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ORIGINAL ARTICLE

Description of extraintestinal pathogenic *Escherichia coli* based on phylogenetic grouping, virulence factors, and antimicrobial susceptibility

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ABSTRACT

Background and Objectives: Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a recently recognized and highly diverse pathotype of *E. coli*. Its significance as a pathogen has increased due to the emergence of hypervirulent and multi-drug-resistant (MDR) strains. The aim of this study was to characterize ExPEC isolates from humans based on their phylogenetic group, virulence factor profile, and antimicrobial susceptibility.

Materials and Methods: The isolates were collected from patients with extraintestinal infections caused by *E. coli*, including urinary tract infections, bacteremia, and surgical site infections. The *E. coli* phylogenetic groups were determined using multiplex PCR. Additionally, the isolates were evaluated for their biofilm-forming abilities, susceptibility to antimicrobial agents, and presence of virulence genes.

Results: In this study, the isolates were classified into four phylogenetic groups: A (48.3%), B2 (25.8%), D (19.35%), and B1 (6.45%). All isolates exhibited at least one of the ten analyzed virulence factors. However, there was no direct evidence linking a specific phylogenetic group to a particular virulence factor. Nevertheless, the presence of the *fimH*, *fyuA*, *ompT*, *traT*, and *kpsMTII* virulence genes was correlated with the production of strong biofilms, multidrug resistance (MDR), and the production of alpha hemolysin.

Conclusion: This study provides a description of the phylogenetic groups in ExPEC and their potential association with virulence factor profiles and antimicrobial susceptibility.

Keywords: *Escherichia coli*; Virulence factor; Antibiotic resistance; Phylogeny; Biofilm

INTRODUCTION

In recent years, the importance of Extraintestinal Pathogenic *Escherichia coli* (ExPEC) as a pathogen has increased due to the emergence of hypervirulent and multidrug-resistant strains that cause community- and hospital-acquired urinary tract and bloodstream infections (1). The differences in the virulence of ExPEC strains and the diverse diseases they cause can be attributed to specific virulence genes

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and the variability in which these genes occur among strains (2). ExPEC strains have a greater ability to cause infections outside the intestine, where they can adhere and form biofilms on the surface of host tissues and medical devices, allowing them to persist and evade the host immune system and antimicrobial treatments (3).

The ability of ExPEC strains to cause infections is often associated with their expression of virulence factors (VFs) such as fimbriae, toxins, and siderophores, which can also contribute to their multidrug resistance; the ExPECs are defined as isolates that contain at least two of the following VFs in their genome: *papA* and/or *papC, sfa/foc, afa/draBC, kpsMTII,* and *iutA* (4). Phylogenetic analysis shows that ExPECs are predominantly in phylogenetic group B2 and less commonly in phylogenetic group D (5, 6).

ExPEC has also emerged as a major player in antibiotic resistance, including resistance to cephalosporins and fluoroquinolones, which is frequently reported in Europe, America, and Asia (7). Some studies have shown that the association of phylogenetic groups, presence of certain virulence determinants, and antibiotic resistance genes are indicators of pathogenicity, but the results showed that certain sequence types have a competitive advantage, great adaptability, and the ability to efficiently colonize the human body, leading to their clonal expansion and dominance over less virulent and/or more susceptible *E. coli* clones (8).

Recent studies have also shown overlapping characteristics between avian pathogenic *E. coli* (APEC) and human ExPEC, including similar serogroups, virulence factors, antibiotic resistance, phylogenetic groups, and sequence types (9). These strains may become reservoirs of virulence genes that can be transferred horizontally, enhancing their genomic background and increasing the likelihood of acquiring new genetic information (7, 10-11). This study aimed to describe ExPEC isolates from humans based on phylogenetic group, virulence factor profile, and antimicrobial susceptibility, in order to better understand the pathogenesis of these bacteria.

MATERIALS AND METHODS

Bacterial strains. In this study, thirty-one *E. coli* isolates were collected in January 2020 from patients with extraintestinal infections including urinary tract infection (26 cases); bacteremia (3 cases); and surgical site infection (2 cases) in two hospitals in Cartagena, Colombia. The strains were identified based on observing colonial morphology on EMB medium and confirmed through biochemical tests (12). The strains were stored at -80°C for further analysis. The study used *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *Staphylococcus aureus* ATCC 25923 as controls.

Phylotyping and virulence genotyping: phylogenetic analysis. The isolates were grown in Lysogeny-Broth (LB) agar and incubated overnight at 37°C. The genomic DNA was extracted from *E. coli* isolates using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) and used for PCR analysis. A specific PCR assay was performed to confirm the identity of the isolates as *E. coli* by detecting the *uidA* gene encoding beta-D-glucuronidase. Phylogenetic analysis was performed using the methods described by Clermont et al. (5), and the isolates were classified into one of four groups (A, B1, B2, D) based on the presence or absence of two genes (*chuA* and *yjaA*) and a DNA fragment (TSPE4. C2).

Virulence genotyping. The presence of nine virulence factors (VFs) was screened through PCR as previously described (14-16). The VFs included two adhesin-encoding genes (*fimH, papAH*), three protectins/serum survival-related genes (*kpsMTII, ompT, traT*), two iron acquisition/uptake systems related genes (*iutA, fyuA*), PAI markers, and the uropathogenic-specific protein (*usp*). The PCR products were analyzed using 1.5% agarose gels with ethidium bromide. After electrophoresis, the gel was photographed under UV light and captured digitally. The molecular size of the PCR products was determined using a 100-bp ladder as a reference. The primer sequences, annealing temperature, and size of the amplified fragments (in base pairs) are listed in Table 1.

Analysis of biofilm formation capacity: Congo red agar method by Freeman et al. (17). The tested strains were suspended in a solid medium composed of brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. The medium was made up of BHI (37 g/L), sucrose (50 g/L), base agar (10 g/L), and Congo red stain (0.8 g/L). The Congo red solution was made as a concentrated aqueous solu-

Table 1. Primer sequences and amplified products for the targeted genes.

Gene	Description	Primer sequence (5´ to 3´)	Amplicon size (Pb)	Optimal annealing temperature (ºC)	Reference
Specie specific					
uidA	Beta-D glucuronidase	F: GCGTCTGTTGACTGGCAGGTGGTGG R: GTTGCCCGCTTCGAAACCAATGCCT	503	56	(13)
Phylogenetic group					
chuA	Direct heme uptake	F: ATGGTACCGGACGAACCAAC R:TGCCGCCAGTACCAAAGACA	279	59	(5)
yjaA	Cellular response to acidic pH	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211		
TspE4.C2	Putative esterase lipase gene	F:GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152		
Virulence factors					
kpsMTII	Group II capsular polysaccharide synthesis	F: GCGCATTTGCTGATACTGTTG R: CATCCAGACGATAAGCATGAGCA	272	61,5	(14)
fimH	Type I fimbriae	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	508	62	
PAI	Marker for pathogenicity associated island	F: GGACATCCTGTTACAGCGCGCA R: TCGCCACCAATCACAGCCGAAC	930	63	
papAH	P fimbriae major and minor structural subunits	F: ATGGCAGTGGTGTCTTTTGGTG R: CGTCCCACCATACGTGCTCTTC	720	63	
fyuA	Yersinia associated siderophore system	F: TGATTAACCCCGCGACGGGAA R: CGCAGTAGGCACGATGTTGTA	880	63	
usp	Uropathogenic specific protein	F: ATGCTACTGTTTCCGGGTAGTGTGT R: CATCATGTAGTCGGGGCGTAACAAT	1000	62	(15)
OmpT	Serum resistance associated outer membrane protein	F: TCATCCCGGAAGCCTCCCTCACTACTAT R: TAGCGTTTGCTGCACTGGCTTCTGATAC	556	58	(16)
traT	Serum resistance associated outer membrane protein	F: GGTGTGGTGCGATGAGCACAG R: CACGGTTCAGCCATCCCTGAG	290		
iutA	Aerobactin iron transport system	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300		

tion, autoclaved separately at 121°C for 15 minutes, and added to the agar when it had cooled to 55°C. The inoculated plates were incubated aerobically for 24 to 48 hours at 37°C.

Screening of morphotypes (Congo Red agar assay). The morphotypes of each strain were determined based on the appearance of their colonies after 24 hours of incubation at 37°C. The plates were visually inspected and the morphotypes were categorized as red, dry and rough (rdar) indicating expression of curli fimbriae and cellulose, brown (bdar) indicating expression of fimbriae but not cellulose, pink (pdar) indicating expression of cellulose but not fimbriae, and smooth and white (saw) indicating expression of neither cellulose nor fimbriae (18).

Microtiter plate assay (Quantitative assays for biofilm formation). A crystal violet staining method was used to assess the biofilm-forming abilities of the isolates (19) with modifications. Each isolate was grown in 1 mL of LB broth overnight at 37°C with constant shaking and then transferred to new culture medium (diluted by 1:100) to achieve an OD 600 between 0.45 and 0.65. The biofilm assay was performed in triplicate for each strain. 30 µL of the bacteria in log phase growth were added to 96-well polystyrene plates containing 100 mL fresh LB broth and incubated at 37°C for 24 hours. The plates were rinsed 3 times with deionized water, and the adherent bacteria cells were stained with 0.5% crystal violet for 30 minutes. After rinsing 3 times with deionized water, the crystal violet was liberated by a mixture of

80% ethanol and 20% acetone following a 15-minute incubation. The OD values were measured at 492 nm. The tested strains were classified into non-biofilm producer (OD ≤ ODc), weak biofilm producer (OD > ODc, but ≤ 2× ODc), moderate biofilm producer (OD > 2× ODc, but ≤ 4× ODc), and strong biofilm producer (OD > 4× ODc) according to the criteria of Stepanovic et al. (20).

Hemolysin production. Hemolysis was determined by streaking *E. coli* isolates onto blood agar containing 5% sheep blood and incubating them at 37°C for 24 h. Following incubation, hemolytic strains were characterized according to their types/extent of hemolysis by each colony onto blood agar plate. Alpha-hemolysin produced a wide zone of complete hemolysis with blurred edges, beta-hemolysin gave a wide zone of partial hemolysis with sharp edges and non-hemolysis was evaluated as gamma hemolysis (21).

Antibiotic susceptibility testing. Antimicrobial susceptibility was determined using the disk diffusion method on Muller-Hinton agar, according to Clinical Laboratory Standards Institute (CLSI) guidelines (22). A total of 23 antibiotics were used: Ampicilline (Amp), Ampicillin/Sulbactam (Sam), Cefotaxime (Ctx), Nitrofurantoin (F), Amikacin (Ak), Ciprofloxacin (Cip), Trimethoprim/Sulfametoxazole (Sxt), Aztreonam (Azm), Cefazolin (Cez), Cefepime (Fep), Cefoxitin (Fox), Ceftazidime (Caz), Ceftriaxone (Cro), Doripenem (Dor), Ertapenem (Etp), Gentamicin (Gen), Meropenem (Mem), Piperacilin/Tazobactam (Tzp), Piperacilin (Pip), Tobramycin (Tob). Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more categories of antibiotics (23). The Multidrug Resistance (MAR) index was calculated to compare the resistance level of the isolates, using the formula: MAR index = (number of resistant antibiotics / number of antibiotics tested) × (number of isolates per sample) (24).

Detection of ESBL: phenotypic screening of ESBL. Isolates were screened for resistance to three oxyimino-cephalosporins: Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg) and the monobactam: Aztreonam (30 µg) by disk diffusion test. Zone diameters were read using CLSI criteria (22). An inhibition zone of ≤17 mm Ceftazidime, ≤22 mm Cefotaxime, ≤19 mm Ceftriazone and ≤17 mm Aztreonam indicated a probable ESBL producing strain requiring phenotypic confirmatory testing. *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control for ESBL tests.

Phenotypic confirmatory method of ESBL. ESBL production was detected by the double disc synergy test (DDST) using Clavulanic Acid-Amoxicillin (20/10 µg) and Ceftazidime (30 µg), Cefotaxime (30 µg), Aztreonam (30 µg) and Cefepime (30 µg) on Mueller Hinton agar as recommended by French Society for Microbiology (http://www.sfm-microbiologie.org). The presence of ESBL was manifested by the synergistic effect of the inhibitor and discs (effect of egg, fish tail or American soccer ball) (25).

RESULTS

All 31 strains were verified as *Escherichia coli* species based on their morphological, genotypic, and physiological characteristics. Table 2 provides details on the presence or absence of virulence factors, hemolysin, biofilm-forming ability, and morphotypes evaluated using red Congo agar. In this research, all strains possessed at least one of the ten virulence factors analyzed (as listed in Table 2). However, no clear relationship was observed between the type of virulence factor and a specific phylogenetic group. Nevertheless, the virulence genes *fimH, fyuA, ompT, traT, iutA,* and *PAI* (Fig. 1) were found in all phylogenetic groups. Four genes (*fyuA, fimH, kpsMTII,* and *iutA*) were present in a high proportion of the studied strains (90.3%) and were found to coexist with *ompT, traT,* and *PAI* genes in all phylogenetic groups. The Biofilm-forming abilities of different isolates were detected by crystal violet on 96-well plates and indicated that biofilm formation ability differed among the isolates the strains were divided into four categories as described above (Table 2).

Most pathogenic *E. coli* strains have multiple systems for acquiring ferric ions. In this study, alpha hemolytic activity was observed in 45.1% of the isolates, beta hemolytic activity was detected in 41.95%, and gamma hemolytic or non-hemolytic strains were found in 12.95% of the isolates.

In the Table 3 displays the results of the antibiotic susceptibility test of the isolates against 20 antibiot-

Table 2. Prevalence of virulence factors in various phylogenetic groups of extraintestinal *Escherichia coli* isolates.

Virulence factors	Phylogenetic Group				Total
	A	B1	B2	D	
	(n= 15) 48.3%	(n=2) 6.45%	(n= 8) 25.8%	(n= 6) 19.35%	(n= 31) 100%
fyuA	(15) 100%	(2) 100%	(6) 75%	(5) 83.3%	(28) 90.3%
fimH	(15) 80%	(1) 50%	(7) 87.5%	(4) 66.7%	(24) 77.4%
ompt	(10) 66.6%	(2) 100%	(6) 75%	(6) 100%	(24) 77.4%
traT	(7) 46.7%	(1) 50%	(3) 37.5%	(5) 83.3%	(16) 51.6%
iutA	(9) 60%	(1) 50%	(8) 100%	(5) 83.3%	(23) 74.1%
PAI	(4) 26.7%	(1) 50%	(4) 50%	(1) 16.7%	(10) 32.3%
papAH	(2) 13.3%	-	(3) 37.5%	(1) 16.7%	(6) 19.3%
kpsMTII	(9) 60%	-	(8) 100%	(4) 66.7%	(20) 64.5%
Usp	-	-	-	(1) 16.7%	(1) 3.22%
Hemolysis					
Alfa	(6) 40%	(1) 50%	(4) 50%	(3) 50%	(14) 45.1%
Beta	(7) 46.7%	(1) 50%	(3) 37.5%	(2) 33.3%	(13) 41.9%
Gamma	(2) 13.3%	-	(1) 12.5%	(1) 16.7%	(4) 12.9%
Biofilm					
Non adherent	(3) 20%	-	(2) 25%	-	(5) 16.1%
Weak	(3) 20%	(1) 50%	(2) 25%	(3) 50%	(9) 29.1%
Moderate	(8) 53.3%	(1) 50%	(3) 37.5%	-	(12) 38.7%
Strong	(1) 6.7%	-	(1) 12.5%	(3) 50%	(5) 16.1%
Pathogenicity					
Pdar	(9) 60%	-	(4) 50%	-	(13) 41.9%
Rdar	-	-	-	(2) 33.3%	(2) 6.45%
Bdar	(6) 40%	(2) 100%	(4) 50%	(4) 66.7%	(16) 51.6%
Saw	-	-	-	-	-

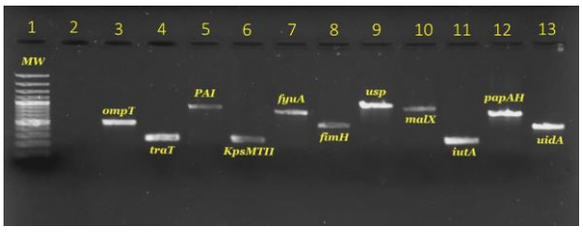

Fig. 1. Virulence Factors, electrophoresis gel: A. Lane 1. DNA Marker ladder. Lane 2. Negative control. Lane 3. *ompT*. Lane 4. *traT*. Lane 5. *PAI*. Lane 6. *kpsMTII*. Lane 7. *fyuA*. Lane 8: *fimH*. Lane 9. *usp*. Lane 10. *malX*. lane 11. *iutA*. Lane 12. *papAH*. Lane 13. *uidA*.

ics. 97.7% of the isolates were found to be sensitive to doripenem and ertapenem, while 95% were sensitive to meropenem. On the other hand, 67.8% of the *E. coli* strains were resistant to ampicillin, 41.9% to ciprofloxacin, 45.2% to piperacillin, and 16.1% to trimethoprim/sulfamethoxazol. 64.5% of the *E. coli* isolates had a Multiple Antibiotic Resistance (MAR) index of 0.2 or higher, indicating the presence of multiresistant strains. The production of Extended-Spectrum Beta-Lactamases (ESBL) was detected in 58% of the isolates, which showed synergistic effects against the antibiotics tested.

The antibiotic resistance pattern was compared with the ability to form a biofilm and distribution groups phylogenetic (Table 4). These results showed an association between highest antibiotic resistance isolates with presented strong and moderate biofilm in most of the strains.

DISCUSSION

Studies have reported that certain strains of ExPECs are not randomly distributed, and may be associated with their geographic distribution. ExPECs can cause disease in various body sites outside of the gastrointestinal tract, including the urinary tract, neonatal meningitis, sepsis, pneumonia, and surgical site infections. The phylogenetic groups B2 and D

Table 3. Antibiotic susceptibility pattern of *E. coli* species isolates.

Antimicrobial Agentes	Disk concentration	Diameter inhibition		%R	%I	%S
		Resistant	Susceptible			
Amikacin	30	≤14	≥17	6.5	-	93.5
Amp/Sulbactam	10	≤11	≥15	35.5	20.5	31.8
Ampicilin	10	≤13	≥17	67.8	-	32.2
Aztreonam	30	≤15	≥22	38.7	6.5	54.8
Cefazolin	30	≤14	≥18	42	3.2	54.8
Cefepime	30	≤14	≥18	41.9	-	58.1
Cefotaxime	30	≤14	≥23	38.7	-	65.3
Cefoxitin	30	≤14	≥18	6.5	3.2	90.3
Ceftazidime	30	≤14	≥18	38.7	-	61.3
Ceftriaxone	30	≤13	≥21	35.5	3.2	61.3
Ciprofloxacin	5	≤15	≥21	41.9	-	58.1
Doripenem	8	≤13	≥21	3.2	-	96.8
Ertapenem	4	≤15	≥21	3.2	-	96.8
Gentamicin	10	≤12	≥15	22.6	-	77.4
Meropenem	10	≤13	≥16	6.5	-	93.5
Nitrofurantoin	300	≤14	≥17	6.5	-	93.5
Pip/tazo	100/10	≤17	≥21	22.5	6.5	71.3
Piperacilin	100	≤17	≥21	45.2	-	54.8
Tobramycin	10	≤12	≥15	19.3	9.7	71
Trimet/sulfa	0.25/23.7	≤10	≥16	50	-	50

Table 4. Distribution groups phylogenetic, antibiotic resistance pattern and ability to form a biofilm.

Group Phylogenetic	Antimicrobial Resistance	Biofilm
A	Ams, Amp, Atm, Cfz, Fep, Ctx, Fox, Caz, Nit, Pip, Sxt	Strong
	Ams, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Cip, Pip, Tob, Sxt	Moderate
	Ams, Amp, Atm, Cfz, Fep, Ctx, Fox, Caz, Tzp, Pip, Sxt	Moderate
	Ams, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Tzp, Pip	Moderate
	Amp, Atm, Cfz, Ctx, Fox, Cro, Cip, Sxt	Moderate
	Amp, Atm, Fep, Mem, Pip, Tob, Sxt	Moderate
	Ams, Atm, Cfz, Fep, Caz, Cro, Tzp	Moderate
	Amp, Cfz, Ctx, Cro, Gen, Sxt	Moderate
	Amp, Cip, Gen, Pip, Stx	Moderate
	Amp, Cip, Gen, Pip, Tob, Sxt	Weak
	Ams, Amp, Pip, Sxt	Weak
	Cip	Non adherent
	Amp	Non adherent
	-	Non adherent
B1	Amk, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro	Strong
	Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Pip, Tob, Sxt	Moderate
B2	Ams, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Cip, Pip, Sxt	Moderate
	Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Cip, Gen, Pip, Tob	Moderate
	Ams, Amp, Cip, Tzp, Tob, Sxt	Moderate
	Cip	Weak
	Amp	Weak

Table 4. Continuing...

	Amp	Weak
	Cip	Non adherent
	-	Non adherent
	Amk, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Cip, Dor, Etp, Gen, Mem, Nit, Tzp, Tob, Sxt	Strong
	Ams, Atm, Cfz, Fep, Caz, Cro, Cip, Gen, Nit, Tzp, Pip, Tob, Stx	Strong
D	Ams, Amp, Atm, Cfz, Fep, Ctx, Caz, Cro, Cip, Gen, Pip, Sxt	Strong
	Ams, Amp, Pip, Stx	Weak
	Amp	Weak
	-	Weak

are primarily found in individuals residing in developed countries such as the United States and Australia (14, 26). On the other hand, we have Phylogenetic group A and B1 strains appear to be predominant in humans living in developing countries as Bolivia and Colombia (27). The high prevalence of non-pathogenic strains of phylogenetic group A in this study can also be attributed to the migration of these intestinal strains into the urinary tract, where they serve as gene reservoirs for virulence factors. This increases the likelihood of acquiring new genetic information through horizontal gene transfer, due to the genetic plasticity of ExPEC strains. As a result, these strains have a higher potential for dissemination.

Ranjbar et al. (28) have reported that the majority of ExPEC strains belong to the B2 and D groups, which are considered the most prevalent and more pathogenic strains. On the other hand, strains of *E. coli* belonging to the A and B1 groups are primarily regarded as commensal. However, in line with our findings, similar results have been reported in studies conducted by Pompilio et al. (29) Bozcal et al. (30) and Ahumada-Santos et al. (31) where they found that the A phylogenetic group is the most frequent. The varying occurrence of phylogenetic groups reported in different studies can be attributed to differences in host characteristics, environmental and geographical conditions, sampling regions or be attributed to the heterogeneity of the ExPEC isolates. These factors can contribute to the diversity and distribution of phylogenetic groups among bacterial isolates.

The virulence factors (VFs) of *Escherichia coli* are a set of genetic elements that enable the bacteria to cause disease. These VFs can include genes encoding toxins, adhesins, and other molecules that help the bacteria to invade host tissues and evade host immunity. The presence of specific VFs can be an indicator of the pathogenic potential of a strain and can be useful for understanding the mechanisms of disease caused by *E. coli*, the VFs help the microorganism to avoid or subvert host defenses, colonize key anatomical sites, and/or incite a noxious host inflammatory response, thereby causing disease (32). These findings align with those reported by Franz et al. (33). This study underscores the importance of the *fyuA* and *fimH* genes as valuable markers for identifying uropathogenic *Escherichia coli* (UPEC) strains, corroborating previous findings by Rezatofighi et al. (34) and García et al. (35) these genes were universally detected in all analyzed UPEC strains, indicating their high prevalence. Furthermore, UPEC strains demonstrate diverse phylogenetic distribution, suggesting their widespread presence. It is noteworthy that UPEC strains consistently possess virulence factors that significantly contribute to their pathogenicity, playing a crucial role in the initiation and progression of urinary tract infections. The severity of UPEC infections can vary, influenced by specific virulence factors, host susceptibility, and the immune response. A comprehensive understanding of the genetic and molecular mechanisms governing UPEC virulence is pivotal for developing effective preventive and therapeutic strategies against these pathogens.

This highlights the significance of iron uptake receptors in biofilm formation in iron-poor environments such as human urine. The results suggest that iron uptake through the yersiniabactin system is crucial for biofilm growth (36). A bacterial biofilm is a complex, three-dimensional community of aggregated cells that are encased in a matrix of exopolysaccharides produced by the cells themselves. The biofilm adheres to both abiotic and biotic surfaces. The matrix of polysaccharides protects the cells from an-

tibacterial agents, antibodies, and white blood cells, and the close proximity of cells within a biofilm promotes the exchange of plasmids, thereby increasing the spread of antimicrobial resistance (37).

The majority of the studied strains exhibited low or no biofilm formation. This observation can be attributed to the predominance of strains belonging to the phylogenetic group A, as reported by Nielsen et al. (38) These findings align with previous studies that have associated the low pathogenicity of group A strains with reduced biofilm-forming abilities. On the other hand, strains belonging to the B2 and D phylogenetic groups displayed a higher capacity for biofilm formation, indicating their heightened virulence. These results are consistent with existing literature highlighting the association between increased virulence and enhanced biofilm-forming abilities in strains from these phylogenetic groups. Thus, the observed differences in biofilm formation among the studied strains can be explained by their phylogenetic classification and are in line with established patterns of pathogenicity and virulence.

The virulence pattern *fimH, fyuA, ompT, traT, kpsMTII* was associated with a strong biofilm production capacity, interestingly the strains belonged to the phylogenetic group D, although strains belonging to this group with only the virulence factor *ompT* presented weak biofilm, this characteristic could be attributed to the fact that this gene is responsible for the stages of adhesion and autoaggregation of biofilm production (39). Congo red binding has been used as a potential virulence marker and differentiate between invasive and non-invasive *E. coli* in this study, 100% of *E. coli* isolates were positive for Congo red binder. This assay evaluates the several expressions of morphotypes: rdar, bdar, pdar and saw, recognized by bacterial colony colour and rugosity, dependents of the expression of virulence factors cellulose and curly associated biofilm production (40), the bdar morphotype was the most prevalent from all isolates (51.6%), followed by pdar (41.9%), and 6.45% rdar. The *saw* morphotype was not present in the isolates.

Antibiotic susceptibility and biofilm formation are two important aspects of bacterial behavior that can have a significant impact on human health. Antibiotic susceptibility refers to the ability of bacteria to grow or survive in the presence of antibiotics, while biofilm formation refers to the ability of bacteria to form communities that are resistant to antibiotics and host defenses. The combination of antibiotic resistance and biofilm formation can lead to persistent and difficult-to-treat infections, especially in healthcare settings.

Certain virulence genes have been positively associated with large, transmissible plasmids encoding multidrug resistance (MDR) in ExPEC, high prevalence of the *fyuA* and *ituA* genes was observed in our study in the strains producing MDR/ESBLs. These results coincide with several investigations, which reported that UPEC fitness is not affected by genomic diversity, presence of virulence factors and production of ESBL, on the contrary, these genetic characteristics are an advantage for the dissemination of some clones of epidemiological importance (41-43).

These results can be attributed to biofilms that contribute significantly to the non-penetration of antibiotics to bacteria, which is why they cannot break down their mechanism of action against these agents antibacterial. Although establishing these relationships not an objective of this study, it is reasonable to suppose that characteristics the studied strains provided advantages to improve the adaptive mechanisms, which potentiated its development, colonization and infection. Therefore, the results of this study highlight the need for ongoing monitoring and control of antibiotic resistance and the importance of developing strategies to prevent and treat infections associated with biofilm-forming bacteria. One limitation of our study was the small sample size, which may have affected the generalizability of our findings to a larger population. Additionally, this could potentially introduce differences in the classification of strains into phylogenetic groups, more accurate and refined results. It is important to consider these limitations when interpreting the results and generalizing them to a broader context.

CONCLUSION

The results in the study reveal that biofilms play a significant role in hindering the penetration of antibiotics, rendering them ineffective against bacteria. The observed relationship between antibiotic resistance and biofilm formation suggests that these characteristics provide a survival advantage to the bacteria, leading to improved colonization and increased risk of infection. Further research is necessary to better understand the spread of these multidrug-resistant ExPEC strains, their clinical impact, and risk factors.

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