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ORIGINAL ARTICLE

Association between *TLR4* polymorphisms (896 A>G, 1196 C>T, − 2570 A>G, − 2081 G>A) and virulence factors in uropathogenic *Escherichia coli*

Natividad Castro-Alarcón[1] · Rubén Rodríguez-García[1] · María Ruíz-Rosas[2] · José Francisco Muñoz-Valle[3] · Iris Paola Guzmán-Guzmán[1] · Isela Parra-Rojas[1] · Mirna Vázquez-Villamar[1]

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Abstract

Escherichia coli is the main etiological agent of urinary tract infections. Its virulence factors are important during the initial interaction stage with the host as they enable colonization of urinary tract tissues. The genetic markers evidencing susceptibility to develop recurrent infections have been previously described. Toll-like receptors are critical sensors of microbial attacks, and they are also effectors of the individual's innate defense for elimination of pathogens. The aim of this study was to evaluate the association between functional polymorphisms (896 A>G, 1196 C>T, − 2570 A>G, − 2081 G>A) and susceptibility to develop urinary tract infections as well as *E. coli* virulence factors. This study includes 100 samples from patients diagnosed with UTI and 100 samples from uninfected subjects. A conventional urine culture was performed and the isolates were identified by using the Vitek automated system. *TLR4* gene polymorphisms were identified by the PCR–RFLP technique. The *hlyA, fimH, papC, iutA* and *cnf1* virulence factors as well as the *E. coli* phylogenetic group were assessed by PCR. In this study, it was observed that the presence of the − 2570 polymorphism represents a risk of UTI ($p < 0.01$), whereas − 2081 confers protection ($p < 0.01$). The 896A>G and 1196C>T polymorphisms were associated with the *E. coli* virulence factors *fimH* and *hlyA*, respectively ($p < 0.05$). The B2 group was the most frequent in clinical isolates (51%), and it displayed more virulence factors regarding other phylogenetic groups ($p \leq 0.05$). An interesting finding was that strains considered as commensals, belonging to groups A and B1, can cause UTI and present virulence factors. Polymorphisms occurring in the TLR4 promoter region are correlated with susceptibility or risk of UTI, whereas structural polymorphisms are associated with the recognition of virulence factors displayed by *E. coli*.

Keywords TLR-4 · Polymorphisms · Uropathogenic *Escherichia coli* · Phylogenetic group · Virulence factors

Introduction

Urinary tract infections (UTI) are among the most common bacterial infections in humans, and they still prevail as the underlying cause of an important proportion of morbidity and mortality. Age, sex, chronical diseases, congenital malformations and antibiotic treatment, among several factors, are factors conferring susceptibility toward recurrent UTI cases that represent a higher risk of pyelonephritis and severe kidney dysfunction [1–3]. The main factors associated with UTI are those involved in innate immune response, the first line of defense against infections [4–6]. When activated, this response triggers several outcomes such as the releasing of chemokines, interferons, as well as antimicrobial substances and proinflammatory cytokines that contribute to eliminating the infection [7, 8].

Toll-like receptors (TLRs) are able to detect and identify different sections of some microbial components linked to the pathogen itself. Currently, it is known that TLR4 molecules are expressed by urothelial cells lining the kidneys and the bladder in the urinary tract. The most important target

✉ Mirna Vázquez-Villamar
mirnavillamar@hotmail.com

[1] Facultad de Ciencias Químico Bilógicas, Universidad Autónoma de Guerrero, Lázaro Cárdenas S/N Col. La Haciendita, C.P. 39090 Chilpancingo, Guerrero, Mexico

[2] Laboratorio Clínico, Área de Microbiología, Clínica Hospital ISSSTE, Chilpancingo, Guerrero, Mexico

[3] Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico

ligand of the TLR4 receptor is lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria such as *Escherichia coli* [9]. Other bacterial components as the fimH adhesin, type I and P fimbriae and even hlyA are also recognized as TLR4 inductors in the host's urinary tract [10–13].

Therefore, TLR4 activation is important for the host's response toward UTI. The proinflammatory activity exerted by LPS is crucial to counteracting bacterial infections by triggering the activation of the immune system, by activating innate mechanisms and subsequently a T-helper 1 (Th1) protective immune response [14]. Interestingly, the infection of mice harboring TLR4 mutations resulted in an asymptomatic carrier status. However, it is important to point out that such mice ultimately died from these infections [15].

TLR4 polymorphisms in the promotor region have been studied associated with modulation of gene expression. These findings suggest genetic variations in the TLR4 promoter could be essential elements for UTI susceptibility. Polymorphisms on the TLR4 extracellular domain, such as 896 A>G and 1196 C>T, have been considered as "loss of function" because they modify the interaction of pathogen-associated molecular patterns (PAMPs) to TLR4 [16].

In this study, we evaluated the association between the 896 A>G, 1196 C>T, − 2570 A>G, − 2081 G>A functional polymorphisms with the susceptibility of patients to develop urinary tract infections, as well as *Escherichia coli* virulence factors.

Materials and methods

Subjects

The samples of patients with medical order for urine culture to discard UTI acquired in the community and blood biometry at the ISSSTE (Instituto de Seguridad y Servicios Sociales de los trabajadores del Estado) clinic (Chilpancingo, Gro.) were selected between August 2016 and February 2017. Instructions were provided before collected the clinical sample. A survey was applied to know the sociodemographic indicators as well as any clinical symptom and risk factors associated with urinary tract infections. Mexican Mestizo individuals up from 8 years old of either sex were included. Mexican mestizos were defined as a person born in Mexico possessing a Spanish last name with Mexican ancestry going back up to three generations, including themselves [17]. Patients were allocated in two groups: 100 subjects with UTI caused by *E. coli* and 100 uninfected subjects. The non-infected subjects were people over 18 years of age who attend a routine medical checkup, for example, pregnant women, diabetics and adults, and the urine culture was negative. Patients with UTI caused by other microorganisms were excluded.

Clinical isolate

A urine culture was performed on conventional media (MacConkey agar, blood agar, mannitol salt agar and Biggy agar). Lactose-fermenting bacteria, among them *Escherichia coli*, were identified in agar MacConkey. Patients displaying a $> 100^3$ CFU/mL count were considered to be affected by a UTI, whereas those showing $> 10^4$ CFU/mL were asymptomatic [18]. The clinical isolates were identified using a Vitek automated system (bioMérieux).

TLR4 gene polymorphisms

The genomic DNA (gDNA) was isolated from peripheral blood using the modified Miller's method [19]. The gDNA was quantified in a nanodrop (Thermo Fisher Scientific). The TLR4 polymorphisms were genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLPs), using primers previously reported [20–22]. The regions containing the 896 A>G (Asp-299Gly, rs4986790), 1196 C>T (Thr399Ile, rs4986791), − 2570 A>G, − 2081 G>A (rs10983755) polymorphisms were amplified by PCR, a pair of specific primers was used for each polymorphism, the reaction mixture to determine all polymorphisms was performed in a final volume of 25 µL using 1 × of GO Taq® Green Master Mix (Promega), 0.5 µM of each oligonucleotide, 1 ng of gDNA and adjusting the final volume with H_2O. The following cycling conditions were used: initial denaturation at 94 °C for 10 min, 35 cycles of 94 °C for 30 s for denaturation, 60 °C for 30 s for annealing, extension at 72 °C for 30 s and, finally, 72 °C for 7 min for an ending extension. The PCR-amplified fragments were observed on a 6% polyacrylamide gel electrophoresis at 90 V for 1 h and stained with silver nitrate, 249 base pairs (bp) for 896 A>G, 405 bp for 1196 C>T, 334 bp for − 2570 A>G and 172 bp for − 2081 G>A. The products were subsequently digested with restriction enzymes, respectively: *AflIII, TspRI, NCoI* and *HinfI* (New England, Biolabs, Ipswich, Massachusetts, USA). For 896 A>G polymorphism, the restriction patterns generated were 249 bp for AA; 249, 223, 26 bp for AG; and 223 and 26 bp for GG genotype. For 1196 C>T polymorphism, the digested fragments were 405 bp for CC; 405, 376, 29 bp for CT; and 376 and 29 bp for TT genotype. For − 2570 A>G polymorphism, the digested fragments were 334 bp for AA; 334, 293, 41 for AG; and 293 and 41 for GG. With respect to − 2081 G>A polymorphism, the genotypes were identified for 116, 56 bp for GG; 172, 116, 56 bp for GA; and 172 bp for AA. To visualize them, an electrophoresis was performed in 6% (29:1) polyacrylamide gels, and the

samples were run at 120 V for 1 h. Seven percent gels (19:1) were used for − 2081 G>A polymorphisms, and they were run at 120 V for 2 h to be finally stained with silver nitrate.

Bacterial DNA extraction

DNA extraction was carried out by thermal shock in the following conditions: 200 µL of sterile water was placed in 1.5-mL tubes. Approximately 3–5 colonies were taken, placed on these tubes and dispersed by vortex. They were placed in cold at 20 °C for 15 min. After this period, they were rapidly placed in boiling water (100 °C) for 10 min. Samples were centrifuged at 13,000 rpm for 10 min, and the supernatant was separately collected to be stored at − 80 °C until further use.

Phylogenetic group assessment

Phylogenetic groups were assessed by quadruplex PCR as described by Clermont 2013. Seven of the previously identified groups were detected (A, B1, B2, C, D, E, F) as well as clade 1. All PCRs were carried out in a 20-µL final volume containing 1 × buffer, 2 µM of each dNTP, 2 U Taq polymerase (Invitrogen, Carlsbad, California, USA), 2 µL DNA (100 ng) and 1 µL of the respective primers. The reaction was performed under the following conditions: 4-min denaturation at 94 °C, 30 cycles of 5 s at 94 °C and 20 s at 57 °C (E group) or 59 °C (quadruplex and C group) and a final extension step of 5 min at 72 °C. Specific primers were used to assess the F, E and C groups. An agarose gel electrophoresis was performed, and the gel was subsequently stained with 2% ethidium bromide. Visualization was carried out using a UV transilluminator.

Detection of virulence genes

A multiplex PCR was carried out for the *fimH, papC* and *iutA* genes, whereas a duplex PCR was performed for *cnf1* and *hlyA*. The reaction mixture was prepared in a 25-µL final volume containing 2 µL DNA, 4 mM $MgCl_2$, 0.8 µM dNTPs, 0.6 µM of each of the previously described oligonucleotides (Stell Adam L.2000; Navia MM, 2002) and 2.5 U of Taq polymerase (Invitrogen, Carlsbad, California, USA. The following conditions were used: initial denaturalization for 5 min at 94 °C, 25 cycles at 94 °C for 30 s, 63 °C for 3 s, 68 °C for 3 min and a final extension at 72 °C for 10 min. An agarose gel electrophoresis was performed, and the gel was subsequently stained with 2% ethidium bromide. Visualization was carried out using a UV transilluminator.

Result analysis

A statistical analysis was performed by using the STATA software version 11.1 for Windows® (StataCorp, College Station, TX, USA) and the GraphPad Prims 6 software (GraphPad Software Inc.; San Diego, CA, USA). Data were presented as simple frequencies. Both, associations and the Hardy–Weinberg equilibrium (HWE) were evaluated by using the χ^2 statistical test. A statistical significance was considered at a p value ≤ 0.05.

Results

Clinical and demographic features

Patients were classified by age and sex, and we observed that the female gender is predominant by 76%. All patients were grouped into four main groups according to age: underage (< 18 years old), young adults (18–30 years old), old adults (31–59 years old) and elder (> 60 years old). Among them, the one that was frequently affected by urinary infections was the elder group with a 46.4% frequency. Within this patient group, the main symptom was burning while urinating up to 19% followed by pain with a 15% frequency. Among the susceptibility factors, we observed that diabetes mellitus was an important factor contributing to the establishment of these infections as 22% of the patients declared to be diagnosed with diabetes. A difference was observed when compared to the control group, $p < 0.01$ (Table 1).

TLR4 896 A>G, TLR4 1196 C>T, TLR4 − 2570 A>G and TLR4 − 2081 G>A genotypic and allelic frequencies

Based on the Hardy–Weinberg law, the control group was in genetic equilibrium ($p > 0.05$), regarding the 896 A>G, TLR41196 C>T, TLR4 − 2570 A>G and TLR4 − 2081 G>A polymorphisms of the *TLR4* gene. After genotyping both study groups, both genotype and allele frequencies were assessed. No association was found between the 896 A>G and 1196 C>T polymorphisms when both groups were compared. However, by comparing genotype and allele frequencies for the − 2570 A>G polymorphism, an association was found ($p = 0.003$) regarding the risk to develop UTI in the presence of the G allele in heterozygotes. This risk increases in the presence of the GG polymorphic genotype ($p < 0.01$). The − 2081 G>A polymorphism was associated with protective effect in the presence of the A allele ($p < 0.01$), in spite of the fact that the polymorphic genotype was not detected in our population (Table 2).

Table 1 Demographic and clinical characteristics in urinary tract infection patients and uninfected subjects

Variables	Infection patients (%)	Uninfected subjects (%)	*p* value
Demographics			
Gender			
Female	76	66	0.09
Male	24	36	
Age (years)			
Underage	4.2	13.9	$p < 0.001$*
Young adults	7.4	22.8	
Old adults	42.1	54.4	
Elder	46.4	8.9	
Clinical			
Symptomatology			
No symptoms	59	17	$p < 0.001$*
With symptoms	41	84	
Most frequent			
Dolor	15	0	
Burning	19	0	
Itch	7	0	
Fever	9	3	0.07
Sickness	3	4	0.71
Chill	5	0	
Low back pain	9	0	
Side pain	0	14	
Susceptibility factors			
Diabetes mellitus (DM)	22	4	$p < 0.001$*
Chronic renal failure (CRF)	7	3	0.07
DM + CRF	5	0	
Pregnancy	1	0	

*Statistically significant *p* values ($p \leq 0.05$). Frequencies were compared by χ^2 test

Association between phylogenetic group and virulence factors of uropathogenic *E. coli*

Based on the amplification of specific genes, *E. coli* strains were classified into eight phylogenetic groups: A, B1, B2, C, D, E, F and clade. The B2 group was the most frequent causing agent of UTI within our population (51%). Interestingly, a high frequency of the A group is also observed (15%), followed by B1 (11%) (Fig. 1). After establishing the association between phylogenetic groups and virulence factors, as expected, the B2 phylogroup exhibited more virulence factors when compared to other groups ($p < 0.05$), as well as group D, also displayed several virulence factors. However, it was also observed that the strains considered as commensals, belonging to groups A and B1, can cause UTI and present virulence factors. The *E. albertii* group expressed none of the five virulence genes (Fig. 2).

Frequency virulence factors of *Escherichia coli* and association with TLR4 896 A>G, TLR4 1196 C>T, TLR4 − 2570 A>G and TLR4 − 2081 G>A genotypic in patients with urinary tract infection

The genes coding for virulence factors identified in uropathogenic *E. coli* strains from clinical isolates are: *hlyA, fimH, papC, iutA* and *cnf1*. The most frequent virulence factor was fimbriae, coded by the *fimH* gene (84%), followed by the *iutA* iron uptake system (66%), *papC* (37%), *cnf1* (24%) and *hlyA* in a lesser extent (15%). The association between TLR4 genotypes and virulence factors was analyzed, and it was found that polymorphisms occurring at the receptor's ligand recognition site are correlated with the presence of virulence genes of *E. coli* causing infection. Specifically, the AA genotype of the 896 A/G polymorphism was associated with the presence of *hlyA* ($p < 0.05$), whereas the CC genotype of the 1196 C/T polymorphism was associated with the presence of *fimH* ($p < 0.05$) (Table 3).

TLR4 896 A>G, TLR4 1196 C>T, TLR4 − 2570 A>G and TLR4 − 2081 G>A genotypic and symptomatology association

When the association between polymorphisms and symptoms was analyzed, it was detected correlation between the presence of polymorphism 896 A>G and clinical symptoms ($p < 0.05$). A higher frequency was observed in the presence of the AA genotype (90.24%), whereas the G allele was detected in patients displaying less symptoms (9.76%). However, no association was found when the 1196 C>T, − 2570 A>G and − 2081 G>A polymorphisms were studied (Table 4).

Discussion

Urinary tract infections are the second most frequent infection in humans, and uropathogenic *Escherichia coli* (UPEC) is the main etiologic agent [23]. Several of the well-known factors contribute to its establishment, although genetic factors have been recently proposed as important risk factors for UTI susceptibility [24].

In our study population, a higher frequency of UTI is observed on females, especially in old adults. Several studies conducted on the country confirm that women are more likely to contract UTI at any age, although the most susceptible groups are the elders, as shown in this population [25, 26].

After comparing both genotype and allele frequencies of these polymorphisms located at the TLR4 promoter region, we observed that only by carrying − 2570 A>G it represents a risk factor for contract UTI, as observed for heterozygotes.

Table 2 *TLR4* 896 A>G, *TLR4* 1196 C>T, *TLR4* −2570 A>G and *TLR4* −2081 G>A genotypic and allelic frequencies

		Infection patients (%)	Uninfected subjects (%)	*p* value	OR (CI 95%)	*p* value
TLR4 896 A>G						
Genotypes	AA[a]	96	97	0.70	1.0	–
	AG	4	3		1.3 (0.2–9.1)	0.7
	GG	0	0		–	–
HWE			$\chi^2=0.02$, $p=0.87$			
Alleles	A	98	98.5	0.70	1.0	–
	G	2	1.5		1.3 (0.2–9.2)	0.7
TLR4 1196 C>T						
Genotypes	CC[a]	99	98	0.56	1.0	–
	CT	1	2		0.4 (0.0–9.6)	0.5
	TT	0	0		–	–
HWE			$\chi^2=0.01$, $p=0.91$			
Alleles	C	99.5	99	0.56	1.0	–
	T	0.5	1		0.4 (0.0–9.6)	0.5
TLR4 −2570 A>G						
Genotypes	AA[a]	14	34	0.003	1.0	–
	AG	66	55		2.9 (1.3–6.4)	$p<0.01$*
	GG	20	11		4.4 (1.5–12.9)	$p<0.01$*
HWE			$\chi^2=2.6$, $p=0.1$			
Alleles	A	47	61.5	0.004	1.0	–
	G	53	38.5		1.8 (1.18–2.73)	$p<0.01$*
TLR4 −2081 G>A						
Genotypes	GG[a]	96	83	0.003	1.0	–
	GA	4	17		0.2 (0.04–0.66)	$p<0.01$*
	AA	0	0		–	–
HWE			$\chi^2=0.86$, $p=0.35$			
Alleles	G	98	91.5	0.004	1.0	–
	A	2	0.5		0.2 (0.05–0.69)	$p<0.01$*

Observed and expected frequencies in all polymorphic sites were in Hardy–Weinberg equilibrium

[a]Genotype reference

*Statistically significant *p* values ($p \leq 0.05$). Genotype and allele frequencies were compared by χ^2 test

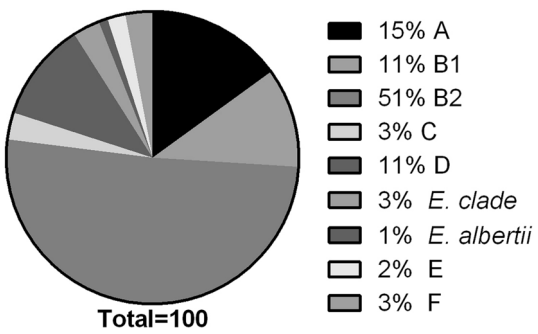

Fig. 1 Frequency of phylogenetic group of strains of *Escherichia coli* causing urinary tract infection

This risk increases in the presence of the GG polymorph phenotype. Conversely, it was found that by only carrying a −2081 G>A polymorphic allele, a protective effect is displayed against UTI in heterozygotes. However, it is not possible to confirm whether this association increases in the presence of the polymorphic genotype as it was not present in our study population.

Ragnarsdottir et al. [20] performed a series of in silico predictions in which they suggest possible mechanisms to explain why the presence of sequence variants within the *TLR4* promoter region may affect protein expression. Interestingly, they observed that the −2570 A>G polymorphism potentially affects a CdxA binding site. The latter is a homeodomain protein, and Tst-1 is a POU (Pit–Oct–Unc) transcription factor that significantly decreases the expression of the TLR4 gene [20].

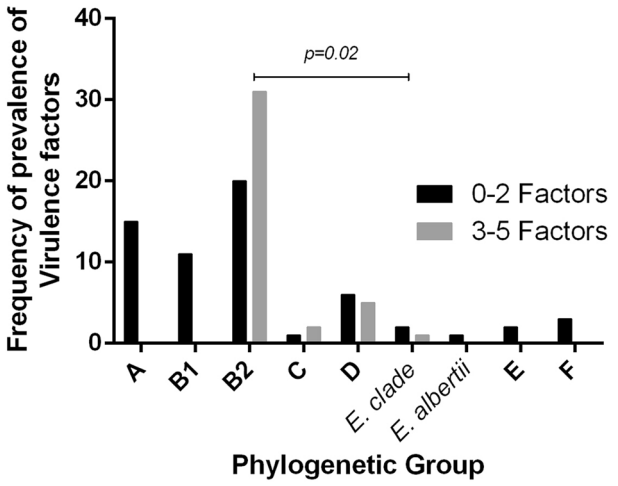

Fig. 2 Association phylogenetic group and virulence factors of uropathogenic *Escherichia coli*. Relationship between groups containing 0–2 and 3–6 virulence genes was determined by χ^2 test. Statistically significant p values ($p \leq 0.05$). The virulence factors evaluated are: *hlyA, fimH, papC, iutA* and *cnf1*

Thus, these findings suggest that the identification of the − 2570 A>G polymorphism as risk factor may be caused by a decreased receptor abundance, based on previous studies that showed an asymptomatic status linked to the attenuation of this gene, in fact Ragnarsdottir et al. [20] suggesting that reduced mucosal Tlr4 function may protect the host against symptomatic infection. Nevertheless, this condition maybe compromises the patient by preventing an adequate immune response for bacteriuria, including a non-medical visit due to lack of symptoms, carrying detrimental consequences to health, as infection may progress toward superior organs as kidneys, thereby generating several damages.

Ragnarsdottir et al. [20] also reported that the presence of the − 2081 G>A polymorphism modifies a possible N-Myc binding site as well. The latter is a transcription factor regulating the expression of several genes. A higher expression of the TLR4 gene has been reported in the presence of the − 2081 G>A polymorphism [20]. Thus, we suggest that our findings indicating − 2081 G>A as a protective factor may be explained by its ability to induce a higher TLR4 expression and therefore it contributes to eliminate the infection by triggering an efficient immune response.

At the TLR4 extracellular domain, the 1196 C>T y 896 A>G polymorphisms have been suggested to modify its function as the T-C transition at the 1196 position may lead to an exchange of isoleucine by threonine at the TLR4 extracellular domain. On the other hand, the G-A transition at the 896 position may lead to an exchange of glycine for aspartic acid. Therefore, these polymorphisms are classified as "loss of function" because they modify the interaction between TLR4 and its ligand [16].

In this study, no associations were found between genotype and allele frequencies of the 896 A>G and 1196 C>T polymorphisms when UTI patients were compared with uninfected subjects. Our results do not agree with those reported by Hawn et al. 27, as they report an association between the G allele of the 896 A>G polymorphism with recurrent UTI. It is worth to mention that within our study population a polymorphic genotype was not found. However, an association between the 896 A>G polymorphism and the presence of *hlya* was observed in *Escherichia coli* isolates in the presence of the wild-type allele (A/A). The latter suggests that such this site may be critical for the recognition of such virulence factor [27]. In fact, previous studies have demonstrated that this factor may directly stimulate TLR4 [13]. *hlya* is pore-forming toxin able to lyse red cells and nucleate host cells. It has been shown that it induces an increased production of IL-6 and IL-8 as well as pyelonephritis. This may explain the association between the 896 A>G polymorphism and symptoms observed in this study [28].

Regarding the 1196 C>T polymorphism, we found no association with UTI and the presence of the polymorphic genotype. Our findings support the observations made by Hawn et al. 27, as they did not identify an association with UTI susceptibility in adult women and they did not report the presence of the polymorphic genotype. However, it is interesting that this study identified an association between the 1196 C>T polymorphism and the presence of the *fimH* gene in the presence of the wild-type allele (C/C) in clinical isolates. This result suggests that this site may be important during the recognition of such virulence factor. This study is the first one to report this previously overlooked finding.

Escherichia coli strains were classified into eight groups: A, B1, B2, C, D, E, F and clade, based on the amplification of specific genes. Both A and B1 strains are considered to possess a low virulent potential, whereas B2 and D strains harbor genes coding virulence factors responsible for colonization, adhesion, invasion and evasion from the defense mechanisms of the human host. The B2 group was the most frequent in our clinical isolates, and they exhibit more virulence genes in comparison with the other strains from the rest of the phylogenetic groups [29, 30]. Nevertheless, it was also observed that strains considered as commensals, such as those from the A and B1 groups, have acquired virulence genes and they may cause UTI.

In this study, the *hlyA, fimH, papC, iutA* and *cnf1* virulence genes were assessed in *Escherichia coli* clinical isolates. A study conducted in Mérida (Venezuela) by Millan et al. reported that the more frequent adhesins were type 1 fimbriae, such as *fimH*, when compared to other adhesins [31]. Miranda-Estrada et al. observed similar results as they reported a high frequency of *fimH* within the central and southeast populations in Mexico. In our study, the

Table 3 Frequency virulence factors of *Escherichia coli* and association with *TLR4* 896 A>G, *TLR4* 1196 C>T, *TLR4* −2570 A>G and *TLR4* −2081 G>A genotypic in patients with urinary tract infection

	Virulence factors									
	papC		*IutA*		*fimH*		*cnf1*		*hlyA*	
	Present 37%	Absent 63%	Present 66%	Absent 34%	Present 84%	Absent 16%	Present 24%	Absent 76%	Present 15%	Absent 85%
TLR4 896 A>G genotypes	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
AA	34 (91.89)	62 (98.41)	62 (93.4)	34 (100)	80 (95.24)	16 (100)	22 (91.67)	74 (97.37)	13 (86.67)	83 (97.65)
AG	3 (8.11)	1 (1.59)	4 (6.06)	0	4 (4.76)	0	2 (8.33)	2 (2.63)	2 (13.33)	2 (2.35)
p value		0.10		0.14		0.37		0.21		$p=0.04$*
TLR41196 C>T genotypes										
CC	37 (100)	62 (98.41)	66 (100)	33 (97.06)	84 (100)	15 (93.75)	24 (100)	75 (98.68)	15 (100)	84 (98.82)
CT	0	1 (1.59)	0	1 (2.94)	0	1 (6.25)	0	1 (1.32)	0	1 (1.18)
p value		0.44		0.16		$p=0.02$*		0.57		0.67
TLR4 −2570 A>G genotypes										
AA	6 (16.22)	8 (12.70)	11 (16.67)	3 (8.82)	12 (14.29)	2 (12.50)	3 (12.50)	11 (14.47)	2 (13.33)	12 (14.12)
AG	24 (64.86)	42 (66.67)	42 (63.64)	24 (70.59)	54 (64.29)	12(75)	16 (66.67)	50 (65.79)	10 (66.67)	56 (65.88)
GG	7 (18.92)	13 (20.63)	13 (19.70)	7 (20.59)	18 (21.43)	2 (12.50)	5 (20.83)	15 (19.74)	3 (20)	17 (20)
p value		0.88		0.56		0.67		0.96		0.99
TLR4 −2081 G>A genotypes										
GG	36 (97.30)	60 (95.24)	63 (95.45)	33 (97.06)	81 (96.43)	15 (93.75)	24 (100)	72 (94.74)	15 (100)	81 (95.29)
GA	1 (2.70)	3 (4.76)	3 (4.55)	1 (2.949	3 (3.57)	1 (6.25)	0	4 (5.26)	0	4 (4.71)
p value		0.61		0.69		0.61		0.25		0.391

*Statistically significant *p* values ($p \leq 0.05$). Frequencies were compared by χ^2 test. Polymorphic genotypes were not found for TLR4 896 A>G, TLR4 1196 C>T, TLR4 − 2081 G>A

Table 4 TLR4 896 A>G, TLR4 1196 C>T, TLR4 − 2570 A>G and TLR4 − 2081 G>A genotypic and symptomatology association

	Symptomatology				
	No symptoms *n* (%)	With symptoms *n* (%)	*p* value	OR	*p*
TLR4 896 A>G genotypes					
AA[a]	59 (100)	37 (90.24)	1.0	–	–
AG	0	4 (9.76)	$p < 0.05$*	0.6	6
GG	0	0	–	–	–
TLR4 1196 C>T genotypes					
CC[a]	59 (100)	40 (97.56)	1.0	–	–
CT	0	1 (2.44)	0.228	0	1.45
TT	0	0	–	–	–
TLR4 − 2570 A>G genotypes					
AA[a]	6 (10.17)	8 (19.51)	1.0	–	–
AG	43 (72.88)	23 (56.10)	0.11	2.4	2.42
GG	10 (16.95)	10 (24.39)	0.68	1.3	0.17
TLR4 − 2081 G>A genotypes					
GG[a]	56 (94.92)	40 (97.56)	1.0	–	–
GA	3 (5.08)	1 (2.44)	0.507	2.1	0.44
AA	0	0	–	–	–

[a]Genotype reference

*Statistically significant *p* values ($p \leq 0.05$). Frequencies were compared by χ^2 test

fimH virulence factor occurs in most of the isolated strains. This factor is essential during colonization as it promotes adhesion to urothelium because of its high affinity toward uroplakin; thus, bacterial settlement is enabled and consequently it is critical for UTI [32]. Moreover, this study shows the association between the *fimH* gene and the 1196 C>T polymorphism.

The *iutA* was the second most frequent virulence factor, and similar results were reported by Munkhdelger 33. This may be caused by its close relationship with iron transport. This element is highly important for survival as the urinary tract lacks iron and other nutrients; thus, the ability to obtain them is essential when infection occurs [33, 34].

It is important to mention that there are several TLR types in the immune system that may interact with virulence factors displayed by pathogenic bacteria causing UTI, and they trigger an inflammatory process. TLR2 is important as they detect and identify UTI caused by pathogenic microorganisms as they recognize atypical LPS among a wide variety of target ligands such as lipoproteins, lipoteichoic acid, peptidoglycans and heat-shock proteins (HSPs). TLR5 activation is induced by the presence of bacterial flagellin. As UPEC is the typical causing agent of UTI, it possesses peritrichous flagella that induce TLR5 expression. Therefore, they are the most suitable molecules among all defense mechanisms in the urinary tract. It will be interesting to perform an integral evaluation regarding the association between these receptors and UTI establishment [35].

An efficient immune response is able to clear the urinary tract of microbial pathogens such as UPEC, even within a few days. Conversely, a malfunction of the innate response may promote UTI progression. In conclusion, the genetic variations existing within the *TLR4* promoter region may be essential elements during UTI susceptibility. On the other hand, variations of the TLR4 receptor are associated with the recognition of virulence factors occurring in *Escherichia coli*. This invaluable information may help us in the future to use TLR4 as biomarker for UTI diagnosis and prognosis.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest related to this study.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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