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Virulence Factors and Pathogenicity Islands in Uropathogenic *Escherichia coli* in Brazilian Children

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Escherichia coli (*E. coli*) is a Gram-negative bacillus responsible for intestinal and extraintestinal infections, and the main cause of urinary tract infection. Its pathogenicity is mediated through virulence factors located in regions of the bacterial chromosome called pathogenicity islands, in addition to the production of extended-spectrum beta-lactamases (ESBL).

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Aims: Investigate the frequency of virulence factors from *E. coli* isolated in the urine of children with suspected urinary tract infection in the community, investigate the types of ESBL and its frequency of virulence factors and perform the phylogenetic classification of the strains.
Study Design: This is an observational, descriptive and cross-sectional study carried out through the analysis of positive urine cultures from children aged 0 to 12 years old.
Place and Duration of Study: The samples were collected in the city of Londrina, state of Paraná (Brazil) and processed from May 1, 2021, to September 9, 2022.
Methodology: 89 *E. coli* samples from urine cultures were included. The PCR method was used for genotypic identification of the main virulence factors of *E. coli*.
Results: The *iutA* iron acquisition system was the most prevalent virulence factor (77.5%). There was a statistical difference in the two groups ESBL or non-ESBL in relation to the *fimH* and *hlyA*. The ESBL most prevalent was CTX-M2. PAI IV_{536} was the most prevalent among all isolates (52.8%) and the only pathogenicity island with a significant result. The most prevalent phylogenetic classification group was B2 (41.6%).
Conclusions: *E. coli* strains have an arsenal of virulence factors that allow them to induce infection, with the statistical difference between the ESBL and non-ESBL groups. PAI IV_{536} was the most prevalent among all isolates, in the same way as phylogenetic group B2. The finding of CTX-M2 like the more prevalent type of ESBL agrees with other studies in the region.

Keywords: ExPEC; UTI pediatrics; virulance factors; phylogenetic classification.

1. INTRODUCTION

The bacterium *Escherichia coli* (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium inhabiting the common microbiota of the human gastrointestinal tract, from where they can emerge and cause extraintestinal infections [1,2]. *E. coli* presents a genetic diversity that includes non-pathogenic commensal intestinal isolates and isolates that can cause intestinal and extraintestinal infections. Pathogenic *E. coli* strains can cause extraintestinal diseases, such as urinary tract infection (UTI), bacteremia, neonatal meningitis and other non-intestinal infections [3].

They are classified into seven phylogenetic groups: A, B1, B2, C, D and F, and clade I. Commensal strains are part of the human intestinal microbiota and play an important role in digestion and the synthesis of certain vitamins. Such strains do not have virulence factors and generally belong to groups A or B1. Pathogenic strains are classified into intestinal *E coli* (intestinal pathogenic *E. coli* - IPEC) and extraintestinal (extraintestinal *E.* coli - ExPEC). IPEC strains responsible for intestinal infections belong to phylogenetic groups A, B1 or D, while ExPEC strains responsible for extraintestinal infections are part of groups B2 or D [4].

ExPEC comprises isolates from infections outside the intestinal tract and are grouped into uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli (NMEC)* and *E. coli* that causes sepsis (SEPEC) [4]. UPEC can invade the bladder and generating substantial changes in its morphology, with a robust immune response. In addition, intracellular bacterial communities (IBC) form within the surface cells of the bladder. IBC are made up of thousands of bacteria, similar to a biofilm, and are important in leading to later infection [5,6].

UTI is among the most frequent bacterial infections in the community and in the hospital environment and, as it presents significant morbidity and mortality, it generates high economic costs for the health system. It is estimated that there are more than 400 million UTI occurrences worldwide annually, impacting countries' health systems. In the USA, the total cost of healthcare used to treat UTI is more than 1,6 billion dollars [5].

ExPEC strains have several virulence factors that make them capable of colonizing the host and spreading. Virulence factors are encoded by genes located on the bacterial chromosome, where they are often located in specific regions called pathogenicity islands (PAI), or in mobile elements, such as plasmids. Virulence factors include adhesion molecules (adhesins), iron acquisition systems, mechanisms to escape the immune system and toxins [6].

The increase in pathogenicity caused by the acquisition of virulence factors increases the risk of therapeutic failure and increases mortality. Added to this is the acquisition of other resistance genes such as extended-spectrum

beta-lactamases (ESBL), one of the most prevalent resistance mechanisms produced by enterobacteria [7]. The ability of *E. coli* to cause mutation, acquire and transmit plasmids and other mobile genetic elements that encode resistance genes has represented a rapidly expanding problem [4].

The main objective of this study was to investigate the frequency of ExPEC virulence factors from the urine of community pediatric patients with suspected UTI. Other objectives were to investigate the types of ESBL in the bacteria studied, to compare the frequency of virulence factors of ESBL-producing strains with non-ESBL-producing strains, to compare the frequency of PAI of ESBL-producing strains with non-ESBL-producing strains, and to carry out phylogenetic classification of the strains studied.

2. MATERIALS AND METHODS

The samples were collected from positive urine cultures of children with suspected UTI aged 0 to 12 years old, carried out in the laboratory of the Municipal Health Authority of the Municipality of Londrina (Centrolab), from May 1, 2021, to September 9, 2022. These samples were collected from out-patient children in the primary care services of the Municipal Health Authority in the city of Londrina, a city in the state of Paraná (Brazil). Of the samples collected, 89 *E. coli* samples from urine cultures were included, of which 44 were samples that showed ESBL and 45, as a control, did not. To identify the isolates, the VITEK ® 2 GN ID card and the VITEK ® 2 AST 239 card were used to evaluate the antimicrobial susceptibility test.

DNA extraction was carried out using the boiling method and the polymerase chain reaction (PCR) was subsequently carried out.

A total of 7 genes of the main virulence factors were analyzed, namely: hemolysin (*hlyA*), aerobactin (*iutA*), yersiniobactin (*fyuA*), type 1 fimbria (*fimH*), P fimbria (*papC*), serum resistance (*traT*), polysaccharide capsule (*KpsMT II*), described in Table 1. The PCR method was used for the genotypic identification of the main virulence factors of ExPEC.

The characterization of ESBL was carried out using the multiplex PCR technique using specific primers for the detection of the *bla* CTX-M genes as described by Woodford, Fagan and Ellington [8]. The PCR reaction was performed using the TopTag ® Master Mix Kit (QUIAGEN).

A total of 3 described UPEC pathogenicity islands were researched (Table 2) and the PCR method was also used for this [9].

The samples were classified into 7 phylogenetic groups (A, B1, B2, C, D, E, F and clade I), based on the presence of the genes *chuA, yjaA, arpA, trpA* and DNA fragments (*TSPE4.C2*) (Table 3) using the PCR method. *E. coli* strains that do not fall between these groups are described as classification unknown.

Table 1. Genes encoding virulence factors

Genes	Sequences (5'-3')	Coded virulence factors	Base pairs
fimH	TGC AGA ACG GAT AAG CCG TGGGCA GTC ACC TGC CC TCC GGT A	Fimbriae type 1	508
papC	GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GCA GGG TGT GGC	P Fimbriae	328
hlyA	AAC AAG GAT AAG CAC TGT TCT GGC ACC ATA TAA GCG GTC ATT CCG GTC	Hemolysin	1.177
iutA	GGC TGG ACA TCA TGG GAA CTG G CGT CGG GAA CGG GTA GAA TCG	Aerobactin siderophore receptor	300
fyuA	TGA TTA ACC CCG CGA CGG AA CGC AGT AGG CAC GAT CTT GTA	Yersiniobactin siderophore receptor	880
traT	GGT GTG GTG CGA TGA GCA CAG GGT GTG GTG CGA TGA GAC CAG	Serum resistance	290
kpsMTII	GCG CAT TTG CTG ATA CTG TTG CAT CCA GAC GAT AAG CAT GAC CA	Group 2 of capsular antigens	272

Source: Johnson and Stell [10]

Table 2. Genes encoding pathogenicity islands

Pathogenicity Islands	Sequences (5'-3')	Base pairs
PAI I_{CFT073}	GGA CAT CCT GTT ACA GCG CGC A TCG CCA ATC ACA GC GAA C	930
PAI II_{CFT073}	ATG GAT GTT GTA TCG CGC ACG AGC ATG TGG ATC TGC	400
PAI IV_{536}	AAG GAT TCG CTG TTA CCG GAC TCG GGC AGC GTT TCT TCT	300

Source: Sabaté et al. [9]

Table 3. Genes used for phylogenetic classification

Genes	Sequences (5'-3')	Base pairs
chuA	ATG GTA CCG GAC GAA CCA AC TGC CGC CAG TAC CAA AGA CA	288
yjaA	CAA ACG TGA AGT GTC AGG AG AAT GCG TTC CTC AAC CTG TG	211
TspE4.C2	CAC TAT TCG TAA GGT CAT CC AGT TTA TCG CTG CGG GTC GC	152
arpA	AAC GCT ATT CGC CAG CTT GC TCT CCC CAT ACC GTA CGC TA	400
trpA	CGG CGA TAA AGA CAT CTT TCA AC GCA ACG CGG CCT GGC GGA AG	301
arpA (group E)	GAT TCC ATC TTG TCA AAA ATA TGC CC GAA AAG AAA AAG AAT TCC CAA GAG	290

Source: Johnson and Stell, [10]

To perform the statistics, qualitative and independent variables were used on the IBM® Statistical software platform Package for the Social Sciences (SPSS)® version 22 for Windows. The *Chi-Square Test* was used to measure the degree of association between the variables. The *P-value* used as a significant difference between groups was 0.05. To evaluate the intensity of association between the variables, *odds ratio* was used with a 95% confidence interval (CI).

3. RESULTS

3.1 Virulence Factors

Among the virulence factors analyzed, the *iutA* iron acquisition system was the most prevalent among the isolates, with a total percentage of 77.5%. The *fyuA* iron acquisition system it was the second most prevalent gene with a total of 75.3%. Next was the *traT* gene (70.8% of the total), a virulence factor responsible for serum resistance. Only in the *fimH*, *kpsMTII* genes and *papC* the percentages of *E. coli* producers were lower than non-ESBL producers.

When comparing the two types of ESBL-producing and non-ESBL-producing UPEC, there was a statistical difference in the two groups in relation to the *fimH* virulence factor, with a predominance of non-ESBL strains (P < 0,001), and *hlyA* (P = 0,007), with a predominance of strains ESBL, which were 4 times more likely to express the gene than non-ESBL-producing *E. coli* (Table 4).

3.2 ESBL Classification

Of the 44 samples of ESBL-producing strains, 21 were identified as ESBL CTX-M2, 13 as ESBL CTX-M1 and 10 as ESBL CTX-M8.

3.3 Pathogenicity Islands

PAI IV_{536} was the most prevalent among all isolates (52.8%) and the only pathogenicity island with a significant result (P < 0.001), with 13 times greater chances of being present in ESBL-producing *E. coli* when compared to the control group of non-producers.

The second most prevalent pathogenicity island in all isolates was PAI I_{CFT073} with 27.0%. Finally, PAI II_{CFT073} was less prevalent, with 10.1% of the total samples (Table 5).

3.4 Phylogenetic Classification

Among the isolates, the most prevalent phylogenetic classification group was B2

(41.6%), followed by D (22.5%), E (19.1%), F (6.7%) and clade I (1,1%). However, none of the *E. coli* isolates from children with UTI analyzed belonged to groups A, B1 and C. The clade I group presented only one isolate, which was non-ESBL producer. Group E had a higher percentage of ESBL-producing *E. coli*, but the other groups (B2, D, F and clade I) had a higher percentage of non-ESBL-producing samples. Furthermore, 9% of the isolates had an unknown classification (Table 6).

Table 4. Percentage of virulence factors of *E. coli* isolated from children with suspected UTI

Genes	ESBL producers n (%)	Non-ESBL producers n (%)	Total n (%)	*P*	OR (CI 95%)
iutA	36 (81.8)	33 (73.3)	69 (77.5)	0.447	1.64 (0.60-4.50)
fyuA	38 (86.4)	29 (64.4)	67 (75.3)	0.130	0.30 (0.05-1.54)
traT	34 (77.3)	29 (64.4)	63 (70.8)	0.245	1.88 (0.73-4.77)
fimH	21 (47.7)	40 (88.9)	61 (68.5)	<0.001	0.11 (0.04-0.03)
kpsMTII	25 (56.8)	29 (64.4)	54 (60.7)	0.52	0.73 (0.31-1.70)
papC	12 (27.3)	13 (28.9)	25 (28.1)	1.00	0.92 (0.37-2.32)
hlyA	14 (31.8)	4 (8.9)	18 (20.2)	0.007	4.78 (1.43-15.99)

Source: authors themselves, 2022

Table 5. Percentage of *E. coli* PAIs isolated from children with UTI

Pathogenicity Islands	ESBL producers n (%)	Non-ESBL producers n (%)	Total	*P*	OD (95% CI)
PAI IV_{536}	36 (81.8)	11 (24.4)	47 (52.8)	<0.001	13.91 (4.99-38.73)
PAI I_{CFT073}	16 (36.4)	8 (17.8)	24 (27.0)	0.06	2.64 (0.99-7.05)
PAI II_{CFT073}	7 (15.9)	2 (4.4)	9 (10.1)	0.09	4.07 (0.80-20.80)

Source: authors themselves, 2022

Table 6. Phylogenetic classification of *E. coli* isolated from children with UTI

Phylogenetic classification	ESBL producers, n (%)	Non-ESBL producers, n (%)	Total
A	-	-	-
B1	-	-	-
B2	18 (40.9)	19 (42.2)	37 (41.6)
C	-	-	-
D	4 (9.1)	16 (35.6)	20 (22.5)
E	14 (31.8)	3 (6.7)	17 (19.1)
F	1 (2,3)	5 (11.1)	6 (6.7)
Clade I	-	1 (2,2)	1 (1,1)
UC*	7 (15.9)	1 (2,2)	8 (9.0)

**Unknown classification*
Source: authors themselves, 2022

4. DISCUSSION

In this study, virulence factors, pathogenicity islands and the phylogenetic classification of *E. coli* isolates from the urine of children with community UTIs were investigated. Virulence factors increase the ability of strains to cause infections, as they are associated with the pathogenicity of ExPEC [11].

They can be chromosomally encoded and are usually located within pathogenicity islands or on plasmids. Specific regions of the bacterial chromosome where virulence genes are located are called PAI. PAI are common among *E. coli* strains that cause extraintestinal infections, mainly in ExPEC phylogroups B2 and D. There is a range of virulence factors, and the combination between them occurs in different strains of ExPEC and can lead to many pathways for the extraintestinal virulence of *E. coli* [11,12].

The most prevalent gene was *iutA* followed by the *fyuA* gene, both express virulence factors associated with the iron acquisition system. Iron, an electron carrier, plays a fundamental role in bacterial metabolism, participating in cellular respiration, DNA replication and oxygen transport. Such virulence factors are fundamental in the pathogenesis of urinary tract infection, as they allow bacterial proliferation in the urinary tract, which is extremely limited in iron [4].

In addition to *uitA* and *fyuA,* other frequent genes were *traT*, *fimH* and *kpsMTII.* According to a study carried out at the University Hospital of Londrina by Daga and others [13], the virulence factors and pathogenicity islands of ExPEC isolated from different clinical materials were analyzed. In this study, urine isolates from inpatient UTI patients obtained the genes *fimH* (77.3%), *fyuA* (54.5%), *iutA* (45.5%), *traT* (40.9%), *kpsMTII* (34.1%), respectively with higher prevalence. For urine isolates from outpatients with UTI, there was a predominance of the genes *fimH* (98.0%), *fyuA* (67.3%), *traT* (51.1%), *kpsMTII* (51.1%) and iutA (46. 9%), corroborating the findings of the present study.

The expression of the *fimH* gene, which was significant in non-ESBL-producing strains in relation to ESBL-producing strains with a value of $p<0.001$ for the samples in this study, is relevant in UPEC due to its participation in the bacterial adhesion process. Adhesion is carried out by adhesins, virulence factors that recognize and bind to receptors present in the bladder epithelium, for the bacteria to establish themselves in the host and cause infection [6]. These adhesins are found at the ends of fimbriae, with type 1 fimbriae containing the adhesin *fimH* mediates the binding of UPEC with uroplakin present in the bladder epithelial cell, allowing the bacteria to resist the mechanical movements of peristalsis and urination [5]. A possible explanation for this finding is that non-ESBL strains would need to express this virulence factor more frequently as they do not have the competitive advantage of this bacterial resistance mechanism (production of beta-lactamases). Some studies have shown that more resistant strains may express fewer virulence factors.

Another finding in this study was that ESBL-producing UPEC strains had a higher frequency of the *hlyA* gene than non-producers, a finding with statistical difference in this study. The toxins produced by *E. coli*, such as hemolysin A encoded by the *hlyA* gene*,* are virulence factors that contribute to cell lysis through the formation of pores in the membranes of urinary epithelial cells [12]. It is reported in the literature that ESBL-producing *E. coli* is linked to the expression of the *hlyA* gene, according to a study by Hasan and Ibrahim [14], 82% of UPEC samples from children with UTI had the *hlyA* gene and 64% of these were positive for ESBL. A possible explanation for this would be that the presence of this virulence factor would be another competitive advantage of ESBL strains. Hasan and Ibrahim also found the same result, with ESBL strains of *E. coli* having a greater presence of *hlyA* gene.

Regarding the finding of three specific types of ESBL, CTX-M2, CTX-M1 and CTX-M8 with a predominance of the first, it agrees with other studies, which show that this is the main type of beta-lactamase in South American countries [15,16].

This study showed that PAI IV_{536} was most prevalent among all isolates and the only pathogenicity island with a significant result ($p<0.001$), with ESBL strains having a 13 higher frequency than non-ESBL strains. The specific locations where virulence genes are concentrated are called pathogenicity islands. Pathogenicity islands are specific regions of the bacterial chromosome where virulence genes accumulate [4]. They are large integrative elements consisting of one or more genes encoding virulence factors that are absent in

non-pathogenic bacteria of the same or related species. Unlike other integrative elements (e.g., bacteriophages, plasmids, or integrative and conjugative elements), PAI do not have the ability to replicate or self-mobilize. Many virulence factors are encoded by PAI and involve fundamental steps in the infectious process [6].

PAI IV_{536} encodes the siderophore system yersiniabactin, responsible for iron uptake. As previously described, iron is essential for bacteria because of its involvement in many metabolic functions, such as oxygen and electron transport. The acquisition of iron is a fundamental need for the survival of UPEC in the urinary tract environment, which is extremely iron-limited. In addition to yersiniabactin, UPEC also expresses the siderophores salmochelin and aerobactin [11].

The fact that ESBL strains present more PAI IV_{536} than non-ESBL-producing strains is probably related to gene regulation through still unknown mechanisms of virulence stimulation and inhibition. It is proven that PAI not only encode virulence factors but are also involved in virulence regulation [6].

According to the phylogenetic classification, phylogroup B2 was the most prevalent, followed by group D. This finding agrees with another study carried out by Tanabe et al in the Brazilian state of São Paulo in 2018 [17], where it was observed that phylogroup B2 was the most prevalent in urine cultures of adult patients from the community.

Phylogenetic groups B2 and D are the groups found most frequently among the ExPEC strains responsible for causing human infections, as they present several virulence genes that allow the induction of extraintestinal infections [4]. Phylogroups A, C and B1 were not identified in the urine isolates analyzed from children with UTI, according to reports in the literature, isolates belonging to groups A and B1 are commonly belonging to commensal strains of the intestinal microbiota and do not have virulence determinants of causing intestinal or extraintestinal diseases [1]. Group C is closely related to group B1, non-pathogenic phylogroups and therefore may not have been identified among the isolates [18].

5. CONCLUSION

According to the results of this study, it was possible to conclude that *E. coli* from children with suspected UTI have an arsenal of virulence factors that allow them to induce infection, with the most expressed virulence factors being related to the iron acquisition system, serum resistance, toxin formation and adhesin production. Such actions are fundamental for the survival, proliferation and pathogenesis of *E. coli* in the urinary tract.

There was a statistical difference between the ESBL and non-ESBL groups in relation to virulence factors *fimH* and *hlyA* probably related to the existence of positive and negative relationships between antibiotic resistance and virulence. It was shown that non-ESBL strains had more of the *fimH* adhesin gene and ESBL strains had more of the *hlyA* gene, responsible for the formation of pores in host cell membranes.

Furthermore, PAI IV_{536} was the most prevalent among all isolates, in the same way as phylogenetic group B2, indicating high pathogenicity of the isolates and corresponding to the virulence potential elucidated.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

As per international standards, parental written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was carried out using bacteria isolated from urine samples of children with suspected urinary tract infections. Therefore, there was no direct contact with the patient. Nevertheless, the study was approved by the Ethics and Research Committee of the State University of Londrina CAAE 66491323.4.0000.5231.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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