *bla*TEM; en 34,6 %, el *bla*CTX-M, y en 23,1 %, el *bla*SHV. Los genes *bla*CTX-M detectados pertenecían a los grupos CTX-M-1 y CTX-M-2. Se demostró la transferencia *in vitro* de plásmidos por conjugación en 17 de los 26 aislamientos productores de BLEE. Los tres tipos de genes detectados se transfirieron a los transconjugantes. La edad mayor de 60 años, las infecciones urinarias con complicaciones y el uso previo de catéter, predispusieron a la infección por cepas de *E. coli* productoras de BLEE.

Conclusiones: La gran frecuencia de aislamientos multirresistentes productores de BLEE debería alertar a las autoridades sanitarias para tomar medidas que reduzcan el riesgo de epidemias causadas por este tipo de bacterias en la comunidad.

Palabras clave: Escherichia coli, enfermedades urológicas, beta-lactamasas, farmacorresistencia bacteriana, infecciones comunitarias adquiridas.

Urinary tract infections affect people of all ages and are one of the most frequently treated pathologies in primary healthcare centers. Although urinary infections can be caused by several species in the Enterobacteriaceae, most cases (both community and hospital-acquired) are produced by *E. coli* [1,2]. Infection rates by extended-spectrum β-lactamases (ESBL)-producing *E. coli* have increased in the last 10 years in Latin America, with some regions showing prevalences of up to 32%. In addition, 11 to 25% of these ESBL-producing *E. coli* infections are now resistant to third generation cephalosporins including important rates of multidrug resistance, and only the antibiotics carbapenems, tigecycline and colistin are showing susceptibility rates >90% [3].

ESBL-producing strains are also often resistant to antibiotics of other classes (sulfonamides, aminoglycosides, quinolones) thus complicating treatment strategies. The screening of ESBL-producing bacteria by antibiotic susceptibility testing is challenging, hence detection of specific genes by PCR is commonly used for final confirmation. The most common ESBL are the SHV, TEM, and CTX-M types, and the association of these three types of enzymes with *E. coli* infections has been investigated in many studies. CTX-M-type enzymes have been shown to be the most prevalent ESBL [4] and have been associated with numerous

outbreaks of infections both in hospitals and in the community, and particularly, along with the SHV and TEM types, in urinary *E. coli* strains isolated from nonhospital settings [5]. This has resulted in a change in epidemiological patterns with ESBL-producing strains appearing outside the hospital environment, thus converting them into an emerging public health problem [2,6]. The frequencies of ESBL found in South America rank amongst the highest in the world with CTX-M dominant possibly due to poorer social and economic situations, hospital overcrowding, lack of antimicrobial stewardship, excessive over-the-counter antibiotic usage, and under-supported infection control practices [6].

In Venezuela, there are several reports of infections by ESBL-producing Enterobacteriaceae species from Caracas [7], Cumaná [8], Ciudad Bolívar [9] and Mérida [10], and the reports from Ciudad Bolívar and Mérida also describe ESBL-producing isolates detected from communities. In this context and given the increasing probability of dissemination of ESBL-producing strains in the community, the aim of the present study was to characterize multidrug-resistant *E. coli* isolates causing community-acquired urinary tract infections and the risk factors associated with the presence of ESBL in Cumaná, Venezuela.

Materials and methods

Bacterial isolates and patients

We evaluated a total of 103 *E. coli* isolates from patients attending the urology outpatient consultation and the emergency unit from January 1st through June 30th, 2014, at the *Hospital Universitario "Antonio Patricio de Alcal*á*"* in Cumaná, Estado Sucre, Venezuela. Urine samples from patients from both genders (20 males and 83 females) and different ages (ranging from 12 to 70 years) who showed urinary tract infection symptoms and indications for urine culture were examined for isolates. All patients were diagnosed with community-acquired urinary tract infection defined as previously suggested [11] as having abundant bacteria and polymorphonuclear leukocytes in the urine sample and positive urine culture with bacterial counts equal to or greater than 103 CFU/ml of urine when *E. coli* was identified. The urinary tract infections were classified as complicated or uncomplicated according to the guidelines established by the Infectious Diseases Society of America [12].

A community-acquired infection was defined according to the criteria proposed by Friedman, *et al.* [13], differentiating it from healthcare-associated infections and hospital-acquired infections, like an infection present at the moment of the consultation and when patients had not received any of the following: intravenous therapy at home, wound care or specialized nursing care administered by a healthcare agency, close family or friends, and self-administered intravenous medical therapy in the 30 days before the infection as well as those patients that did not attend a hospital or hemodialysis clinic, or received intravenous chemotherapy in

the previous 30 days, or had been hospitalized in an acute care hospital for two or more days in the previous 90 days, or resided in a nursing home or long-term care facility.

Microbial susceptibility tests and detection of ESBL

Antimicrobial susceptibility and interpretation were evaluated using the disk diffusion susceptibility test according to the Clinical Laboratory Standards Institute [14]. The antimicrobials tested (Oxoid Ltd., UK) were ampicillin (10 µg), cefalotin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (30 µg), ampiciliin-sulbactam (10/10 µg) amoxicillin-clavulanic acid (2:1) (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), tobramycin (10 µg) and trimethropim-sulfamethoxazole (25 µg).

ESBL phenotypes were confirmed using the combined disc method [14] using *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 as the control strains. Multidrug resistance was defined as those isolates that showed resistance to ≥1 agents in ≥3 antimicrobial categories according to the consensus of a group of international experts [15].

Detection of the genes coding for β-lactamases

DNA was isolated by extraction using the Wizard Genomic purification kit™ (Promega). Resistant genes were identified by means of a PCR assay for *bla*TEM, *bla*SHV and *bla*CTX-M using the primers described elsewhere [16-18]. The PCR products were electrophoresed in 2% agarose gel and stained with GelRed Nucleic Acid Stain™ (Biotium). A 100 bp DNA ladder (Fermentas, Lithuania) was used as the molecular weight marker.

Transfer of resistance

Conjugation assays were done in a solid medium using *E. coli* j62- 2 (resistant to rifampicin, CVCM 131) as the recipient [7]. The clinical and recipient strains were grown separately in Luria-Bertani broth (Hardy Diagnostics, Santa Maria, CA, USA) at 37°C for 18 hours, and then mixed in a ratio of 1:4 (clinical isolates: recipient). Transconjugants were selected on MacConkey agar containing 100 µg rifampicin and 2 µg cefalotin.

Differentiation of the CTX-M group by PCR-RFLP

Restriction enzyme digestion was carried out according to Mahboobeh [19].The members of the CTX-M group were identified according to the fragments generated by double digestion by the *Pst*I and *Pvu*II enzymes. *E. coli* 2944NR was used as an RFLP positive control since it contains

aCTX-M-1 type β-lactamase while *E. coli* J62-2 was used as a negative control. The RFLP products were observed in a 2% agarose gel containing GelRedNucleic Acid Stain™ (Biotium).

Statistical analyses

The risk factors considered in this study were: Nasogastric tube use, Foley catheter use, chronic kidney disease, cerebral vascular accident, bedridden, immunosuppression treatment, pregnancy, presence of any functional or structural urinary abnormality, diagnosis of a urinary tract infection within one year, antimicrobial treatment within three months, hospitalization within the previous three months but not within the previous 30 days, diabetes, and age over 60.

The risk factors associated with infection by an ESBL-producing *E. coli* isolate were determined firstly by using a binary logistic regression for each variable followed by multivariate analysis for those variables showing best relevance, in which the variables were considered as predictors of ESBL.

The analysis was performed using a stepwise selection of variables, and the area under the ROC curve was calculated to determine the model's predictive ability. The statistical analyses were carried out using the statistical package for the social sciences (SPSS™, version 18, IBM Corporation).

Ethical aspects

The patients gave their written consent after they were informed of the risks and benefits of participating in the study and had answered an epidemiological questionnaire.

The treatment of the patients, the analysis of the isolates, and the information generated were conducted according to the bioethical and biosafety guidelines set out by the *Comisión de Ética, Bioética y Biodiversidad* of the *Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas «Dra. Susan Tai"* at the *Universidad de Oriente.*

Results

The *E. coli* isolates (n=103) evaluated in this study showed high rates of resistance for the antimicrobials frequently used to treat patients with urinary infections, such as ampicillin (96.1%), cefalotin (59.4%) and ampicillin-sulbactam (24.8%), amoxicillin-clavulanate (25.7%), trimethoprim-sulfamethoxazole (39.6%) and ciprofloxacin (29.7%).

The isolates from the uncomplicated urinary tract infections (30.1%) showed significantly lower resistance compared to those from complicated urinary tract infections (figure 1).
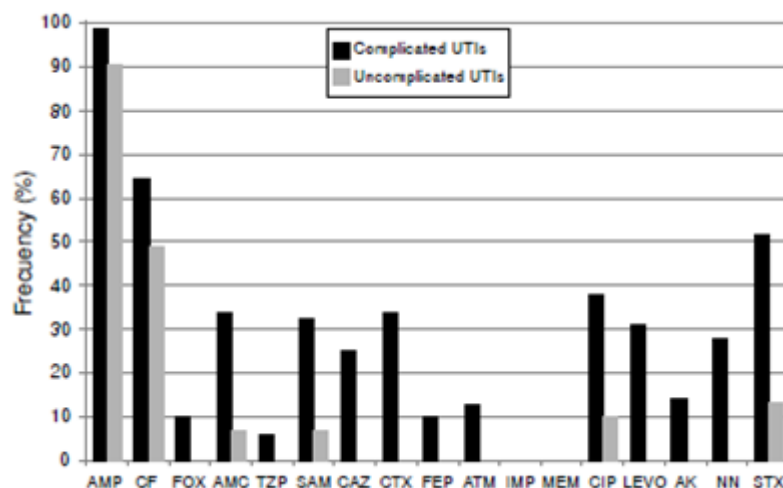
Figure 1

CF Frequency of resistant *Escherichia coli* isolates from patients with community-acquired urinary tract infections AMP: Ampicillin CF: Cefalotin; FOX: Cefoxitin; AMC: Amoxacillin-clavulanic acid; TZP: Piperacillin-tazobactam; SAM: Ampicillin-sulbactam; CAZ: Ceftazidime; CTX: Cefotaxime; FEP: Cefepime; ATM: Aztreonam; IMP: imipenem; MEM: meropenem; CIP: Ciprofloxacin; LEVO: Levofloxacin; AK: Amikacin; STX: Trimethoprim-sulfamethoxazole Note: Isolates with intermediate resistance were classified as resistant.

A total of 26 (25.2%) isolates were multidrug resistant (table 1), of which 21 (20.4%) showed production of ESBL. Of the 26 multidrug resistant isolates, 18 showed resistance to six or more antimicrobial categories, and the categories most associated with resistance were penicillins, cephalosporins, both with and without extended-spectrum, and folate pathway inhibitors (table 1). ESBL-producing isolates also showed a high frequency (66.7%) of simultaneous resistance to trimethoprim-sulphamethoxazole, fluoroquinolones and aminoglycosides compared to non-producing isolates (2.4%).

Table 1

Phenotypic and molecular characteristics of multidrug resistant *Escherichia coli* isolates. Here we show the DDST phenotypes of the bacterial isolates from patients with community-acquired urinary tract infections and their transconjugants, when appropriate, as well as the genes detected by PCR.

Isolates	ESBL	Resistance	Bla genes	Resistance in transconjugants	Bla genes transferred
EC14	+	AMP, CF, CAZ, CTX, STX	bla_{TEM}		
EC33	-	AMP, CF, FOX, CTX, ATM, CIP, LEVO, STX	bla_{SHV}		
EC37	-	AMP, CF, CTX, CIP, STX	-		
EC49	-	AMP, CF, AMC, CAZ, CTX, ATM, CIP, LEVO, AK, TOB, STX,	bla_{TEM}		
EC95	+	AMP, CF, AMC, CAZ, CTX, CIP, LEVO, STX	bla_{SHV}		
EC190	-	AMP, CF, CAZ, NAL, CIP, LEVO, STX	bla_{TEM}		
EC305	-	AMP, CF, AMC, CTX, ATM, SAM, CIP, LEVO, AK, TOB, STX	bla_{TEM}		
EC372	+	AMP, CF, CAZ, CTX, FEP, CIP, STX	bla_{TEM}		
EC919	+	AMP, CF, CAZ, CTX, FEP, CIP, LEVO	bla_{TEM}		
EC16	+	AMP, CF, CAZ, CTX, SAM, STX	bla_{TEM}	AMP, CF, CAZ, CTX, STX	blaTEM
EC30	+	AMP, CF, CTX, SAM, CIP, TOB, STX	bla_{TEM}, bla_{CTX-M}	AMP, CF, CTX, TOB, STX	blaTEM, blaCTX-M
EC61	+	AMP, CF, AMC, FOX, CAZ, CTX, SAM, CIP, LEVO, AK, TOB, STX	bla_{TEM}, bla_{CTX-M}	AMP, CF, CTX, AK, TOB, STX	blaTEM, blaCTX-M
EC79	+	AMP, CF, CTX, SAM, CIP, LEVO, TOB, STX	bla_{TEM}	AMP, CF, CTX, TOB, STX	blaTEM
EC94	+	AMP, CF, CTX, SAM, CIP, TOB, STX	bla_{TEM}	AMP, CF, CTX, STX	blaTEM
EC129	+	AMP, CF, CAZ, CTX, FEP, CIP, LEVO, TOB, STX	bla_{SHV}	AMP, CF, CAZ, CTX, FEP, TOB, STX	blaSHV
EC204	+	AMP, CF, AMC, CTX, ATM, CIP, LEVO, TOB, STX	bla_{TEM}, bla_{SHV}, bla_{CTX-M}	AMP, CF, CTX, TOB, STX	blaTEM, blaCTX-M
EC339	+	AMP, CF, AMC, FOX, CAZ, CTX, ATM, SAM, CIP, LEVO, AK, TOB, STX	bla_{TEM}, bla_{SHV}, bla_{CTX-M}	AMP, CF, CAZ, CTX, ATM, AK, TOB, STX	blaTEM, blaCTX-M
EC347	+	AMP, CF, AMC, CAZ, CTX, FEP, SAM, CIP, LEVO, AK, TOB	bla_{CTX-M}	AMP, CF, CAZ, CTX, FEP, NAL, CIP, AK, TOB	blaCTX-M
EC349	+	AMP, CF, AMC, CAZ, CTX, FEP, SAM, CIP, LEVO, TOB, STX	bla_{TEM}, bla_{SHV}	AMP, CF, CAZ, CTX, FEP, TOB, STX	blaTEM, blaSHV
EC395	+	AMP, CF, AMC, FOX, CAZ, CTX, ATM, SAM, CIP, LEV, TOB	-	AMP, CF, CAZ, CTX, TOB	-
EC514	+	AMP, CF, AMC, FOX, CAZ, CTX, ATM, SAM, CIP, LEVO, TOB, STX	bla_{CTX-M}	AMP, CF, CAZ, CTX, ATM, TOB, STX	blaCTX-M
EC779	+	AMP, CF, CAZ, CIP, LEVO, STX	-	AMP, CF, CAZ	-
EC869	+	AMP, CF, AMC, CTX, CIP, LEVO, STX	bla_{TEM}, bla_{CTX-M}	AMP, CF, AMC, CTX	blaTEM, blaCTX-M
EC876	+	AMP, CF, CAZ, CTX, PTZ, FEP, CIP, LEVO, AK, TOB, STX	bla_{TEM}	AMP, CF, CAZ, CTX, FEP, AK, TOB, STX	blaTEM
EC926	+	AMP, CF, AMC, CAZ, CTX, ATM, SAM, CIP, LEVO, AK, TOB, STX	bla_{TEM}, bla_{CTX-M}	AMP, CF, CAZ, CTX, NAL, CIP, AK, TOB, STX	blaTEM, blaCTX-M
EC983	+	AMP, CF, AMC, CAZ, CTX, ATM, FEP, CIP, LEVO, AK, TOB, STX	bla_{CTX-M}	AMP, CF, CAZ, CTX, ATM, FEP, AK, TOB, STX	blaCTX-M

ESBL: Extended-spectrum β-lactamases; AMP: Ampicillin; CF: Cefalotin; AMC: Amoxacillin-clavulanic acid; FOX: Cefoxitin; CAZ: Ceftazidime; CTX: Cefotaxime; ATM: Aztreonam; SAM: Ampicillin-sulbactam; TZP: Piperacillin-tazobactam; FEP: Cefepime; CIP: Ciprofloxacin; LEVO: Levofloxacin; AK: Amikacin; TOB: Tobramycin; STX: Trimethoprim-sulfamethoxazole

The *bla*TEM gene was found in 17 isolates (65.4%) while *bla*CTX-M was detected in nine isolates (34.6%) and *bla*SHV in six isolates (23.1%). The *bla*TEM and *bla*SHV genes were found in *E. coli* isolates, ESBL-producing or not, while *bla*CTX-M was only found in ESBL-producing isolates. More than one gene was amplified in seven isolates: The most frequent combination was *bla*TEM + *bla*CTX-M (four isolates), and a three-gene combination was found in two ESBL producing isolates (table 1). The RFLP results revealed that five of the *bla*CTX-M genes were identified as belonging to the CTX-M-2 group and four to the CTX-M-1 group (figure 2).
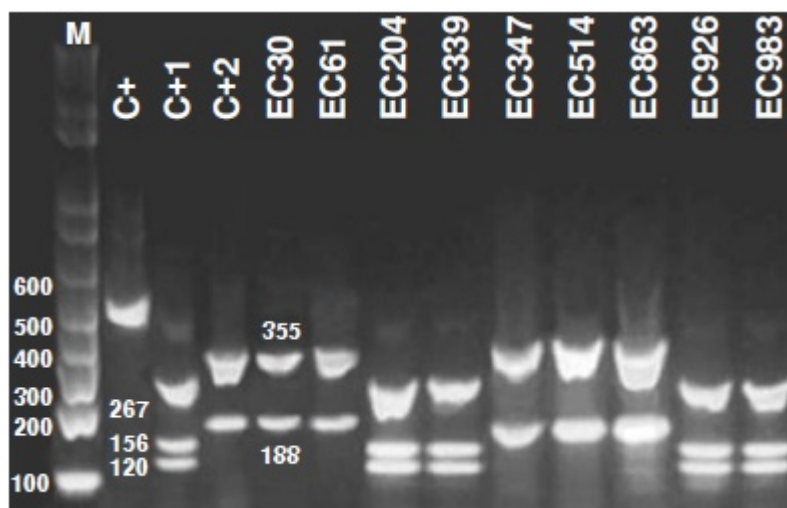
Figure 2

Digestion profile of the *bla*CTX-M gene detected in *Escherichia coli* isolated from patients with community-acquired urinary infections M: Molecular weight marker (1 kb Invitrogen); C+: Undigested blaCTX-M-2 fragment; C+1: Control blaCTX-M-1, strain 2944NR; C+2: Control blaCTX-M-2 Kp 22; lines 5-13: Isolates of *Escherichia coli* containing the blaCTX-M gene

Of the 26 multidrug resistant isolates resistant to third generation cephalosporins and subjected to *in vitro* bacterial conjugation, only 17 were able to transfer plasmids with resistance genes at a mean transfer rate of 10-7clinical isolates-transconjugant. Phenotypic analysis of the transconjugants demonstrated that part of the resistance pattern present in the clinical isolates was also observed in the transconjugants (table 1). All of the *bla*CTX-M genes were transferred to the transconjugant strain from the clinical isolates while only 64.7% (11/17) of the *bla*TEMgene and 33.3% (2/6) of the *bla*SHV were transferred (table 1). In the transconjugants of the two clinical isolates containing the three genes (204 and 339), the *bla*SHV gene was not transferred. These results demonstrate the transmissibility of *bla* genes to other *E. coli* strains.

When analyzing the risk factors for ESBL-producing *E. coli*, we found that an age over 60 years, complicated urinary tract infection, and urinary catheterization (more than 30 days previous to the infection) predisposed patients to an infection by a multidrug resistant/ESBL-producing *E. coli* strain (table 2) with relative risks (RR) 3.1 (95% CI: 1.5-6.4), 4.1 (95% CI: 1.1- 16.5),and 3.3 (95% CI: 1.6-6.8) times higher, respectively, for patients with those conditions compared to those without them.

Table 2

Epidemiological and clinical variables associated with ESBL-producing *Escherichia coli*

Risk factors	n	ESBL + (n=21)	ESBL - (n=82)	P	RR (95% CI)
Male gender	20	9	11	0.962	
Age >60 years	20	9	11	0.002*	3.1 (1.5-6.4)
Complicated urinary tract infection	72	19	53	0.048	4.1 (1.1-16.5)
Recurrent urinary tract infection	38	5	33	0.183	
Previous (30 days) urinary catheterization	11	6	5	0.001*	3.3 (1.6-6.8)
Previous empiric treatment with first line beta-lactams[a,b]	35	13	28	0.134	
Treatment with extended spectrum cephalosporins[a,c]	28	5	23	0.313	
Renal lithiasis	32	7	25	0.603	
Renal impairment	12	2	10	0.493	
Diabetes	29	9	20	0.389	

ESBL: Extended spectrum β-lactamases; RR: Relative risk; CI: Confidence interval

* Statistically significant

a Within three months

b First line β-lactams: AMP, SAM, and CF

c Extended spectrum cephalosporins: 3rd and 4th generation cephalosporins and monobactams (ATM)

Other risk factors, such as nasogastric tube use, cerebral vascular accident, bedridden patients, immunosuppression treatment, pregnancy, the presence of a structural urinary abnormality, and hospitalization within the previous three months were not analyzed since no ESBL-producing isolate was found in any of the patients showing these conditions.

Of the 20 male patients with complicated urinary tract infections, seven were aged over 60 with functional problems due to their prostate or kidneys and frequently attended the urology consultation for their control. All seven patients had urinary tract infections caused by ESBL-producing isolates. Of the 83 female patients with urinary tract infections, 53 were classified as complicated due to any of the abovementioned factors but with recurrent urinary tract infections within a year being the most frequent condition.

Discussion

The resistance of *E. coli* isolates from complicated urinary tract infections to many of the antimicrobials tested here indicates that treatment should only be given after susceptibility testing. High resistant rates to third-generation cephalosporins and aztreonam of clinical isolates of another bacteria, *K. pneumoniae*, have also been found in a previous study undertaken in Cumaná [12]. The relatively high frequency of multidrug resistant *E. coli* found in the community and the high proportion of isolates showing resistance to ≥6 antimicrobials indicates the need to revise the treatment guidelines for urinary tract infections in Venezuela. A significant increase in the prevalence of multidrug resistant *E. coli* has also been reported in the US with rates raising from 9.1% in 2001 to 17.0% in 2010 [20] and resistance to three, four or five antimicrobial

agents. In the same study, widespread resistance was observed for trimethoprim-sulfamethoxazole (62.6%, 88.6%, and 97.9%, respectively) and ciprofloxacin (48.9%, 84.3%, and 98.2%, respectively).

Uncomplicated community-acquired urinary tract infections are normally treated empirically without undertaking a urine culture or an antibiogram unless they recur repeatedly [21]. Our findings, however, demonstrate the importance of monitoring this empirical use of antimicrobials at a community level as recommended by the Infectious Diseases Society of America in order to select an appropriate empirical treatment [22].

β-lactam antibiotics are commonly the first choice of treatment against many microorganisms including uropathogens. Nevertheless, in recent years there has been a progressive increase in the frequency of *E. coli* strains from outpatients that characteristically show reduced susceptibility to penicillins and third generation cephalosporins due to the production of ESBL [23]. This is now considered an emergent problem at the community level especially as changes in epidemiological patterns have been observed: While SHV and TEM have been shown to be the most prevalent enzymes in nosocomial strains, CTX-M is reported as predominant in community-acquired strains [24].

In this study, phenotypic characterization revealed that an important fraction of the isolates studied produced ESBLs. Here, the *bla*TEM gene was the most prevalent ESBL gene indicating the possible circulation of TEM-type ESBLs in strains from the community that have not been detected in nosocomial strains from the *Hospital General de Cumaná* [12]. We also found variations in the antibiotic resistance of isolates containing only *bla*TEM, which may suggest the presence of clavulanic-acid inhibited ESBLs not characterized in this study, or the presence of other *bla*TEM type genes. The non-ESBL-producing isolates that did contain the *bla*TEM gene are probably TEM-1 that confers resistance to penicillins and first-generation cephalosporins. Resistance to third-generation cephalosporins could then be due to other resistance mechanisms, for example, OXA-type enzymes which have been identified from some *E. coli* strains isolated from patients with community-acquired urinary tract infections in India [25]. The *bla*SHV gene has been found to be less frequent in community-based strains than *bla*TEM and *bla*CTX-M [26].

The identification of the specific CTX-M enzyme type from the susceptibility pattern proved to be difficult due to the overlapping of the ceftazidime and cefotaxime phenotypes and the fact that several genes were present. The wide dissemination of CTX-Ms around the world has been referred to as the "CTX-M pandemic" since this enzyme is the most common β-lactamase carried by the Enterobacteriaceae that cause community onset urinary tract infections as shown in reports from different continents [2,24,27-31].

In Venezuela, there have been very few reports that identify the type of *bla* genes found in bacterial strains causing urinary infections. The CTX-

M-32 enzyme (CTX-M-1) was identified in an *E. coli* strain isolated from a patient with diabetes from the Mérida state who was also suffering from a community-acquired urinary tract infection, which is the first report of this variant in South America [32].

It is important to note that some CTX-M enzymes are associated with specific countries: CTX-M-9 in Spain; CTX-M-1 in Italy and the United Kingdom, and CTX-M-2 in Israel, Japan, and most South American countries [24,33]. In contrast, variants such as CTX-M-15 (CTX-M-1), which originated from the Asp240Gly substitution in CTX-M-3 and CTX-M-14 have been detected all over the world [24,34]. The seasonal appearance and spread of these enzymes in different habitats may partially explain their epidemiology. The use of antimicrobials and other risk factors in different regions, as well as the characteristics of different geographical compartments, may have contributed to the current situation surrounding CTX-M.

The *in vitro* bacterial conjugation assays demonstrated that the genes coding for ESBL are localized in transferable plasmids which also carry other resistance determinants affecting antimicrobials, such as aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones. Furthermore, the transfer of resistance to ciprofloxacin and nalidixic acid indicates that these strains contain a resistance mechanism against this group of antimicrobials that can disseminate horizontally as well as vertically. In India, 94% of the CTX-M-producing isolates were able to transfer their genes by conjugation with plasmids ranging in size from 120 to 272 kbp and half of these plasmids also carried the TEM-1 gene [25]. This dissemination of ESBL in conjugative plasmids increases the possibility of an accelerated rate of proliferation [2].

Few studies have evaluated risk factors for community-onset ESBL infections and some of the studies previously undertaken in the U.S.A. were conducted prior to the emergence of community-wide multidrug-resistant *E. coli* (35). Here we identified age over 60 years, complicated urinary tract infections, and urinary catheterization as risk factors for infection with ESBL-producing *E. coli* strains. Other studies have also shown older age [35-40], urinary catheterization [36,37,39] and complicated urinary tract infections [40] as factors that increase the risk of acquiring a urinary tract infection by multidrug resistant bacteria.

In conclusion, our results reveal the presence of multidrug resistant isolates that cause community-acquired urinary infections. This is of great epidemiological importance and requires the intervention of the health authorities in the region to prevent further dissemination of these types of strains.

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References

1. Laupland KB, Ross T, Pitout JD, Church DL, Gregson DB. Community-onset urinary tract infections: A population-based assessment. Infection. 2007;35:150. https://doi.org/10.1007/s15010-007-6180-2

2. Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: The worldwide emergence of clone ST131 O25: H4. Int J Antimicrob Agents. 2010;35:316-21. https://doi.org/10.1016/j.ijantimicag.2009.11.003

3. Guzmán-Blanco M, Labarca JA, Villegas MV, Gotuzzo E. Extended spectrum β-lactamase producers among nosocomial Enterobacteriaceae in Latin America. Braz J Infect Dis. 2014;18:421-33. https://doi.org/10.1016/j.bjid.2013.10.005

4. Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: Origin and diffusion. Front Microbiol. 2012;3:110. https://doi.org/10.3389/fmicb.2012.00110

5. Bonnet R. Growing group of extended-spectrum β-lactamases: The CTX-M enzymes. Antimicrob Agents Chemother. 2004;48:1-4. https://doi.org/10.1128/AAC.48.1.1-14.2004

6. Dhillon RH, Clark J. ESBLs: A clear and present danger? Crit Care Res Pract. 2012;2012:625170. https://doi.org/10.1155/2012/625170

7. Narváez P, Pedroza R, Alonso G, Rodríguez-Lemoine V. Caracterización de plásmidos de resistencia a antibióticos en aislados nosocomiales del Hospital Universitario de Caracas. Rev Soc Ven Microbiol. 2005;25:29-34.

8. Guzmán M, Alonso G. Caracterización de ß-lactamasas de espectro extendido (BLEE) en cepas nosocomiales de *K. pneumoniae*. Sucre-Venezuela. Invest Clin. 2009;50:419-31.

9. Guevara A, Machado S, Manrique E. Urinary infections acquired in community: Epidemiology, resistance to antibiotics and therapeutic options. Kasmera. 2011;39:87-97.

10. Hernández E, Araque M, Millán Y, Millán B, Vielma S. Prevalencia de β-lactamasa CTX-M-15 en grupos filogenéticos de *Escherichia coli* uropatógena aisladas en pacientes de la comunidad en Mérida, Venezuela. Invest Clin . 2014;55:32-43.

11. de Cueto M. La microbiología en el diagnóstico de la infección del tracto urinario. In: Pigrau C, editor. Infección del tracto urinario. Primera edición. Madrid: Salvat; 2013. p. 11-22.

12. Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis. 2005;40:643-54. https://doi.org/10.1086/427507

13. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, *et al*. Health care-associated bloodstream infections in adults: A reason to change the accepted definition of community-acquired infections. Ann

Intern Med. 2002;137:791-7. https://doi.org/10.7326/0003-4819-137-10-200211190-00007

14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-four informational supplement, M100-S24. Wayne, PA: CLSI; 2014. p. 226.

15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268-81. https://doi.org/10.1111/j.1469-0691.2011.03570.x

16. Eckert C, Gautier V, Saladin-Allard M, Hidri N, Verdet C, Ould-Hocine Z, *et al.* Dissemination of CTX-M- type beta-lactamases among clinical isolates of Enterobacteriaceae in Paris, France. Antimicrob Agents Chemother . 2004;48:1249-55. https://doi.org/10.1128/AAC.48.4.1249-1255.2004

17. Haeggman S, Löfdal S, Poauw A, Verhoef J, Brisse S. Diversity and evolution of the class A chromosomal beta-lactamase gene in *Klebsiella pneumoniae.* Antimicrob Agents Chemother . 2004;48:2400-8. https://doi.org/10.1128/AAC.48.7.2400-2408.2004

18. Eldestein M, Pimkin M, Palagin I, Eldestein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob Agents Chemother . 2003;47:3724- 32. https://doi.org/10.1128/AAC.47.12.3724-3732.2003

19. Mahboobeh N, Forghanifard M, Moshrefi S. Prevalence and molecular characterization of plasmid-mediated extended-spectrum β-lactamase genes (*bla*TEM, *bla*CTX and *bla*SHV) among urinary *Escherichia coli* clinical isolates in Mashhad, Iran. Iran J Basic Med Sci. 2012;15:833-9.

20. Sánchez GV, Baird AM, Karlowsky JA, Master RN, Bordon JM. Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary *Escherichia coli* from US outpatients. J Antimicrob Chemother. 2014;69:3259-62. https://doi.org/10.1093/jac/dku282

21. Alos JI. Epidemiología y etiología de la infección urinaria comunitaria. Sensibilidad antimicrobiana de los principales patógenos y significado clínico de la resistencia. Enferm Infecc Microbiol Clin. 2005;23:3-8. https://doi.org/10.1157/13091442

22. Spellberg B, Talbot G, Brass E, Bradley J, Boucher H, Gilbert D, *et al.* Position paper: Recommended design features of future clinical trials of antibacterial agents for community-acquired pneumonia. Clin Infect Dis . 2008;47(Suppl.3):S249-65. https://doi.org/10.1086/591411

23. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. Clin Inf Dis. 2001;32(Suppl.2):S94-103. https://doi.org/10.1086/320182

24. Canton R, Coque T. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol. 2006;9:466-75. https://doi.org/10.1016/j.mib.2006.08.011

25. Hussain A, Ewers C, Nandanwar N, Guenther S, Jadhav S, Wieler LH, *et al.* Multiresistant uropathogenic *Escherichia coli* from a region in India where urinary tract infections are endemic: Genotypic and

phenotypic characteristics of sequence type 131 isolates of the CTX-M-15 extended-spectrum-beta-lactamase-producing lineage. Antimicrob Agents Chemother . 2012;56:6358. https://doi.org/10.1128/AAC.01099-12

26. Ben-Ami R, Rodríguez-Baño J, Arslan H, Pitout JD, Quentin C, Calbo ES, *et al*. A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis . 2009;49:682-90. https://doi.org/10.1086/604713

27. Ahmed O, Alfadel O, Asghar H, Elhassan M. Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella* spp. urinary isolates from Sudan with confirmed ESBL phenotype. Life Sci J. 2013;10:191-5.

28. Radice M, Power P, Di Conza J, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. Antimicrob Agents Chemother . 2002;46:602-4. https://doi.org/10.1128/AAC.46.2.602-604.2002

29. Wang H, Kelkar S, Wu W, Chen M, Quinn J. Clinical isolates of Enterobacteriaceae producing extended-spectrum b-lactamases: Prevalence of CTX-M-3 at a hospital in China. Antimicrob Agents Chemother 2003;47:790-3. https://doi.org/10.1128/AAC.47.2.790-793.2003

30. Yamasaki K, Komatsu M, Yamashita T, Shimakawa K, Ura T, Nishio H, *et al*. Production of CTX-M-3 extended-spectrum beta-lactamase and IMP-1 metallo beta-lactamase by five Gramnegative bacilli: Survey of clinical isolates from seven laboratories collected in 1998 and 2000, in the Kinki region of Japan. J Antimicrob Chemother . 2003;51:631-8. https://doi.org/10.1093/jac/dkg103

31. Kung CH, Ku WW, Lee CH, Fung CP, Kuo SC, Chen TL, *et al*. Epidemiology and risk factors of community-onset urinary tract infection caused by extended-spectrum β-lactamase-producing Enterobacteriaceae in a medical center in Taiwan: A prospective cohort study. J Microbiol Immunol Infect. 2015;48:168-74. https://doi.org/10.1016/j.jmii.2013.08.006

32. Araque M, Millán B, Mendoza E, Salas M. CTX-M-32 beta-lactamase-producing uropathogenic *Escherichia coli* isolated in Latin America. J Infect Dev Ctries. 2013;7:432-5. https://doi.org/10.3855/jidc.3488

33. Coque T, Baquero F, Cantón R. Increasing prevalence of ESBL-producing enterobacteriaceae in Europe. Eurosurveillance. 2008;47:19-29. https://doi.org/10.2807/ese.13.47.19044-en

34. Rossolini G, D'Andrea M, Mugnaioli C. The spread of CTX-M-type extended-spectrum beta-lactamases. Clin Microbiol Infect . 2008;14:33-41. https://doi.org/10.1111/j.1469-0691.2007.01867.x

35. Banerjee R, Strahilevitz J, Johnson JR, Nagwekar PP, Schora DM, Shevrin I, *et al*. Predictors and molecular epidemiology of community-onset extended-spectrum β-lactamase-producing *Escherichia coli* infection in a Midwestern community. Infect Control Hosp Epidemiol. 2013;34:947-53. https://doi.org/10.1086/671725

36. Blanco VM, Maya JJ, Correa A, Perenguez M, Muñoz JS, Motoa G, *et al*. Prevalencia y factores de riesgo para infecciones del tracto urinario de inicio en la comunidad causadas por *Escherichia coli* productor de betalactamasas de espectro extendido en Colombia. Enferm Infecc

Microbiol Clin . 2016;34:559-65. https://doi.org/10.1016/j.eimc.2015.1 1.017

37. Kronenberg A, Hilty M, Endimiani A, Muhlemann K. Temporal trends of extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in in- and outpatients in Switzerland, 2004 to 2011. Euro Surveill. 2013;18:20484. https://doi.org/10.2807/ese.18.21.20484 -en

38. Anesi JA, Lautenbach E, Nachamkin I, Garrigan C, Bilker WB, Wheeler M, *et al*. Clinical and molecular characterization of community-onset urinary tract infections due to extended-spectrum cephalosporin-resistant Enterobacteriaceae. Infect Control Hosp Epidemiol . 2016;37:1433-9. h ttps://doi.org/10.1017/ice.2016.225

39. Bours PH, Polak R, Hoepelman AI, Delgado E, Jarquin A, Matute AJ. Increasing resistance in community-acquired urinary tract infections in Latin America, five years after the implementation of national therapeutic guidelines. Int J Infect Dis. 2010;14:e770-4. https://doi.org/10.1016/j.ij id.2010.02.2264

40. Seija V, Fratchez V, Ventura V, Pintos M, González M. Factores asociados al desarrollo de infección urinaria de origen comunitario causada por *Escherichia coli* resistente a fluoroquinolonas. Rev Chilena Infectol. 2014;31:400-5. https://doi.org/10.4067/S0716-10182014000400004

Notes

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Conflict of interest declaration

| Conflict of interest | None of the authors report any conflicts of interest. |