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Characterization of *Escherichia coli* clinical isolates causing urinary tract infections in the community of Chilpancingo, Mexico

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Summary. *Escherichia coli* is the main cause of urinary tract infections (UTI) in ambulatory patients, especially strains belonging to the B2 phylogenetic group and ST131 clonal group. Antibiotic treatment is usually administered empirically; however, it is not always effective due to bacterial multidrug resistance and the production of extended spectrum β-lactamases (ESBLs). The aim of this study was to characterize *E. coli* clinical isolates from patients with UTI in a community of the State of Guerrero, Mexico. From January to August 2014, 134 clinical isolates of *E. coli* were recovered. Strain identification and antibiotic susceptibility were performed using the Vitek automated system. Phylogenetic and O25b-ST13 groups were determined by multiple PCR. Identification of the bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes was performed by conventional PCR. We found that over 50% of the isolates were resistant to betalactams and quinolones, while 0 to 33% were resistant to aminoglycosides and nitrofurans, and 56.49% of the strains were ESBL producers. B2 phylogenetic group was the most predominant (43%) compared to the other groups. The prevalence of *bla* genes was: bla_{CTX-M} 64.3%, bla_{SHV} 41.4%, and bla_{TEM} 54.3%. These results show a high percentage (55%) of multidrug-resistant strains isolated from UTI patients from the community in the city of Chilpancingo, Guerrero, Mexico. [**Int Microbiol** 19(4): 209-215 (2016)]

Keywords: *Escherichia coli* · urinary tract infections · β-lactamases · multidrug resistance · Chilpancingo (Mexico)

Introduction

Urinary tract infections (UTIs) are defined by the presence of bacterial pathogens in the urinary tract. They are the second most prevalent infectious disease, comprising around one fourth of all infections [13]. In Mexico, they are the first cause of medical outpatient consultation of women in reproductive age, and in 2010 they were the third cause of morbidity [4]. UTIs are classified according to the site of infection, in uncomplicated and complicated [25]. In most cases, uncomplicated UTIs are treated effectively by empirical antibiotic therapy, without performing urine culture unless the empirical therapy fails. This empirical therapy, coupled with the high

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rate of infections per year worldwide, and the indiscriminate use of antibiotics, has led to an increase in antibiotic resistance in UTI-causing bacteria [1]. Thus, treatments have become more complex, since there has been an increase in antibiotic resistance along with the occurrence of extended-spectrum β-lactamases (ESBLs) [21].

ESBLs are plasmid-encoded enzymes produced by many Gram-negative bacteria. They confer resistance to penicillins, broad-spectrum cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime) and monobactams (e.g., aztreonam), but not to cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem). They are usually located on large plasmids, which also carry genes for resistance to other antibiotics, including fluoroquinolones, aminoglycosides, and cotrimoxazole. These β-lactamases, unlike AmpC β-lactamases, are inhibited by clavulanic acid, tazobactam or sulbactam [19,29]. ESBL-producing strains also show co-resistance to aminoglycosides, fluoroquinolones, tetracyclines, nitrofurantoin, and trimethoprim-sulfamethoxazole [30]. The most common β-lactmases are the TEM and SHV types, which are mainly expressed in *Escherichia coli* and *Klebsiella pneumoniae*, respectively, and also the CTX-M, which was described later [3].

Escherichia coli is the leading cause of urinary tract infections, it being responsible for 75−90% of UTIs in ambulatory patients [24]. This bacterium has been classified into seven main phylogenetic groups (A, B1, B2, C, D, E, and F), and one *Escherichia* cryptic clade I, based on the combination of four genetic markers: *arpA*, *chuA*, *yjaA*, and the DNA fragment TspE4.C2 [7]. Uropathogenic strains usually belong to groups B2 and to a lesser extent to group D, whereas commensal strains belong to groups A and B1 [2,27]. Among B2 pathogenic strains, *E. coli* sequence type 131 (ST131) is considered an emerging important pathogen. Strains belonging to this group are resistant not only to most β-lactam antibiotics, but also to aminoglycosides and fluoroquinolones [17]. Most ST131 strains belong to the O25:H4 serotype, they having the specific O25b type. However, ST131 strains with serotype O16:H5 have been recently identified, as well as some others that are non typeable for O and H antigens [11,20].

The aim of this work was to analyze a total of 134 *E. coli* clinical isolates from ambulatory patients with UTI from the community in the city of Chilpancingo, Guerrero, Mexico in order to determine their antibiotic susceptibility, ESBLs production and phylogenetic group.

Material and methods

***Escherichia coli* isolates and antibiotic susceptibility testing.** Clinical urine samples from 131 ambulatory patients from the ISSSTE clinic in Chilpancingo, Guerrero, Mexico where analyzed. Samples were collected from January to August of 2014 and only one isolate per patient was examined. Identification of isolates and antimicrobial susceptibility testing was performed by Vitek automated system. Antibiotics assayed were: ampicillin, ampicillin/sulbactam, cefazolin, ceftriaxone, cefepime, aztreonam, amikacin, gentamicin, tobramycin, ciprofloxacin, moxifloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole. Isolates resistant to antibiotics of three or more different classes were classified as multidrug-resistant (MDR).

ESBLs production. ESBLs production was confirmed by the double-disk synergy test (DDST) following the CLSI guidelines [Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 16th informational supplements. CLSI Document M2-A9, Wayne PA: 2006]. Disks containing ceftazidime (30 µg) or cefotaxime (30 µg) with or without 10 µg clavulanic acid were used. The plates were incubated at 37 °C for 18 h. *Escherichia coli* strain ATCC 25922 was used as negative control, and *K. pneumoniae* strain ATCC 700603 was used as positive control.

Conventional phylogenetic grouping. Extraction of total bacterial DNA from isolated *E. coli* colonies was performed by heat shock, boiling 2 to 3 colonies resuspended in 100 µl of distilled water for 5 min. Samples were cooled in ice for 5 min, boiled again, and centrifuged at 10,000 rpm for 2 min. The supernatant was recovered and stored at −20 °C until use. Assignment of *E. coli* phylogenetic groups was performed using the quadruplex phylo-group assignment PCR assay described by Clermont et al. [7] using primers for genes *arpA*, *chuA* and *yjaA*, and the TspE4.C2 DNA fragment. PCR was performed in a final volume of 20 µl using 2.5 U of Taq polymerase, 2 mM dNTP's, 25 mM $MgCl_2$, 2 µl 10X buffer, 20 pmol of each primer, and 200 ng of DNA. The conditions used were: 5 min at 94 °C (1 cycle), 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C (30 cycles), 7 min at 72 °C. The amplicons were analyzed by 2% agarose gel electrophoresis.

The detection of O25b/ST131 clonal group was done only with isolates belonging to the B2 phylogenetic group by multiplex PCR using primers O25pabBspe.F (5′-TCCAGCAGGTGCTGGATCGT-3′) and O25pabBspe.R (5′-GCGAAATTTTTCGCCGTACTGT-3′) [8] , and rfb1bis.f (5′-ATACCGACGACGCCGATC-3′) and rfbO25b.r (5′-TGCTATTCATTATGCGCAGC-3′) [9]. PCR was performed as follows: 2 min at 94 °C (1 cycle), 30 s at 94 °C, 10 s at 60 °C, 30 s at 72 °C (30 cycles), 2 min at 72 °C. The amplicons were analyzed by 2% agarose gel electrophoresis.

Identification of *bla* genes. The identification of *bla* genes was performed by PCR using specific primers for TEM, SHV and CTX-M types: TEMF (5′-CCTTCCTGTTTTTGCTCACCCA-3′), TEMR (5′-TACGATACGGGAGGGCTTAC-3′) [16], SHVF (5′-ATGCGTTATATTCGCCTGTG-3′), SHVR (5′-TTAGCGTTGCCAGTGCTCGAT-3′) [18] CTX-M/F′ (5′-TTTGCGATGTGCAGTACCAGTAA-3′), and CTX-M/R′ (5′-CGATATCGTTG GTGGTGCCATA-3′) [12]. PCR was performed in a final volume of 25 µl using 5 U of Taq polymerase, 10 mM dNTPs, 25 mM $MgCl_2$, 10X buffer, 10 pmol of each primer and 5 µl of DNA. The amplicons were analyzed by 2% agarose gel electrophoresis. *Escherichia coli* strain ATCC 25922 was used as negative control, ESBLs-producing *E. coli* strains carrying genes SHV-2, TEM-1 and CTX-M-15 were used as positive controls.

Conjugation experiments and plasmid isolation. A resistance transfer experiment using a conjugation assay based on the method described by Miller (1992) was performed for 5 strains carrying ESBLs genes. Sodium azide-resistant *E. coli* J53 was used as the recipient strain. Transconjugants were selected in selective media containing sodium azide (150 µg/ml) (Sigma) plus ampicillin (AMP) or ceftazidime (CAZ), and replicated afterwards in media M9 and MM. Plasmid DNA was extracted from clinical isolates and transconjugants by the method described by Kieser [15]. Purification of the 6-kb plasmids was performed with the Zymo Plasmid Miniprep kit according to the manufacturer's instructions. DNA was visualized after electrophoresis in 1% agarose gels stained with ethidium bromide.

Minimum inhibitory concentration (MIC). The isolates that were positive for ESBLs production were confirmed by the MIC reduction test using the broth dilution method according to the CLSI guidelines. The ranges of concentrations tested were 256–0.03 µg/ml for CAZ, and 256–0.015 µg/ml for CTX in Mueller-Hinton broth. Cut points: CTX-S (sensitive): ≤8; I (intermediate): 10–32; R (resistant): ≥64; CAZ-S: ≤8; I: 16; R: ≥32. Plates were incubated at 35 °C for 18 h. The *E. coli* ATCC 25922 strain was used as a control to validate susceptibility tests.

Statistical analysis. All statistical analyses were performed using the Stata-Transfer V.12.0 software. Correlation between ESBLs production and antibiotic susceptibility was analyzed with the Chi-squared test. Phylogenetic

Table 1. Correlation between antibiotic susceptibility patterns and ESBL production of isolated *Escherichia coli* strains

Antibiotic resistance		ESBL– n (%)	ESBL+ n (%)	Total %	*P* value
Ampicillin	Susceptible	19 (15.2)	0 (0)	15.2	< 0.001*
	Resistant	36 (28.8)	70 (56)	84.8	
Ampicillin/Sulbactam	Susceptible	21 (16.8)	21 (16.8)	33.6	0.522
	Resistant	34 (27.2)	49 (39.2)	66.4	
Cefazolin	Susceptible	48 (38.4)	1 (0.8)	39.2	<0.001*
	Resistant	7 (5.6)	69 (55.2)	60.8	
Ceftriaxone	Susceptible	53 (42.4)	1 (0.8)	43.2	<0.001*
	Resistant	2 (1.6)	69 (55.2)	56.8	
Cefepime	Susceptible	52 (41.6)	1 (0.8)	42.4	<0.001*
	Resistant	3 (2.4)	69 (55.2)	57.6	
Aztreonam	Susceptible	52 (41.6)	2 (1.6)	43.2	<0.001*
	Resistant	3 (2.4)	68 (54.4)	56.8	
Gentamicin	Susceptible	48 (38.4)	36 (28.8)	67.2	<0.001*
	Resistant	7 (5.6)	34 (27.2)	32.8	
Tobramycin	Susceptible	51 (40.8)	34 (27.2)	68	<0.001*
	Resistant	4 (3.2)	36 (28.8)	32	
Ciprofloxacin	Susceptible	41 (32.8)	14 (11.2)	44	<0.001*
	Resistant	14 (11.2)	56 (44.8)	56	
Moxifloxacin	Susceptible	42 (33.6)	14 (11.2)	44.8	<0.001*
	Resistant	13 (10.4)	56 (44.8)	55.2	
Nitrofurantoin	Susceptible	49 (39.2)	64 (51.2)	90.4	0.634
	Resistant	6 (4.8)	6 (4.8)	9.6	
Trimethoprim/Sulfamethoxazole	Susceptible	26 (20.8)	17 (13.6)	34.4	0.008*
	Resistant	29 (23.2)	53 (42.4)	65.6	
Multiresistant	No MDR	42 (33.6)	14 (11.2)	44.8	<0.001*
	MDR	13 (10.4)	56 (44.8)	55.2	

* *P* value < 0.05, statistically significant.

groups and *bla* genes were compared using Fisher's exact test. All *P* values > 0.05 were considered statistically significant.

Results

Identification and susceptibility patterns. Of the 131 isolates tested, 4 were identified as *Escherichia fergusonii* and 2 as *E. albertii*, leaving us with 125 *E. coli* strains. Of the 125 strains isolated, 84.8% were resistant to ampicillin, 66.4% to ampicillin/sulbactam, 60.8% to cefazolin, 56.8% to ceftriaxone, 57.6% to cefepime, 56.8% to aztreonam, 32.8% to gentamycin, 32% to tobramycin, 56% to ciprofloxacin, 55.2% to moxifloxacin, and 65.6% to trimethoprim/sulfamethoxazole. Note that all of the isolates were sensitive to amikacin and carbapenems, while 90.4% were sensitive to nitrofurantoin. More than half of the isolates (55.2%) were classified as MDR, since they were resistant to 3 or more classes of antibiotics. To determine if the isolated strains produced ESBLs, a DDST assay was performed. Of the 125 isolated strains, 70 (56%) were positive for ESBLs production. The correlation between susceptibility rates of the isolated strains and ESBLs production are shown in Table 1. MDR is related to ESBLs production, obtaining statistically significant *P* values.

Phylogenetic grouping. Of the 125 clinical isolates ,12.8% belonged to group A, 6.4% to group B1, 43.2% to group B2, 10.4% to group C, 10.4% to group D, 2.4% to group E, 7.2% to group F, and 7.2% to clade I, group B2 being the most prevalent. We then analyzed strains in group B2 to determine if they belonged to the O25b-ST131 group. PCR results showed amplified products with both pairs of primers in 37% of the clinical isolates, meaning that they belonged to the O25b-ST131 group; 59.3% showed products only with primers O25pabBspe, meaning that they belonged to the sequence type ST131, and 3.7% were positive only for serogroup O25b.

Table 2. Correlation between *Escherichia coli* phylogenetic groups and ESBLs production

Phylogenetic group (n = 125) *	ESBL		
	Positive [n (%)]	Negative [n (%)]	Total (n)
A	8 (11.4)	8 (14.5)	16
B1	2 (2.8)	6 (10.9)	8
B2	37 (52.8)	17 (30.9)	54
C	6 (8.5)	7 (12.7)	13
D	5 (7.1)	8 (14.5)	13
E	0 (0)	3 (5.4)	3
F	7 (10.0)	2 (3.6)	9
Clade I	5 (7.1)	4 (7.2)	9
Total	70 (100)	55 (100)	125

* *P* value = 0.042, calculated with Fisher's exact test.

Table 3. *Escherichia coli* phylogenetic groups and *bla* genes prevalence

Phylogenetic group	TEM[a] [n (%)]	SHV[b] [n (%)]	CTX-M* [n (%)]
A	4 (10.5)	4 (13.8)	4 (8.9)
B1	1 (2.6)	2 (6.9)	0
B2	19 (50)	12 (41.4)	27 (60)
C	3 (7.9)	1 (3.4)	6 (13.3)
D	4 (10.5)	2 (6.9)	4 (8.9)
F	5 (13.2)	6 (20.7)	1 (2.2)
Clade I	2 (5.3)	2 (6.9)	3 (6.7)
Total	38 (100)	29 (100)	45 (100)

*P value = 0.005 (statistically significant), calculated with Fisher's exact test.
[a] (P = 0.513), [b] (P = 1.00).

ESBLs identification and horizontal transfer. As shown in Table 2, 52.8% of ESBLs-producing strains belonged to group B2, while the rest were distributed among the other groups, except group E. The PCR was performed for the 70 positive strains to detect the *bla* gene bla_{CTX-M}. This gene was present in 64.3% of the isolated strains. The *bla* genes bla_{TEM} and bla_{SHV} were also detected, they being present in 54.3% and 41.4%, respectively. After identifying the *bla* genes, we correlated them with the phylogenetic groups (Table 3). Our results showed that none of the two strains identified as B1 were positive for bla_{CTX-M}, while it was the most prevalent in strains from group B2. Group B2 also had the most strains with bla_{TEM} and bla_{SHV}. Of the strains belonging to group O25b-ST131, 75% contained the bla_{CTX-M} gene.

As most ESBLs genes have been shown to be encoded in plasmids [26], we performed conjugation assays with 5 ESBL-producing strains. As shown in Table 4, two strains (2890 and 3361) could transfer large-sized plasmids, although strain 2890 only transferred one, while the rest of the strains trans-

Table 4. Genotypic and phenotypic characteristics of *Escherichia coli* isolates and their transconjugants

Strain	Phylog. group	Plasmid (kb)	ESBL	MIC (μg/ml)		Trans-conjugant	Plasmid (kb)	ESBL	MIC (μg/ml)	
				CAZ	CTX				CAZ	CTX
3332	B2	130, 6	CTX-M, SHV, TEM	64	>256	T3332	6	CTX-M, SHV, TEM	128	>256
2806	B2	110, 6	CTX-M, TEM	>256	>256	T2806	6	CTX-M, TEM	128	>256
2136	B2	130, 6	CTX-M, SHV, TEM	128	>256	T2136	6	SHV, TEM	0.06	0.25
2890	C	100, 90	CTX-M, SHV, TEM	>256	>256	T2890	90	SHV, TEM	0.5	0.25
3361	D	130, 100	CTX-M, SHV	>256	>256	T3361	130, 100	CTX-M, SHV	>256	>256

MIC: minimum inhibitory concentration. CAZ: ceftazidime. CTX: cefotaxime.

ferred only a 6-kb plasmid. To determine which *bla* genes were transferred, we performed PCR with purified plasmids from the transconjugants. Three strains received all the *bla* genes from the donor strains (T3332, T2806 and T3361), while the other two did not receive the bla_{CTX-M} gene. The two strains that did not receive the bla_{CTX-M} gene were sensitive to CAZ and CTX, suggesting that CTX-M should be the functional ESBLs.

Discussion

Escherichia coli is the most common cause of uncomplicated and community acquired UTIs. Our results showed that multidrug resistance strains of *E. coli* were present in UTI patients from Chilpacingo, Guerrero, and that those strains belonged mainly to the phylogenetic group B2, specifically the sequence type ST131, which has been identified as the main etiological agent of community-acquired UTI, with resistance conferred by CTX-M-type β-lactamase enzymes [10]. Of the 94.54% of strains positive for ST131, only 36.36% belonged to serogroup O25b. The group O25b-ST131 is well recognized as an international pandemic clonal group [28], and in 2011 was identified in Mexico City [23] and Chilpancingo, Guerrero [22]. The strains that were positive for ST131 but negative for O25b might belong to serogroup O16, which has also been associated with the clonal group B2-ST131, and can also be detected with the Clermont *pabB* PCR test, because the O16-ST131 group carries the same *pabB* allele (*pabB*74) [20].

We also observed that 56.7% of the isolated strains were MDR, and of these, 45.5% produced ESBLs. However, to determine which ESBLs-type enzyme is involved in this phenotype, it would be necessary to sequence all the *bla* genes identified, since this is essential to discriminate between the non-ESBLs enzymes (e.g., TEM-1 or SHV-1) from the different variants of TEM and SHV ESBLs (e.g. TEM-3, SHV-2, etc.) [3], especially in the case of the transconjugants that, despite being positive for TEM and SHV *bla* genes, were sensitive to CAZ and CTX.

Consistently with the fact that the group O25b-ST131 has been associated with the presence of the ESBLs type CTX-M-15, it appears to be a correlation between O25b-ST131 strains (36.36%) and the presence of the CTX-M enzyme (38.2%). However, the sequencing of the bla_{CTX-M} genes is necessary to determine that they effectively belong to the CTX-M-15 type. Note that the prevalence of the CTX-M-type ESBLs reported in this work is the lowest found in this region since 2003, when Castro et al. [5] found this gene with a prevalence of 43%, and by 2010 it had increased to 50% [6].

Regarding antibiotic resistance, our results are consistent with previous reports that show that Mexican strains have the highest rates of ampicillin-resistant bacteria (almost 80%), and trimethoprim (61%) [14]. In fact, 85% of the strains we

analyzed were ampicillin resistant and 68% were trimethoprim/sulfamethoxazole resistant. ESBL-producing strains are known to be resistant to all cephalosporins, broad-spectrum penicillins and aztreonam, as were our isolated strains, which showed between 57% and 66% resistance to these antibiotics. In addition, resistance to trimethoprim/sulfamethoxazole and aminoglycosides is generally transferred in the same plasmid, which makes therapeutic options limited. This seems to be the case for trimethoprim/sulfamethoxazole, for which we detected a high percentage of resistant strains, of which 43.3% were ESBLs positive. However, the resistance to aminoglycosides was low, ranging from around 30% for gentamycin and tobramycin to 0% for amikacin. These results indicate that in Chilpancingo, Guerrero, Mexico, it is still possible to use aminoglycosides to treat UTIs (especially amikacin), along with nitrofurantoin, for which only 10% of our strains were resistant.

Note that, in 3 of 5 transconjugants, only a 6-kb plasmid was transferred, not the high molecular weight plasmids. Apparently, 2 of these 3 plasmids were R-plasmids, since they transferred *bla* genes, and the recipient strains became CAZ and CTX resistant. Although the third plasmid transferred the bla_{TEM} and bla_{SHV} genes, it did not transfer $bla_{CTX\text{-}M}$, and thus the recipient strain was not resistant. Actually, the $bla_{CTX\text{-}M}$ gene was the only one that was not transferred in 100% of the cases, it being present in only 60% of the transconjugants. However, our results suggest that in our study population the CTX-M-type was the main ESBL.

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Competing interests. None declared.

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