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Original Article

Characterization of auto-agglutinating and non-typeable uropathogenic *Escherichia coli* strains

Ulises Hernández-Chiñas[1,2], Alejandro Pérez-Ramos[1,2], Laura Belmont-Monroy[1,2,3], Maria E Chávez-Berrocal[1,2], Edgar González-Villalobos[1,2,3], Armando Navarro-Ocaña[4], Carlos A Eslava[1,2], Jose Molina-López[1,2]

[1] Unidad Periférica de Investigación Básica y Clínica en Enfermedades Infecciosas; Departamento de Salud Pública/División de Investigación, Facultad de Medicina, UNAM, Ciudad de México, México
[2] Laboratorio de Patogenicidad Bacteriana, Unidad de Hemato-Oncología e Investigación. Hospital Infantil de México Federico Gómez/Facultad de Medicina UNAM, Ciudad de México, México
[3] Departamento de Microbiología. Escuela Nacional de Ciencias Biológicas, Ciudad de México, México
[4] Laboratorio de Bacteriología, Departamento de Salud Pública, Facultad de Medicina, Ciudad de México, México

Abstract

Introduction: Uropathogenic *Escherichia coli* (UPEC) are the main etiological agent of urinary tract infections (UTIs). Association between different serotypes and UTIs is known, however, some strains are incapable to be serotyped. The aim of this work was to study bthe phenotypical and genotypical characteristics of 113 non-typeable (NT) and auto-agglutinating (AA) *E. coli* strains, isolated from UTIs in children and adults.
Methodology: The 113 UPEC strains were analyzed by PCR assays using specific primers to determine their serogroups, *fimH*, *papC*, *iutA*, *sat*, *hlyCA* and *cnf1*, virulence associated genes, and *chuA*, *yjaA* and TSPE4.C2 for phylogroup determination. Additionally, the diffusion disk method was performed to evaluate the antimicrobial resistance to 18 antimicrobial agents.
Results: Using the PCR assay, 63% (71) of the strains were genotyped showing O25 and O75 as the most common serogroups. The virulence genes *fimH* (86%) and *iutA* (74%) were the most prevalent, in relation to the phylogroups the commensal (A and B1) and virulent (B2 and D) showed similar frequencies ($P > 0.05$). The antimicrobial susceptibility test showed a high percentage (73%) of multidrug-resistant strains.
Conclusions: The genotyping allowed identifying the serogroup in many of the strains that could not be typed by traditional serology. The strains carried virulence genes and were multidrug-resistant in both, commensal and virulent phylogroups. Our findings revealed that, in addition to the classical UPEC serogroups, there are pathogenic serogroups not reported yet.

Key words: Urinary tract infections; serogroup; uropathogenic *Escherichia coli*; *E. coli* non-serotypeable; *E. coli* auto-agglutinating.

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Introduction

In order to define that an *Escherichia coli* (*E. coli)* strain corresponds to the uropathogenic group (UPEC), the bacteria should be isolated from a patient with symptoms of urinary tract infections (UTIs), be included in the classic UPEC serogroups with phylogenetic background and have specific virulence factors and antimicrobial resistance [1]. These UPEC strains are the main etiologic agent of UTIs within the community (60-90%) as well as in hospitals (50-70%) [2,3].

The traditional serotyping of *E. coli* strains includes agglutination assays detecting immunodominant segment of the lipopolysaccharides (LPS) molecule or somatic "O" (187) and flagellar "H" (56) antigens by means of specific antisera [4]. The bacterial colonies expressing LPS are defined as smooth; while those that express an incomplete LPS are defined as rough mutants or as auto-agglutinating [5]. The UPEC serogroups O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83 are the most frequently associated with UTI [6-8]. Traditional serotyping is a labor-intensive procedure that is not feasible in all laboratories. Conversely, when an isolate belongs to the rough phenotype, an auto-agglutinating reaction is observed that does not allow the identification of the bacterium serogroup. The application of typing methods based on molecular procedures defined as genotyping has been proposed for the identification of UPEC strains isolated from UTI patients [1,9,10]. A multiplex PCR was developed by Li. *et al.* [7] to identify those serogroups commonly occurring in UPEC by using the *wzx* (flippase), *wzy* (polymerase) and *orf486* target genes, as they are linked to the

synthesis of the antigen "O" and are clustered within the *E. coli* chromosome.

E. coli is the main facultative bacterium part of the intestinal biota, however, in the evolutionary process associated with horizontal gene transfer different groups of clones emerged. By different analyzes it has been possible to establish the existence of phylogenetic groups A and B in which the commensal strains are included, and the B2 and D phylogroups which include strains that produce extraintestinal infections such as the case of UPEC [11-13].

An important characteristic of UPEC strains is the presence of genes that code for the expression of fimbriae, toxins, iron uptake system and other virulence properties [2,6,13,14]. Due to the clinical impact of ITUs, treatment with antimicrobials is of great importance. However, its inappropriate use has contributed significantly to the increase in the number of multi resistant mutants, that has influenced the recurrence and chronicity of the disease [2,3].

Although in the laboratory there are 187 antisera against the varieties of the somatic antigen and 56 against the flagellar antigen, in many cases a large number of strains are not recognized by the different antisera used so they are defined as non-typeable (NT). In other cases, the strains do not express a complete LPS which causes autoagglutination reactions (AA); both situations hinder the typing of the strains. The aim of this paper was to analyze by PCR the presence of UPEC classic serogroups in *E. coli* strains isolated from patients with UTIs that displayed either the AA or NT phenotypes. Also, we evaluated if the isolates carried genes that code for virulence properties of UPEC strains and their behavior against antimicrobials used in UTIs treatment. In different studies, the presence of non-classical serotypes has been reported in strains of *E. coli* isolated from patients with UTI, so that in the present work might not be possible to identify the serotype in all the strains that will be studied.

Methodology

Bacterial strains

A total of 113 *E. coli* strains, including 87 isolated from adult patients with acute non-complicated UTIs (did not exhibit arterial hypertension, immune system impairment, pregnancy and were ambulatory) from Clinic No. 61 of Instituto Mexicano del Seguro Social and 26 from children with chronic UTIs (CUTIs) who participated in a longitudinal study approved by the research and ethics committee (HIM/2014/022SSA.1122) of the Hospital Infantil de México "Federico Gómez" (HIMFG), were evaluated. In 69 (61%) of the NT strains, the agglutination test was not possible using 186 anti-O serum samples (SERUNAM, Mexico City, Mexico), and 44 (39%) showed AA.

DNA obtention

The DNA of each strain was obtained with the InstaGene Matrix kit (Bio-Rad, USA) according to the manufacturer's instructions.

PCR serogroups identification

A multiplex PCR performed according to Li. *et al.* [7] with specific primers for the *wzx* fragment for O1, O4, O7, O16, O18, O21, O22, and O83; *wzy* for O2, O6, O15, O25, O75 serogroups; and *orf486* for the O8 serogroup were used in the assay.

UPEC virulence-related genes

Genes associated with adherence (*fimH* and *papC*), iron uptake systems (*iutA*) and toxin expression (*sat, hlyCA* and *cnf1*) were analyzed by PCR (Table 1S). The genes were individually amplified by using a reaction mixture containing 3 µL of DNA template, 0.4 µL (10 µM) of the respective primer, and 5 µL of 2X PCR Master Mix (Kapa Biosystems, Inc., Boston, USA) adjusted to a 10 µL final volume with H_2O.

Phylogenetic analysis

The phylogenetic origin of the strains was evaluated by multiplex PCR using primers corresponding to *ChuA, yjaA* and *tspE4.C2* (Table 1S) as reported by Clermont. *et al.* [11].

Antimicrobial Susceptibility

Eighteen antimicrobial agents were utilized for susceptibility by the diffusion disk method using commercially available disks (Oxoid, Hampshire, UK), and the results were interpreted based on the Clinical and Laboratory Standards Institute Manual criteria [15]. *Escherichia coli* ATCC 25922 strain was used as a control in the assay. Based on the criteria reported by Magiorakos. *et al.* [16], the strains were classified as multidrug-resistant (MDR) for those resistant to at least one agent of three or more antimicrobial categories or extensive drug-resistant (XDR) for those that were non-susceptible to at least one agent in all but two or less antimicrobial categories.

Results

Genotyping to determine serogroups

The method developed by Li *et al.* [7] allowed the serogroup identification of 71 (63%) of the 113 NT or

Table 1. Serogroups identified by PCR assays in NT and AA *Escherichia coli* strains isolated of children and adults with UTIs.

***E. coli* serogroups**	***E. coli* strains**				
	Total	**NT[1]**	**AA[2]**	**Children**	**Adults**
	N[3] = 113	**N = 69**	**N = 44**	**N = 26**	**N = 87**
O1	5	3	2	0	5
O2	5	3	2	2	3
O4	3	2	1	0	3
O6	6	5	1	1	5
O7	3	2	1	0	3
O8	4	4	0	0	4
O15	3	1	2	1	2
O18	6	4	2	4	2
O21	6	1	5	0	6
O22	5	4	1	1	4
O25	12	9	3	3	9
O75	10	2	8	0	10
O83	3	3	0	0	3
Total N	**71**	**43**	**28**	**12**	**59**

[1]Non-typeable, [2]Auto-agglutinating; [3] Strains Number.

AA *E. coli* strains isolated from 12 children with CUTIs and 59 adults with acute UTIs (Table 1); the strains were named PCR-genotypeable *E. coli* (PCR-GT-*E. coli*), and the remaining 42 (37%) were strains that did not amplify the genes, named PCR-non genotypeable *E. coli* (PCR-NGT-*E. coli*). An analysis of the PCR-GT-*E. coli* strains characteristics showed that 43 of them corresponded to NT, and 28 corresponded to AA. The serogroups O25 (17%) and O75 (14%) were the most common, followed by O6, O18, O21 (8%); O1, O2, O22 (7%); O8 (6%); and O4, O7, O15, O83 (4%). Considering the patients, O75 (17%) and O25 (15%) were more common in adults than in children, while O18 (33%) and O25 (25%) were more common in children with CUTIs (Table 1).

Phylogenetic analysis

The phylogenetic group assay showed the phylogroups A (46%) and B2 (31%) as the more frequent of the 113 studied *E. coli* strains, and D (17%) and B1 (6%) as the less frequent. However, in PCR-NGT-*E. coli* the phylogroup B2 is the most frequent (Table 2). The analysis between genotypeable and non-genotypeable strains showed that the first are included mainly ($P < 0.05$) in B2 and A phylogroups (data not shown). However, when the analysis was performed between the commensal and virulent phylogroups not significant differences ($P > 0.05$) were observed (data not shown). Considering the origin of the strains was observed that the isolates obtained from children were included mainly in the A and B1 ($P < 0.05$) commensal phylogroups. The same analysis with strains isolated

Table 2. Association between PCR serogroups and phylogroups of *Escherichia coli* strains isolated from patients with UTIs.

***E. coli* serogroups**	**Phylogenetic group**			
	A	**B1**	**B2**	**D**
	N = 52	**N** = 7	**N** = 35	**N** = 19
O1	0	1	1	3
O2	1	0	4	0
O4	1	0	2	0
O6	3	1	1	1
O7	1	0	1	1
O8	4	0	0	0
O15	0	0	2	1
O18	2	0	1	3
O21	5	0	1	0
O22	4	0	0	1
O25	3	0	9	0
O75	1	1	6	2
O83	2	0	1	0
Total N (%)	**27 (52)**	**3 (43)**	**29 (83)**	**12 (63)**

Table 3. Correlation of phylogroups and virulence genes of *Escherichia coli* strains isolated from children and adults with UTIs.

Phylogroups	Children N = 26	Adults N = 87	P-Value[1]
	N (%)	N (%)	
A	16 (62)	36 (42)	0.007
B1	0	7 (8)	0.0068
B2	6 (23)	29 (33)	> 0.05
D	4 (15)	15 (17)	> 0.05
Virulence genes[2]			
fimH	16 (62)	81 (93)	0.0001
sat	19 (73)	18 (21)	0.0001
iutA	9 (35)	75 (86)	0.0001
papC	7 (27)	17 (20)	> 0.05
cnf1	5 (19)	15 (17)	> 0.05
hlyCA	3 (12)	5 (6)	> 0.05

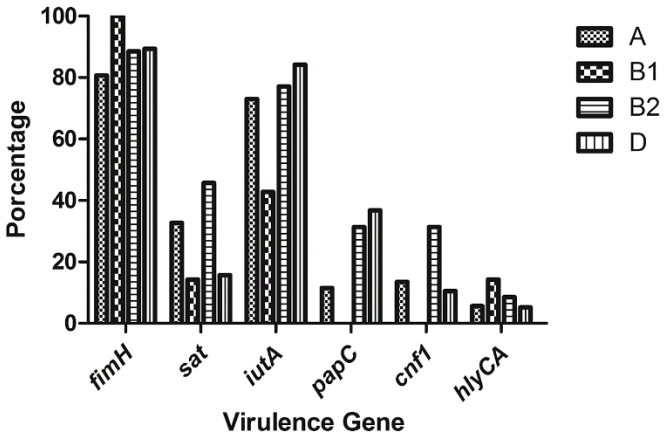
[1]Comaprison of children vs adults; P < 0.05, Fisher's exact test; [2]Some strains amplified more than one gene.

from adult patients showed the same proportion of commensal and virulent phylogroups (Table 3).

Virulence genes

The virulence gene analysis showed that 98 (87%) of the studied strains amplified between one and three virulence genes; *fimH* (86%) and *iutA* (74%) were more common in both groups (children and adults), *sat* was more frequent in children (73%), and *iutA* was more frequently isolated from adults (86%) (Table 3). The analysis between phylogenetic groups and virulence genes showed that *fimH* and *iutA* were identified in both commensal and virulent phylogroups. However, *sat* was predominant in the A and B2 groups, *papC* in B2 and D, *cnf1* in B2, and *hlyCA* was found in 8 (7%) strains distributed in the 4 phylogroups (Figure 1). There was no direct relationship between the virulence genes and the commensal and extraintestinal phylogroups (P > 0.05) (data not shown). PCR-GT-*E. coli* strains harbored between four and six virulence genes with significant differences (P < 0.05) when compared with the PCR-NGT-*E. coli* (Table 4). The *fimH*, *papC*, *cnf1* and *hlyCA* genes were predominant among the PCR-GT-*E. coli* strains but *sat* and *iutA* showed a homogenous distribution between genotypeable and non-genotypeable strains (Table 4).

Figure 1. Distribution of virulence genes detected in 4 phylogenetic groups *of Escherichia coli* strains.



The correlation of serogroups and virulence gene number showed one O25 strain with 6 genes; the serogroups O4, O6, O18, O25 and O75 with five genes; and O1, O18, O22, and O25 serogroups with four genes (data not shown).

Antimicrobial susceptibility

The susceptibility assay showed that a considerable number of the analyzed strains were sensitive to amikacin (91.1%), ofloxacin (94.7%), meropenem

Table 4. Correlation between virulence genes and Genotyped and Non-Genotyped *E. coli* strains.

Virulence genes	GT-*E. coli* N = 71		NGT-*E. coli* N = 42		P-Value[1]
	N	(%)	N	(%)	
fimH	65	(91)	32	(76)	0.0014
Sat	24	(34)	13	(31)	> 0.05
iutA	54	(76)	30	(73)	> 0.05
papC	20	(28)	4	(10)	0.0019
cnf1	18	(25)	2	(5)	0.0001
hlyCA	8	(12)	0	(0)	0.0248

[1]Comparison of the two groups of strains (*E. coli*-GT-PCR vs *E. coli*-NGT-PCR); P < 0.05, Fisher's exact test.

(89.3%), nitrofurantoin (83.2%) and cefepime (79.6%). However, 50% of the *E. coli* strains evaluated were resistant to seven antimicrobials, including ampicillin, nalidixic acid, trimethoprim/sulfamethoxazole, ciprofloxacin, carbenicillin, cefuroxime and norfloxacin (Table S2). The resistance analysis showed 98 different antibiotic-resistant phenotypes, with 66 of them in PCR-GT-*E. coli* strains. One PCR-NGT-*E. coli* strain exhibited resistance to 17 of the 18 antibiotics evaluated in this study, and two O25 strains were resistant to 15 antibiotics. The antibiotic resistance profile showed that 73% of the studied strains were MDR, 21% were XDR and 6% of the strains were sensitive. Comparative analyses between PCR-GT-*E. coli* and PCR-NGT-*E. coli* groups showed that the MDR results and sensitivity were homogenous ($P > 0.05$). Nonetheless, XDR strains were mainly included in the PCR-NGT-*E. coli* group ($P < 0.05$) (Table 5). The correlation between serogroup and antimicrobial susceptibility showed that the O1 serogroup was the least resistant, and the remaining serogroups were MDR (79%), with the exception of O22 and O25, which showed an XDR profile in 60% and 34% of the strains, respectively.

Discussion

The traditional serotyping of *E. coli* is a standard method for subtyping strains for epidemiological studies and enhancing phylogenetic studies. The serotyping of clinical isolates of this bacterium is under constant development, and it is usually possible to identify the isolated strains. In some cases, however, it is not possible to properly characterize the strain with available monospecific polyclonal antisera, either due to autoagglutination or because the isolated *E. coli* strain is novel, and an appropriate antiserum has not been developed [1]. Both situations make it necessary to search for alternatives in the characterization of *E. coli* related to UTIs [1,7]. Data in the literature show that approximately 8-18% of UPEC strains cannot be typified by traditional serology [6,8,17-19] an alternative in this regard is the use of molecular procedures. A PCR assay to identify genes associated with the expression of 14 somatic O antigens of UPEC strains isolated from urine was evaluated in this work. With this procedure, the serogroup was determined in 71 (63%) of 113 non-serotyped strains; however, the assay was designed for the identification of regular UPEC serogroups. The results suggest that the other 42 (37%) strains that did not amplify with the UPEC primers may represent serogroups with local distribution.

In this regard, some studies of UTI carried out in China, India, Japan and Germany observed that *E. coli* serogroups O12, O15, O21, O26, O39, O42, O46, O51, O60, O74, O76, O77, O78, O83, O92, O102, O105, O117, O120, O135, O145, O149, O150, and O153 are not included in the classic UPEC serogroups [6,20-22]. Some of these serogroups and others, such as O9, O11, O17, O29, O35, O70, O96, O100, O101, O127, O138, O152, and O154, have been reported in different studies in Mexico [17-19]. The presence of different serogroups in different countries could be related to autochthonous bacteria that are part of the intestinal biota and that incidentally may cause UTI [23].

Based on genotyping, as on serotyping, O25 and O75 are included among the most frequent serogroups associated with UTI in our work and in others carried out in Mexico [17-19], China [6], India [20], Italy [24], Spain [25] Iran [26]. This suggests the importance, along with their wide distribution in different countries, of both serogroups and their epidemiological impact.

The existence of alternatives to characterize microorganisms causing diseases is of great importance to understand their epidemiology [1,7]. Our observations support the use of PCR genotyping as a good alternative, mainly because it allows the identification of strains with LPS alterations.

Although *E. coli* is usually a non-pathogenic bacterium, several clones have acquired specific virulence factors through the horizontal acquisition of genetic material, which increases their ability to adapt to new niches. Different virulence genes have been identified in UPEC that allow it to colonize, invade and survive in the epithelium of the urinary tract of the host [3]. *fimH* (type I fimbriae) and *iutA* (iron uptake system) were the genes most frequently identified in the evaluated *E. coli* strains. Similar results have been

Table 5. Resistance profile of Genotype and Non-Genotype *E. coli* strains.

Resistance profile	N (%)	GT-*E. coli* N (%)	NGT-*E. coli* N (%)	P-value[4]
XDR[1]	27 (21.3)	12 (16.9)	15 (35.7)	0.0037
MDR[2]	82 (72.5)	56 (78.8)	27 (64.3)	> 0.05
SEN[3]	7 (6.2)	3 (4.2)	4 (9.5)	> 0.05
Total	**113**	**71**	**42**	

[1] XDR (Extensive drug resistant); [2]MDR (Multidrug-resistant); [3]SEN (Sensitive); [4]Comparison between *E. coli*-GT-PCR vs *E. coli*-NGT-PCR.

reported in other studies, where *fimH* (90%) and *iutA* (41% to 83%) were identified in *E. coli* strains isolated from stools or urine [6,19,26-28]. In relation to the toxins *sat*, *cnf1* and *hlyCA* and adhesin *papC* the prevalence is similar to the one reported in other study [29].

However, the relationship between virulence genes and genotypes (serogroups) has been evaluated by other authors [6,26]. The results obtained in this study showed that PCR-GT-*E. coli* strains belonging to serogroups O4, O25, O75 and O18 display virulence genes more frequently than PCR-NGT-*E. coli*. However, Gao *et al.* [6] does not find a difference in the number of virulence genes between the strains that have the classical serogroups, other serogroups and non-typeable ones. In this regard, studies have been conducted *in vivo* for the virulence of UPEC strains of uropathogenic and non-uropathogenic serogroups, where chicks were used to test the lethality of strains. Their results showed that virulence depended on the serogroup and the number of virulence genes that these strains expressed [6].

Studies conducted in Mexico and other countries found that UPEC serogroup strains are integrated mainly in the virulent phylogroups (B2 and D) [17,25,30-32]. In the phylogroup analysis, the PCR-GT-*E. coli* strains showed a higher frequency of strains in the virulent phylogroups (B2 and D) with significant differences ($P < 0.05$) when compared with PCR-NGT-*E. coli* strains. These results confirm the clinical and epidemiological impact of the UPEC strains, although the O antigen is not expressed.

Phylogroups A and B1 include commensal strains [11]. The PCR-NGT- *E. coli* strains showed a higher prevalence in both phylogroups in this study, which suggests that this group is composed of commensal strains of serogroups not described before as UPEC. The participation of commensal strains in the etiopathogenesis of UTI has been previously documented [23] as well as the presence of virulence genes [6], which suggests the opportunistic participation of *E. coli* commensals in UTI.

The resistance of antimicrobials has significantly increased in recent years, which has led to its consideration as a public health problem. The high resistance to ampicillin, amoxicillin, trimethoprim/sulfamethoxazole, ciprofloxacin and nalidixic acid is a consequence of empirical treatment, which is not efficient to control infections [17,25,33,34].

The results of sensitivity to antimicrobials showed the existence of multidrug-resistant strains in both PCR-GT-*E. coli* and PCR-NGT-*E. coli* strains in this study. In other studies, different results have been reported, observing that serotypeable UPEC strains have a higher percentage of resistant antibiotics of the family of β-lactam, aminoglycosides, quinolones and folate pathway inhibitors [26]. Additionally, the commensal strains show high resistance profiles and the *E. coli* strains show the same characteristics in the pathogenesis of UTI [35,36].

Conclusion

PCR genotyping was of great utility to identify the serogroups of 63% of NT or AA strains associated with UTI. On the other hand, it is possible that the 37% of the strains that did not amplify correspond to autochthonous UPEC serogroups. One of the limitations of the procedure is that it only includes the 14 described classic UPEC serogroups, showing the same limitations that have been observed with the serotyping. The study showed the importance of the integral genotypic and phenotypic characterization of strains isolated from clinical samples, since it allowed to identify that a significant number of commensal strains carry virulence genes of UPEC and are resistant to most antimicrobials of routine use in the treatment of UTIs.

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Authors' contributions

MLJ, ECA and HCU conceived of the study and experimental design. NOA identified the serotype by traditional serology. PRA and BLM obtained DNA and performed PCR assays to identify the serogroup and antimicrobial susceptibility assays. GVE and CBME performed PCR assays to identify the phylogenetic origin of the strains and virulence genes. HCU wrote the first draft of the manuscript, which was reviewed and commented upon by MLJ and ECA, who reviewed it critically for important intellectual content.

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References

1. Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM, Feng P (2016) Advances in molecular serotyping and subtyping of *Escherichia coli*. Front Microbiol 7: 644.
2. Terlizzi ME, Gribaudo G, Maffei ME (2017) UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol 8: 1566.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13: 269-284.
4. Orskov F, Orskov I, Evans DJ Jr, Sack RB, Sack DA, Wadström T (1976) Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. Med Microbiol Immunol 162: 73-80.
5. Kunin CM, Beard MV (1963) Serological studies of o antigens of *Escherichia coli* by means of the hemagglutination test. J Bacteriol 85: 541-548
6. Gao Q, Zhang D, Ye Z, Zhu X, Yang W, Dong L, Gao S, Liu X (2017) Virulence traits and pathogenicity of uropathogenic *Escherichia coli* isolates with common and uncommon O serotypes. Microb Pathog 104: 217-224.
7. Li D, Liu B, Chen M, Guo D, Guo X, Liu F, Feng L, Wang L (2010) A multiplex PCR method to detect 14 *Escherichia coli* serogroups associated with urinary tract infections. J Microbiol Methods 82: 71-77.
8. Vosti KL (2007) A prospective, longitudinal study of the behavior of serologically classified isolates of *Escherichia coli* in women with recurrent urinary tract infections. J Infect 55: 8-18.
9. Lacher DW, Gangiredla J, Jackson SA, Elkins CA, Feng PC (2014) Novel microarray design for molecular serotyping of shiga toxin- producing *Escherichia coli* strains isolated from fresh produce. Appl Environ Microbiol 80: 4677-4682.
10. DebRoy C, Fratamico PM, Roberts E (2018) Molecular serogrouping of *Escherichia coli*. Anim Health Res Rev 19: 1-16.
11. Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66: 4555-4558.
12. Johnson JR, Stell AL (2000) Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 181: 2122.
13. Zhao R, Shi J, Shen Y, Li Y, Han Q, Zhang X, Gu G, Xu J (2015) Phylogenetic distribtion of virulence genes among ESBL-producing uropathogenic *Escherichia coli* isolated from long-term hospitalized patients. J Clin Diagn Res 9: DC01-4.
14. Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM, Koo SH, Oh MH, Kim HJ, Choi CH (2016) Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. Lett Appl Microbiol 62: 84-90.
15. Clinical and Laboratory Standards Institute (CLSI) (2017) Performance Standards for Antimicrobial Susceptibility Testing. 27th ed CLSI supplement M100 (ISBN 1-56238-1-56238-805-3)
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281.
17. Molina-López J, Aparicio-Ozores G, Ribas-Aparicio RM, Gavilanes-Parra S, Chávez-Berrocal ME, Hernández-Castro R, Manjarrez-Hernández HÁ (2011) Drug resistance, serotypes, and phylogenetic groups among uropathogenic *Escherichia coli* including O25-ST131 in Mexico City. J Infect Dev Ctries 5: 840-849. doi: https://doi.org/10.3855/jidc.1703
18. Belmont-Monroy L, Ribas-Aparicio RM, Navarro-Ocaña A, Manjarrez-Hernández HÁ, Gavilanes-Parra S, Aparicio-Ozores G, Cauich-Sánchez PI, Garza-Ramos U, Molina-López J (2017) Characterization of *Escherichia coli* causing community acquired urinary tract infections in Mexico City. Diagn Microbiol Infect Dis 87: 193-195.
19. Morales-Espinosa R, Hernandez-Castro R, Delgado G, Mendez JL, Navarro A, Manjarrez A, Cravioto A (2016) UPEC strain characterization isolated from Mexican patients with recurrent urinary infections. J Infect Dev Ctries 10: 317-328. 317-328. DOI 10.3855/jidc.6652
20. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, Gandham N, Wieler LH, Ewers C, Ahmed N (2011) Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* from a semi urban locality in India. PLoS One 6: e18063.
21. Terai A, Yamamoto S, Mitsumori K, Okada Y, Kurazono H, Takeda Y, Yoshida O (1997) *Escherichia coli* virulence factors and serotypes in acute bacterial prostatitis. Int J Urol 4: 289-294.
22. Toval F, Köhler CD, Vogel U, Wagenlehner F, Mellmann A, Fruth A, Schmidt MA,Karch H, Bielaszewska M, Dobrindt U (2014) Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients with urinary tract infection. J Clin Microbiol 52: 407-418.
23. Sokurenko E (2016) Pathoadaptive mutations in uropathogenic *Escherichia coli*. Microbiol Spectr 4:
24. Musumeci R, Rausa M, Giovannoni R, Cialdella A, Bramati S, Sibra B, Giltri G, Viganò F, Cocuzza CE (2012). Prevalence of plasmid-mediated quinolone resistance genes in uropathogenic *Escherichia coli* isolated in a teaching hospital of northern Italy. Microb Drug Resist 18: 33-41.
25. Blanco M, Alonso MP, Nicolas-Chanoine MH, Dahbi G, Mora A, Blanco JE, López C, Cortés P, Llagostera M, Leflon-Guibout V, Puentes B, Mamani R, Herrera A, Coira MA, García-Garrote F, Pita JM, Blanco J (2009). Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 63: 1135-1141.
26. Momtaz H, Karimian A, Madani M, Safarpoor Dehkordi F, Ranjbar R, Sarshar M, Souod N (2013) Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob 12: 8.
27. Paniagua-Contreras GL, Monroy-Pérez E, Rodríguez-Moctezuma JR, Domínguez-Trejo P, Vaca-Paniagua F, Vaca S (2015) Virulence factors, antibiotic resistance phenotypes and O-serogroups of *Escherichia coli* strains isolated from community-acquired urinary tract infection patients in Mexico. J Microbiol Immunol Infect 50: 478-485.
28. López-Banda DA, Carrillo-Casas EM, Leyva-Leyva M, Orozco-Hoyuela G, Manjarrez-Hernández ÁH, Arroyo-Escalante S, Moncada-Barrón D, Villanueva-Recillas S,

Xicohtencatl-Cortes J, Hernández-Castro R (2014) Identification of virulence factors genes in *Escherichia coli* isolates from women with urinary tract infection in Mexico. Biomed Res Int 2014: 959206.
29. Najafi A, Hasanpour M, Askary A, Aziemzadeh M, Hashemi N (2018). Distribution of pathogenicity island markers and virulence factors in new phylogenetic groups of uropathogenic *Escherichia coli* isolates. Folia Microbiol 63: 335-343.
30. Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, Nolan LK (2005) Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology 151: 2097-2110.
31. Robino L, García-Fulgueiras V, Araujo L, Algorta G, Pírez MC, Vignoli R (2014) Urinary tract infection in Uruguayan children: Aetiology, antimicrobial resistance and uropathogenic *Escherichia coli* virulotyping. J Glob Antimicrob Resist 2: 293-298.
32. Cyoia PS, Rodrigues GR, Nishio EK, Medeiros LP, Koga VL, Pereira AP, Vespero EC, Houle S, Dozois CM, Nakazato G, Kobayashi RK (2015) Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic *Escherichia coli* isolates from Brazil. J Infect Dev Ctries 9: 1068-1075. doi: 10.3855/jidc.6683.
33. Ochoa SA, Cruz-Córdova A, Luna-Pineda VM, Reyes-Grajeda JP, Cázares-Domínguez V, Escalona G, Sepúlveda-González ME, López-Montiel F, Arellano-Galindo J, López-Martínez B, Parra-Ortega I, Giono-Cerezo S, Hernández-Castro R, de la Rosa-Zamboni D, Xicohtencatl-Cortes J (2016) Multidrug- and extensively drug-resistant uropathogenic *Escherichia coli* clinical strains: Phylogenetic groups widely associated with integrons maintain high genetic diversity. Front Microbiol 7: 2042.
34. Manjarrez-Hernández A, Molina-López J, Gavilanes-Parra S, Hernández-Castro R (2016) *Escherichia coli* clonal group A among uropathogenic infections in Mexico City. J Med Microbiol 65: 1438-1444.
35. Huang IF, Lee WY, Wang JL, Hung CH, Hu HH, Hung WY, Hung YJ, Chen WC, Shen YT, Cheng MF (2018) Fecal carriage of multidrug-resistant *Escherichia coli* by community children in southern Taiwan. BMC Gastroenterol 18: 86.
36. Qin X, Hu F, Wu S, Ye X, Zhu D, Zhang Y, Wang M (2013) Comparison of adhesin genes and antimicrobial susceptibilities between uropathogenic and intestinal commensal *Escherichia coli* strains. PLoS One 8: e61169.
37. Johnson JR, Stell AL (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis
38. Ananias M, Yano T (2008). Serogroups and virulence genotypes of *Escherichia coli* isolated from patients with sepsis. Braz J Med Biol Res 41: 877-883
39. Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O (1995). Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. FEMS Immunol Med Microbiol 12: 85-90.
40. Usein CR, Damian M, Tatu-Chitoiu D, Capusa C, Fagaras R, Tudorache D, Nica M, Le Bouguénec C (2201). Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. J Cell Mol Med 5: 303-310.

Corresponding author

Jose Molina-López
Hospital Infantil de México Federico Gómez, Dr Márquez 162
Col. Doctores, Alcaldia Cuauhtemoc, CP 06720, CDMX, México.
Phone: (52) 55 52289917
Fax: (52) 55 52289917
Email: joseml@unam.mx

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Annex – Supplementary items

Supplementary Table 1. Oligonucleotides for detection specific to phylogenetic group and virulence factors.

Genes	Sequences 5'-3'	Size in base pairs (pb)	References
ChuA	F- GACGAACCAACGGTCAGGAT R-TGCCGCCAGTACCAAAGACA	279	[15]
yjaA	F-TGAAGTGTCAGGAGACGCTG R-ATGGAGAATGCGTTCCTCAAC	211	
tspE4.C2	F-GAGTAATGTCGGGGCATTCA R-CGCGCCAACAAAGTATTACG	152	
fimH	F-TCGAGAACGGATAAGCCGTGG R-GCAGTCACCTGCCCTCCGGTA	508	[40]
iutA	F-GGCTGGACATCATGGGAACTGG R-CGTCGGGAACGGGTAGAATCG	302	
sat	F-GTTGTCTCTGGCTGTTGC R-AATGATGTTCCTCCAGAGC	501	[41]
papC	F-GACGGCTGTACTGCAGGGTGTGGCG R-ATATCCTTTCTGCAGGGATGCAATA	328	[42]
cnf1	F-AAGATGGAGTTTCCTATGCAGGAG R-CATTCAGAGTCCTGCCCTCATTATT	498	
hlyCA region	F-AGATTCTTGGGCATGTATCCT R-TTGCTTTGCAGACTGTAGTGT	556	[43]

Supplementary Table 2. Distribution of antimicrobial resistance among PCR-GT-*E. coli* and PCR-NGT-*E. coli*.

Antimicrobial	Strains N = 113 N (%)	PCR-GT-*E. coli*[1] N = 71 N (%)	PCR-NGT-*E. coli*[1] N = 42 N (%)	P-Value[2]
Ampicillin	105 (93)	67 (94.3)	38 (90.4)	> 0.05
Carbenicillin	64 (56.6)	24 (33.8)	40 (95.2)	0.0001
Amoxicillin-Clavulanate	34 (30)	28 (39.4)	16 (38)	> 0.05
Piperacillin-Tazobactam	44 (39)	32 (45)	12 (28.5)	> 0.05
Trimethoprim-Sulfamethoxazole	82 (72.5)	49 (69)	33 (78.5)	> 0.05
Nalidixic Acid	82 (72.5)	48 (67.6)	34 (81)	> 0.05
Ciprofloxacin	72 (63.7)	40 (56.3)	32 (76.1)	0.0432
Norfloxacin	57 (50.4)	39 (55)	18 (42.8)	> 0.05
Ofloxacin	6 (5.4)	5 (7)	1 (2.4)	> 0.05
Cefuroxime	69 (61)	40 (56.3)	29 (69)	> 0.05
Ceftazidime	47 (42)	26 (32.4)	21 (50)	> 0.05
Ceftriaxone	30 (26.5)	14 (19.7)	16 (38)	0.0467
Cefepime	23 (20)	11 (15.5)	12 (28.5)	> 0.05
Tobramycin	49 (43.3)	32 (45)	17 (40.4)	> 0.05
Gentamicin	44 (39)	28 (39.4)	16 (38)	> 0.05
Amikacin	10 (8.8)	5 (7)	5 (12)	> 0.05
Nitrofurantoin	19 (16.8)	12 (17)	7 (16.6)	> 0.05
Meropenem	12 (10.6)	8 (11.2)	4 (9.5)	> 0.05

[1] Serogroups identified by PCR. [2]Comparison of the two groups of strains (*E. coli*-GT-PCR vs *E. coli*-NGT-PCR); P < 0.05, Fisher's exact test.