Infection and Drug Resistance

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ORIGINAL RESEARCH

Association Between Uropathogenic *Escherichia coli* Virulence Genes and Severity of Infection and Resistance to Antibiotics

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Purpose: Urinary tract infection (UTI) is the most frequent bacterial infection. Some uropathogenic *Escherichia coli* (UPEC) genes have been associated with disease severity and antibiotic resistance. The aim was to determine the association of nine UPEC virulence genes with UTI severity and antibiotic resistance of strains collected from adults with community-acquired UTI.

Patients and Methods: A case-control study (1:3) (38 urosepsis/pyelonephritis and 114 cystitis/urethritis) was conducted. The *fimH, sfa/foc, cvaC, hlyA, iroN, fyuA, ireA, iutA*, and *aer* (the last five are siderophore genes) virulence genes were determined by PCR. The information of antibiotic susceptibility pattern of the strains was collected from medical records. This pattern was determined using an automated system for antimicrobial susceptibility testing. Multidrug-resistant (MDR) was defined as resistance to three or more antibiotic families.

Results: *fimH* was the most frequently detected virulence gene (94.7%), and *sfa/foc* was the least frequently detected (9.2%); 55.3% (83/150) of the strains were MDR. The evaluated genes were not associated with UTI severity. Associations were found between the presence of *hlyA* and carbapenem resistance (Odds ratio [OR] = 7.58, 95% confidence interval [CI], 1.50–35.42), *iutA* and fluoroquinolone resistance (OR = 2.35, 95% CI, 1.15–4.84, and *aer* (OR = 2.8, 95% CI, 1.20–6.48) and *iutA* (OR = 2.95, 95% CI, 1.33–6.69) with penicillin resistance. In addition, *iutA* was the only gene associated with MDR (OR = 2.09, 95% CI,1.03–4.26).

Conclusion: There was no association among virulence genes and UTI severity. Three of the five iron uptake genes were associated with resistance to at least one antibiotic family. Regarding the other four non-siderophore genes, only *hlyA* was associated with antibiotic resistance to carbapenems. It is essential to continue studying bacterial genetic characteristics that cause the generation of pathogenic and multidrug-resistant phenotypes of UPEC strains.

Keywords: uropathogenic *E. coli*, antibiotic resistance, virulence factor, urinary tract infection

Introduction

Urinary tract infection (UTI) is the most frequent bacterial infection, and it affected more than 404.6 million people worldwide in 2019, resulting in 5.2 million Disability-Adjusted Life-Years.[1] In addition, the high frequency of this infection implies great economic losses due to work absenteeism.[2] In the US, the annual cost of treating this disease exceeds $3.5 billion.[3] UTIs have different clinical forms, and there are several classifications. According to the anatomical division, UTIs can be high (acute pyelonephritis, bacterial nephritis, intrarenal abscess, and perinephric abscess) or low (cystitis, urethritis, and acute prostatitis), and they can be uncomplicated or complicated which are associated with risk factors such as urinary tract abnormalities or immune system compromise.[3–5] UTIs can also be classified by the source of infection as community-acquired, hospital-acquired, and healthcare-associated.[6]

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A broad spectrum of pathogens causes UTIs. However, the most frequent agent of community-acquired UTI is uropathogenic *E. coli* (UPEC), accounting for 80% to 90% of cases.[7] UPEC has generated great interest due to its high antimicrobial resistance. UTIs caused by UPEC occur when fecal contamination of the periurethral area enters through the urethra. Subsequently, the expression of pili and adhesins allows invasion of superficial bladder cells. The host inflammatory response begins to eliminate the extracellular bacteria. However, some bacteria that evade the immune system multiply and form biofilms. In addition, the bacteria produce toxins and proteases that induce damage to host cells, releasing essential nutrients that promote bacterial survival and ascent to the kidneys. Without proper treatment, UPEC crosses the tubular epithelial barrier in the kidneys allowing UTIs to progress to bacteremia.[3]

UPEC strains produce virulence factors encoded in pathogenicity islands (PAI), plasmids, and transposons that allow them to colonize the urinary tract and persist there despite the action of the host immune system. These are divided into 1) cell surface-associated virulence factors, such as adhesins and invasins, and 2) secreted virulence factors, such as toxins and siderophore systems.[8] The adhesion factors of UPEC are fimbriae, and the most common ones are type 1, type P, type S, and F1C, encoded by the *fim, pap, sfa*, and *foc* operons, respectively.[9] The most crucial virulence factor secreted by UPEC is α-hemolysin (HlyA), a proinflammatory toxin that promotes the release of IL-6 and IL-8, increasing the UTI severity.[6] Another relevant toxin is colicin V, encoded by the *cvaC* gene, which is produced under stress conditions and acts as a protectin.[10,11]

In addition, siderophore systems encoded by the *iutA, ireA, fyuA, iroN*, and *aer* genes have been associated with the occurrence and severity of UTIs.[12,13] Siderophores allow *Escherichia coli* the acquisition of iron from the host, as well as its colonization and survival; at the same time, siderophores protect *Escherichia coli* from the toxic potential of this metal.[14] Also, the plasmid-encoded *iutA* gene is the most frequently associated with strains resistant to different antibiotics as it is found on the same plasmid that contains resistance determinants.[15] Although antibiotic therapy is usually adequate for treating UTIs, the increase in antimicrobial resistance, as well as the initiation of an inappropriate antibiotic scheme, facilitates a therapeutic failure that can lead to a complicated UTI.[16] All of the above justifies studies to expand knowledge about the pathophysiology of this disease. Therefore, this study aimed to determine the frequency of nine virulence genes of UPEC and the relationship of these genes with the severity of community-acquired UTI, and antimicrobial resistance.

Materials and Methods

Design and Study Population

A case-control study (1:3) was conducted. The study included men and women 18 years and older, who lived in the Metropolitan Area of Bucaramanga (AMB), Colombia, and went to the Hospital Local del Norte (HLN) or the Hospital Universitario de Santander (HUS) with a clinical suspicion of UTI, and a urine culture of $\geq 10^5$ CFU/mL of *E. coli*. Pregnant women, patients with polymicrobial infection, HIV, cancer, immunosuppressive treatment, patients with a urinary catheter in the last seven days, or in-patients in the last 72 hours before symptom onset were excluded.

The sample size was calculated for each of the nine genes, considering a confidence level of 95%, a power of 80%, a case-control ratio of 1:3, and the frequency of the genes reported in the literature (Supplementary Table S1). The genes with the highest requirement were *fyuA* and *sfa/foc*, for which the required size was 45:138 and 36:123, respectively. We used the open-source software OpenEpi (version 3.01) to calculate the sample size. However, during the study period, February 2015 to May 2018, 38 cases were collected, for which 114 controls were randomly selected.

The cases were patients with urosepsis or pyelonephritis (severe UTI). Pyelonephritis was defined by the presence of urinary or general symptoms and a positive fist percussion sign of the kidney on physical examination, or by the definition of the treating physician. The controls were patients with low UTI (cystitis or urethritis) defined by the presence of low urinary symptoms (dysuria, pollakiuria, nocturia, urinary urgency, bladder urgency, or pain in the hypogastrium).

Isolation, Antimicrobial Susceptibility Test (AST), and Microbial Growth *E. coli*

E. coli strains were collected by laboratory professionals from the HLN and HUS. Any colony identified as *E. coli* by the Vitek® 2 compact system (bioMérieux, France) at the HLN or by the Phoenix™ 100 automated microbiology system (BD, USA) at the HUS was cultured on Luria Broth agar for transport to Laboratorio de Investigaciones Biomédicas y Biotecnológicas (LIBB) at Universidad de Santander. These devices also perform the AST. Subsequently, a culture was performed on Luria Broth agar to grow the strain at the LIBB. After 16 hours, all colonies were picked with a calibrated 0.1 µL sterile loop. Genomic DNA was extracted by the boiling method and stored at −20°C for preservation until PCR test processing.

Molecular Characterization of *E. coli*

E. coli identity was confirmed by detecting the *uidA* gene.[17] The conditions for this PCR test were heating at 94°C for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, and the final extension at 72°C for 5 minutes, using the Platinum™ Taq DNA Polymerase (Invitrogen™, Thermo Fisher Scientific, USA). The *sfa/foc* and *fyuA* genes were analyzed using multiplex PCR test, while the remaining seven genes were analyzed using single PCR tests (Supplementary Table S2). Electrophoresis was performed on 1.5% agarose gel stained with SYBR® safe DNA Gel Stain (Invitrogen™, Thermo Fisher Scientific, USA) for 30 minutes between 80 and 90 volts in a horizontal electrophoresis chamber. The gel was analyzed on a MiniBIS Pro UV transilluminator (DNR Bio-Imaging Systems, Israel). The 1 kb molecular weight marker GeneRuler™ DNA Ladder (Thermo Fisher Scientific, USA) was used.

To obtain a positive control strain, pools of five consecutive samples were made. For the first positive pool, individual PCR was performed on all samples. The identity of the *sfa/foc, fyuA, iroN, fimH, iutA, ireA*, and *cvaC* genes was confirmed in the amplification of one sample by Sanger sequencing, and it was taken as a positive control in the subsequent assays. For the *hlyA* and *aer* genes, confirmation by Sanger sequencing was not performed. Also, *E. coli* ATCC 25922 was used as a negative control strain, and each PCR reaction had a negative reagent control.

Data Collection and Analysis

Demographic and clinical variables were obtained from the medical records. Qualitative variables were described using absolute and relative frequencies. For quantitative variables, median and interquartile range (IQR) were reported because these variables did not show normal distribution. The normal distribution was tested using the Skewness Kurtosis test. The antibiotic susceptibility pattern of the strains (susceptible, intermediate, and resistant) and the gene frequencies were also described using absolute and relative frequencies. Subsequently, univariate analysis was performed comparing the frequency of *E. coli* genes between cases and controls, as well as the frequency of clinical and laboratory characteristics using the Fisher's exact test. In addition, quantitative variables were compared between cases and controls with the Mann–Whitney *U*-test.

The relationship between the presence of virulence genes and the resistance to each family of antibiotics, as well as multidrug resistance (MDR), was also analyzed using the Fisher's exact test. Each strain was classified dichotomously (susceptible or intermediate/resistant) for each antibiotic family. MDR was defined as resistance to three or more antibiotic families in those strains (n = 150) in which between six and twelve antibiotic families were evaluated (see Supplementary Material).[18] In all cases, statistical significance was set at $p < 0.05$. In addition, Odds Ratios (OR) were calculated. The analysis was conducted using the Stata 16/SE (StataCorp, USA).

Results

Sociodemographic and Clinical Characteristics of Cases and Controls

Thirty-eight cases (31 pyelonephritis and 7 urosepsis) and 114 controls (cystitis) were included. The age median was 50 years in cases and 55.5 years in controls. More women were in the control group (90.4% vs 76.3%). Fourteen of the pyelonephritis cases had positive fist percussion sign, and the others were classified as cases according to the clinical criteria of the treating physician as well as the urosepsis cases. The most frequent symptoms in cases were dysuria (68.4%), fever (57.9%), pollakiuria (50%), and flank pain (50%). The main comorbidities were diabetes mellitus and

hypertension. Also, recurrent UTI was the most frequent pathological antecedent, with no significant difference between the two groups. The history of urinary tract surgery and antibiotic intake in the two months before the symptom onset were similar in both groups (Table 1).

Hematocrit percentage was significantly lower in cases, while the total leukocyte count was significantly higher. In the urinalysis, only urobilinogen was found more frequently in cases. Positive nitrites, leukocytosis, and bacterial count per field did not show a significant difference between cases and controls (Table 2).

Frequency of Virulence Genes of UPEC Strains and Association with the UTI Severity

fimH was the most frequent gene (94.7% of strains), and *sfa/foc* (9.2%) was the least frequent. *fyuA* was the most frequent siderophore gene (77.7%) followed by *aer* (71.6%). There was no association between UTI severity and the genes evaluated (Table 3).

Relationship Among Virulence Genes

Regarding the relationship among genes, *cvaC* was positively related to three of five siderophore genes (*iroN, aer*, and *iutA*). In addition, *hlyA* was positively related to *sfa/foc* ($p < 0.001$). Moreover, all strains that had *iutA* gene (n = 71; $p< 0.001$) also had *aer* gene. Similarly, all strains that had *cvaC* gene also had *aer* gene (n = 18; $p= 0.002$). Except for *fimH*, all genes evaluated were positively related to at least one of the other genes (Table 4).

Table 1 Demographic Characteristics, Symptoms, and Medical History of Cases and Controls

Symptoms	Cases n=38	Controls n=114	p[b]
Age Median (IQR)	50 (39–69)	55.5 (43–72)	0.210
Sex Female n (%)	29 (76.3)	103 (90.4)	**0.027**
Dysuria	26 (68.4)	96 (81.6)	0.088
Fever	22 (57.9)	43 (37.7)	**0.030**
Pollakiuria	19 (50.0)	35 (30.7)	**0.031**
Flank pain	19 (50.0)	9 (7.9)	**<0.0001**
Costovertebral pain	18 (47.4)	8 (7.0)	**<0.0001**
Hypogastric pain	15 (39.5)	14 (12.3)	**<0.0001**
Urinary frequency	14 (36.8)	24 (21.0)	0.052
Urinary urgency	13 (34.2)	8 (7.0)	**<0.0001**
Nocturia	8 (21.0)	10 (8.8)	**0.042**
Hematuria	4 (10.5)	5 (4.4)	0.170
Diabetes mellitus	6 (15.8)	15 (13.2)	0.680
High blood pressure	9 (23.7)	13 (11.4)	0.060
Recurrent UTI	25 (65.8)	73 (64)	0.800
Antibiotic use in the last two months[a]	12 (31.6)	29 (25.4)	0.460
Urinary tract surgery	2 (5.3)	9 (7.9)	0.600

Notes: [a]Patients who reported antibiotic use in the last two months before the onset of symptoms. [b]Cases and controls were compared using Fisher's exact test and Mann–Whitney *U*-test. Statistical significance is in bold.
Abbreviation: IQR, Interquartile range.

Table 2 Hemogram, Creatinine and Urinalysis Count of Cases and Controls

Test	n (Cases vs Controls)	Cases n=38	Controls n=114	*p*[a]
Hematocrit Median (IQR)	23 vs 36	37.3 (35.1–39.5)	39.9 (38.6–41.1)	**0.028**
Hemoglobin (g/dl)	23 vs 35	12.4 (11.6–13.2)	13.2 (12.7–13.6)	0.059
Total leukocytes $x10^3$	23 vs 35	14 (10.4–22.6)	8.9 (6.4–13.4)	**0.002**
Platelets $x10^6$	22 vs 31	2.9 (2.3–3.8)	2.9 (2.4–3.5)	0.960
Creatinine (mg/dl)	17 vs 19	0.9 (0.8–2.0)	0.9 (0.8–1.1)	0.270
Urinalysis				
Density	28 vs 46	1015 (1010–1020)	1015 (1010–1020)	0.510
pH	28 vs 46	5 (5–7)	5.5 (5–6)	0.820
Nitrite n (%)	11 vs 17	11 (39.3)	17 (35.4)	0.700
Blood	17 vs 23	17 (68.0)	23 (48.9)	0.120
Leukocyte esterase	21 vs 41	13 (61.9)	24 (58.5)	0.800
Protein	25 vs 46	6 (24.0)	6 (13.0)	0.230
Glycosuria	17 vs 39	2 (11.8)	3 (7.7)	0.600
Ketonuria	24 vs 45	2 (8.3)	3 (6.7)	0.800
Urobilinogen	25 vs 46	5 (20.0)	2 (4.4)	**0.035**
Bilirubin	25 vs 46	2 (8.0)	1 (2.2)	0.240
Leukocyte/hpf ≤5 6–10 11–20 >20	28 vs 49	 6 (21.4) 2 (7.1) 6 (21.4) 14 (50.0)	 14 (28.6) 7 (14.3) 11 (22.5) 17 (34.7)	0.530
Red blood cells/hpf ≤5 6–10 >10	22 vs 39	 15 (68.2) 4 (18.2) 3 (13.6)	 32 (82.0) 4 (10.3) 3 (7.7)	0.430
Bacteria/hpf + ++ +++ ++++	27 vs 49	 5 (18.5) 7 (25.9) 14 (51.9) 1 (3.7)	 4 (8.2) 11 (22.5) 31 (63.3) 3 (6.1)	0.520
Urine mucus 0 + ++ +++	23 vs 48	 3 (13.0) 14 (60.9) 5 (21.7) 1 (4.35)	 4 (8.3) 30 (62.5) 14 (29.2) 0	0.430

Notes: [a]Cases and controls were compared using Fisher's exact test and Mann–Whitney *U*-test. Statistical significance is in bold.
Abbreviations: IQR, Interquartile range; hpf, high power field.

Antibiotic Resistance of UPEC Strains

UPEC strains showed a high rate of resistance to ampicillin (67.6%), ciprofloxacin (51.7%), and trimethoprim-sulfamethoxazole (SXT) (34%), and low resistance to nitrofurantoin (10.2%), piperacillin/tazobactam (6.2%), and amikacin

Table 3 Comparison of Virulence Genes Between Cases and Controls

Gene	Total n=152	Cases n=38	Controls n=114	*p*[a]	OR (95% CI)
cvaC	19 (12.5)	4 (10.5)	15 (13.2)	0.784	0.77 (0.16–2.67)
sfa/foc	14 (9.2)	4 (10.5)	10 (8.8)	0.750	1.22 (0.26–4.59)
fimH	144 (94.7)	34 (89.5)	110 (96.5)	0.108	0.31 (0.55–1.77)
hlyA	24 (15.8)	7 (18.4)	17 (14.9)	0.613	1.29 (0.41–3.65)
iroN	73 (48.0)	16 (42.1)	57 (50.0)	0.456	0.72 (0.32–1.62)
aer	101 (71.6)	24 (75.0)	77 (70.6)	0.824	1.24 (0.48–3.56)
iutA	71 (46.7)	16 (42.1)	55 (48.3)	0.576	0.78 (0.34–1.74)
ireA	27 (17.8)	7 (18.4)	20 (17.5)	1	1.06 (0.34–2.93)
fyuA	118 (77.7)	30 (79.0)	88 (77.2)	1	1.11 (0.43–3.14)

Note: [a]Cases and controls were compared using Fisher's exact test.
Abbreviations: OR, Odds ratio; CI, confidence interval.

Table 4 Relationship Among Virulence Genes in UPEC Strains

Gene	Virulence Genes Samples with Both Genes (*p*-value)[a]							
	***sfa/foc* n=14**	***fimH* n=144**	***iroN* n=73**	***aer*[a] n=101**	***hlyA* n=24**	***iutA* n=71**	***ireA* n=27**	***cvaC* n=19**
fyuA n=118	10(0.517)	112(1)	53(0.176)	87(**0.001**)	21(0.288)	61(**0.031**)	22(0.800)	15(1)
sfa/foc n=14	–	13(0.547)	9(0.265)	9(1)	9(<**0.001**)	6(0.787)	8(**0.001**)	3(0.386)
fimH n=144	–	–	69(1)	96(1)	24(0.357)	70(0.068)	27(0.352)	18(1)
iroN n=73	–	–	–	50(1)	9(0.276)	36(0.626)	14(0.677)	14(**0.025**)
aer[a] n=101	–	–	–	–	18(0.614)	71(<**0.001**)	22(0.148)	18(**0.002**)
hlyA n=24	–	–	–	–	–	12(0.825)	10(**0.002**)	2(0.739)
iutA n=71	–	–	–	–	–	–	15(0.369)	16(<**0.001**)
ireA n=27	–	–	–	–	–	–	–	6(0.110)

Notes: [a]Fisher's exact test; [b]*aer* was evaluated in 141/152 strains, this gene was detected in 101/141 strains. Statistical significance is in bold.

(1.3%). Resistance to cefuroxime was more frequent in controls than in cases (37.5% vs 7.1%; p= 0.04). Resistance to ertapenem increased over time, being 0% in 2015, 2.9% in 2017, and 21.1% in 2018. On the other hand, resistance to SXT decreased from 58.5% in 2015 to 15.8% in 2018. Resistance to nalidixic acid decreased from 55.3% in 2015 to 26.9% in 2016. However, the sustainability of this trend could not be assessed because the resistance to this antibiotic was not evaluated in strains collected in 2017 and 2018.

Considering the resistance of the twelve antibiotic families in strains in which at least six different families were evaluated (n = 150), only 11.3% (n = 17) did not show resistance to any of the antibiotic families. No UPEC was resistant to more than ten antibiotic families. However, 55.3% (83/150) of the strains were MDR (Figure 1).

Relationship Between UPEC Virulence Genes and Resistance to Antibiotic Families

A higher frequency of the *fyuA* gene was found in strains resistant to cefepime, as well as of *hlyA* in those resistant to carbapenems, of *iutA* in those resistant to fluoroquinolones, and of *aer* and *iutA* in those resistant to penicillin. On the other hand, a higher frequency of the *hlyA* gene was found in strains sensitive to fluoroquinolones (p= 0.001), as well as

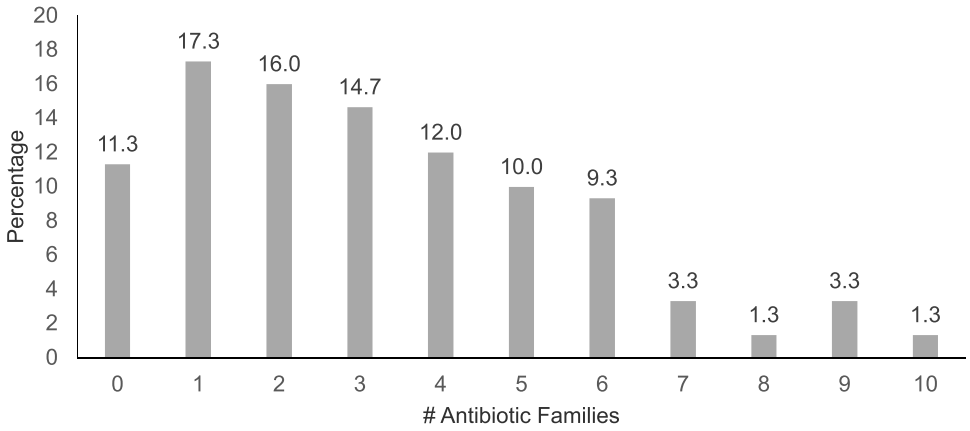

Figure 1 Frequency of UPEC strains resistant to different antibiotic families (n=150).
Notes: Two strains were not considered in this analysis because there was not enough information for antibiotic resistance. X-axis represents the quantity of the antibiotic families to which the strains were resistant, and y-axis represents the percentage of strains resistant.

of *ireA* in strains sensitive to penicillin (p = 0.033). Although, *sfa/foc* was more frequent in strains resistant to carbapenems and *iroN* was more frequent in strains resistant to fluoroquinolones, these differences were not significant (p= 0.058 and p=0.050, respectively). *fimH* and *cvaC* genes were not related to resistance to any of the antibiotic families (Table 5). Additionally, only *iutA* was significantly more frequent in MDR strains (Table 6).

Regarding the association between genes and resistance to antibiotic families, a positive association was found between the presence of *hlyA* and resistance to carbapenems (OR = 7.58, 95% CI, 1.50–35.42), *iutA* and resistance to fluoroquinolones (OR = 2.35, 95% CI, 1.15–4.84), and between *aer* (OR = 2.8, 95% CI, 1.20–6.48) and penicillin resistance as well as *iutA* (OR = 2.95, 95% CI, 1.33–6.69) and penicillin resistance. In contrast, a negative association was found between the presence of *hlyA* and fluoroquinolone resistance (OR = 0.18, 95% CI, 0.05–0.55) and the presence

Table 5 Relationship Between Virulence Genes and Antibiotic Resistance

Antimicrobial Agent	Virulence Genes *p* value[a]								
	cvaC	***sfa/foc***	***fimH***	***hlyA***	***iroN***	***aer***	***iutA***	***ireA***	***fyuA***
1st generation cephalosporin	0.431	1	0.661	0.647	0.605	0.846	0.304	0.647	0.835
2nd generation cephalosporin	0.425	0.410	0.547	0.106	0.379	0.527	0.772	0.714	0.474
3rd generation cephalosporin	1	1	0.435	1	0.704	1	1	0.454	0.111
Cefepime	0.756	0.167	0.583	0.504	1	1	0.830	0.414	**0.043**
Aztreonam	0.615	0.658	0.131	0.615	0.706	1	0.535	1	0.710
Penicillins	1	0.380	1	1	0.377	**0.010**	**0.004**	**0.033**[b]	0.203
Carbapenems	1	0.058	1	**0.006**	1	1	0.544	0.090	1
Aminoglycosides	1	1	0.202	0.198	0.853	0.396	0.062	0.620	1
Nalidixic acid	0.449	1	1	0.127	0.138	0.161	0.127	0.745	0.779
Fluoroquinolones	0.470	0.405	1	**0.001**[b]	0.050	0.852	**0.013**	0.667	0.846
Nitrofurantoin	1	0.690	1	1	0.622	0.574	1	0.335	0.766
SXT	1	0.560	0.422	0.244	0.229	0.426	0.082	1	0.839

Notes: [a]Fisher's exact test; [b]Negative relationship (higher resistance in those who do not have the virulence gene). The specific antimicrobial agents for each family of antibiotics are in the Supplementary Material. Statistical significance is in bold.
Abbreviation: SXT, Trimethoprim-sulfamethoxazole.

Table 6 Frequency of UPEC Virulence Genes and Their Relationship with MDR

Gene	No MDR %	MDR %	p^a
cvaC	11.9	13.3	1
sfa/foc	11.9	7.2	0.401
fimH	95.5	95.2	1
hlyA	17.9	14.5	0.656
iroN	50.8	47.0	0.743
aer	68.3	73.7	0.573
iutA	37.3	55.4	**0.033**
ireA	19.4	15.7	0.665
fyuA	71.6	81.9	0.170

Notes: [a]Fisher's exact test. Statistical significance is in bold.
Abbreviation: MDR, Multidrug resistance.

of *ireA* and penicillin resistance (OR = 0.36, 95% CI, 0.14–0.96). In addition, *iutA* was the only gene associated with MDR (OR = 2.09, 95% CI, 1.03–4.26).

Discussion

Frequency of UPEC Virulence Genes and Their Relationship with the UTI Severity

Most studies report that the most frequent genes in UPEC strains are *fimH* (68% to 96%), *iutA* (54% to 62%), and *aer* (47% to 66%). We found similar frequencies (*fimH* 94.7%, *iutA* 46.7% and *aer* 71.6%).[7,14,19,20] It has been explained that the variation in the frequency of these genes is due to strains belonging to different phylogenetic groups.[21,22] Hyun et al reported that, in UPEC strains, *fimH, sfa/foc, hlyA, cvaC* and *fyuA* genes were more frequent in the B2 phylogenetic group compared to group D.[21] However, we did not perform phylogenetic classification of the strains.

We did not find a relationship between the presence of the virulence genes evaluated and the UTI severity. Contrary to our findings, others have reported differences in the frequency of *iutA, ireA* and *cvaC* genes in strains from patients with cystitis and pyelonephritis,[12] and a higher frequency of *sfa/foc, fimH, hlyA*, and *aer* in isolates that cause pyelonephritis when compared with isolates that cause cystitis.[23] Also, Karam et al, found a higher frequency of *iutA* and *fyuA* in UPEC strains causing cystitis or pyelonephritis than in commensal strains.[24] These differences can be explained by the characteristics of each population. For example, Johnson et al included only women, and they had a lower prevalence of prior UTI history (35%).[12] In addition, Tarchouna et al included children,[23] and Karam et al studied younger women with a mean age of 39.5 years (range 20 to 60 years).[24]

On the other hand, we found a positive relationship between the *iutA, aer, cvaC*, and *iroN* genes, which can be explained because they are contained in the ColV plasmid. The *cvaC* gene is in the variable region of ColV, whereas *aer, iutA*, and *iroN* are in the conserved region.[25] Karam et al,[24] unlike us, found a negative relationship between *iroN* and the *iutA* and *ireA* genes, which may be due to the loss of some operons in certain UPEC strains.

Antibiotic Resistance in UPEC Strains

We found a high rate of resistance to ciprofloxacin and SXT, more likely because these were the first-line antibiotics used for UTI treatment in the region.[26] Antibiotic resistance varies markedly in different regions of the world. Europe has a low resistance to antibiotics such as ciprofloxacin and SXT ranging from 3 to 31%, while resistance to nitrofurantoin reaches a maximum of 6.7%.[27] In East Asian countries, resistance to ampicillin ranges from 54 to 80%, as well as resistance to ciprofloxacin (between 25 and 70%), and SXT (between 40 and 50%), while antibiotics such as piperacillin-

tazobactam and amikacin maintain their effectiveness with very low resistances from 1 to 15%.[21,28,29] Moreover, according to different studies conducted in Iran, ampicillin resistance can reach up to 80%, while resistance to SXT and ciprofloxacin was found in similar ranges to those found in our study (from 45 to 61%).[24,30–33]

In contrast, Allami et al found a different antibiotic resistance pattern, with SXT resistance of 87%, nitrofurantoin resistance of 45%, and ciprofloxacin resistance of 27%, while antibiotics such as amikacin and imipenem had low resistance (11% and 5%, respectively).[7] Also, a study conducted in Cameroon in 2012 showed how the pattern of antibiotic resistance changes even between cities in the same country. Thus, in the city of Buea, low resistance was found for ampicillin, SXT, and ciprofloxacin (from 0 to 6.7%) and very high for nitrofurantoin (80%), while in the city of Bamenda, high resistance was reported for all first-line antibiotics such as ampicillin (73.9%) and SXT (82.6%). Moreover, ciprofloxacin had the least resistance (47.2%).[34]

In addition, higher resistance to ampicillin (92.5%) and SXT (70.1%) has been reported in Mexico, while resistance to nitrofurantoin and amikacin was around 14%,[20] similar to our report. Also, in Colombia, the outpatient diabetic population with community-acquired UTI was 65.5% resistant to ampicillin, 44.8% to SXT, and 32.6% to ciprofloxacin, while resistance to nitrofurantoin and amikacin remained less than 5%.[35] We found MDR in 55.2% of the isolates similar to Jomehzadeh et al that found 51.6% of MDR in UPEC strains in Southwestern Iran.[32,33] However it was higher than reported in Portugal (23.3%),[36] but lower than reported in India (78%).[37]

It is important to highlight that this variability in the antibiotic resistance profiles of UPEC strains is influenced by the local use of antibiotics and their rotation over time.

Relation Between Virulence Genes in UPEC Strains and Antibiotic Resistance

Our data showed a positive association between penicillin and fluoroquinolone resistance and MDR with the presence of the *iutA* gene, whereas UPEC with *ireA* and *hlyA* were more frequent in penicillin- and fluoroquinolone-sensitive strains. In addition, four of the five iron uptake genes (siderophores) were related to resistance to at least one antibiotic family, observing a positive association between penicillin resistance and the presence of more than three iron genes in the strains (47.7% vs 71.1%; p= 0.008). Also, a positive association was found between carbapenem resistance and the presence of *hlyA* (a gene unrelated to iron uptake).

It has been suggested that the high frequency of the *iutA* gene facilitates bacterial growth in urine, which is an iron-deficient medium in which iron uptake systems are crucial for the development of UTI. Also, it has been proposed that this gene is more frequent in antibiotic-resistant strains because it is part of the ColV plasmid that is believed to contain antibiotic resistance determinants.[15] Likewise, *iutA* has been associated with resistance to various antibiotics such as amoxicillin-clavulanic acid, ampicillin, cephalothin, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, tetracycline, and SXT.[15,38] Other authors have reported an association between the *iutA* gene and the blaCTX-M-1-group beta-lactamase in UPEC strains, including some septicemia-causing strains.[4] Other authors have reported UPEC strains with the *fimH, iutA*, and *fyuA* genes that are resistant to cefotaxime and ceftazidime, which could indicate that these three genes have a strong relationship with resistance to third- and fourth-generation cephalosporins.[7,20,24]

On the other hand, it has been described that there seems to be a correlation between antimicrobial resistance and decreased virulence since resistant strains have a lower presence of virulence genes.[22,29] The mechanism by which this association exists is not precise, and it has been proposed that the loss of incompatible pathogenicity islands in highly resistant strains may contribute to this phenotype. However, this has not yet been demonstrated.[29]

Limitations of the Study

This study has several limitations, one of which is the lack of information, inherent to retrospective studies using medical records. For example, some patients did not have all signs, symptoms, or laboratory results recorded. Therefore, in patients where the clinical information was incomplete, the treating physician's diagnosis was considered to classify the severity of the UTI. Another limitation was that the antibiotic panel used to measure resistance was not the same for all isolates. This decreased the sample size to make comparisons among the different groups. Despite this, it was evident the antimicrobial resistance and the relationship with several of the genes evaluated in our study. Also, only 38 cases could be recruited during the study period, corresponding to 84% and 95% of the sample size calculated

to evaluate the *fyuA* gene (between 40 and 45 cases, Supplementary Table S1). However, considering that the frequency of this gene among cases and controls was very similar (79% vs 77%), we think this did not impact the conclusion regarding this gene.

Conclusion

To the best of our knowledge, this is the first study in Colombia that evaluate the relationship between virulence genes of UPEC and the severity of community-acquired UTI, and antimicrobial resistance. None of the genes evaluated in UPEC strains were associated with the severity of community-acquired UTI. However, the frequency of the main virulence genes (*fimH, iutA* and *aer*) was similar to that reported in other countries. In addition, we found that the four ColV plasmid genes evaluated (*iutA, aer, iroN*, and *cvaC)* were positively related to each other. Also, an association was found between the presence of *hlyA* and carbapenem resistance, between *iutA* and fluoroquinolone resistance, and between *aer* and *iutA* and penicillin resistance. However, the *iutA* gene was the only one associated with MDR. The high frequency of resistance to antibiotics widely used in the treatment of UTI and MDR represents a public health problem. These findings support the decision of health authorities and health professionals to continue monitoring local antibiotic resistance of UPEC strains.

Ethics Approval and Consent

Proceedings were approved by the Institutional Review Boards of Universidad de Santander, Hospital Local del Norte, and Universidad Industrial de Santander. Since the researchers did not have any contact with the participants and did not collect sensitive information, the informed consent was not required by ethics committees according to Colombian legislation (Resolución 8430 DE 1993 "Por la cual se establecen las normas científicas, técnicas y administrativas para la investigación en salud"). In addition, this study was conducted in accordance with the Declaration of Helsinki.

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Disclosure

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