First detection of virulence factors in *Escherichia coli* isolated from urogenital tract and correlation with antimicrobial resistance at the National Public Health Laboratory (Brazzaville)

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Abstract

Escherichia coli is the most frequently isolated pathogen of urogenital infections. Its virulence gives it the ability to evade host defences and develop resistance to antibiotics. The aim of this work is to detect virulence factors and correlate them with antibiotic resistance. The virulence genes were detected by multiplex PCR. Susceptibility to antibiotics was tested by diffusion of agar discs. Of the 102 isolated strains, 43.14% had at least one of the three virulence factors tested. The sfa/foc gene was the most predominant, with a rate of 59.10%. The antibiotic susceptibility test showed the overall resistance of the strains tested, ranging from 20 to 80%. The correlation between antibiotic resistance and the expression of virulence factors showed that strains carrying the afa and sfa/foc genes were resistant to amoxicillin/clavulanic acid at rates of 83.3% and 61.5%, respectively; strains carrying the pap gene were 95% resistant to imipenem. Statistically significant P values were obtained, respectively, 0.001 for sfa/foc, 0.034 for afa, and 0.000 for pap. Other statistically significant results were also obtained. These factor detection rates and their antibiotic resistance profiles should lead us to question the hygiene measures to be taken to avoid contamination of the urogenital tract by these factors.

Keywords: *E. coli*, antimicrobial resistance, virulence factors.

1. Introduction

Escherichia coli is the most common pathogen found in genital tract infections [**1**]. Its virulence gives it the ability to evade host defences and develop resistance to antibiotics, which is linked to the creation and spread of resistance factors [**2**]. Antimicrobial resistance (AMR) is a natural phenomenon linked to the creation and spread of antibiotic-enhanced resistance factors. Thus, bacterial infections are currently one of the main public health problems throughout the world, and the particular emergence of new pathogens continues to remind us of their importance. Among the most common infectious diseases of bacterial origin in humans, urinary tract infections are the most common infections encountered in daily practice, both in community and hospital settings [**3**]. Urinary tract infections (UTI) affect both men and women and frequently occur at different ages [**4**]. Approximately 150 million new cases of UTIs are reported each year [**3**]. Many bacterial pathogens are known to be the causative agents of urinary tract infections. However, Escherichia coli species takes the lead with approximately 80 to 90% of community urinary tract infections and 30 to 50% of healthcare-associated urinary tract infections [**3**].

Urinary tract infection (UTI) caused by uropathogenic *E. coli* (UPEC) is a process that begins with the elimination of the epithelial immune system and the successful colonisation of UPEC in the urinary epithelium. UPECs are responsible for approximately 90% of urinary tract infections (UTI) [**5**]; their ability to cause urinary tract infections is related to the expression of many virulence factors [**2**], among which adhesion molecules play an important role [**6**]. These molecules allow E. coli to attach to host cells, colonise the urinary system, resist the flow of urine, and even use antibiotics [**7**]. These virulence properties allow them to be free from host defence mechanisms in order to establish themselves in new ecological niches and express their pathogenicity [**8**]. Structural factors such as outer membrane proteins, fimbria, and flagella are involved in this colonisation and adhesion process. UPECs express several fimbriary and

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afimbriary adhesins, such as P-type fimbriae (PAP), S-type (SFA), F-type fimbriae (FOC) and surface afimbriae (AFA), which help prevent urine evacuation and allow infection by the bacterium [**9**]. The S-type (sfa) and F-type (foc) fimbriae have the same binding specificity and possess high sequence homology [**5**].

Each strain can express different types of adhesins depending on their genetic content and the phases in which the bacterium is located [**5**]. These are found on the bacterial chromosome and are necessary for the bacterium to promote colonisation and adhesion to epithelial cells; therefore, they play an important role in their ability to infect the host [**10,31,32**].

Bacteria with these virulence factors are capable of triggering spontaneous infections of the digestive tract, specifically in humans and certain animals [33,34]. If they manage to cross the intestinal mucosa (through a lesion of the intestinal wall), they can become pathogenic and cause extra digestive infections, including urinary tract and genital infections [**11**]. In this case, they behave like opportunistic pathogens. In the literature, the presence of bacteria with virulence factors, even at low levels, in the female genital tract has been shown to cause genital infections [**12**]. Furthermore, vaginal carriage of *E. coli* has been correlated with the risk of preterm delivery [**13**].

In recent years, many strains of *E. coli* isolated from the urogenital tract have shown high rates of antibiotic resistance, which is of great concern for therapeutic management [**14**]. Previous studies have shown that in addition to the expression of virulence factors, *E. coli* strains isolated from the urogenital tract are multidrug resistant. Antibiotic resistance and expression of virulence factors are major determinants of UPECs [**15**].

The objective of this study is to isolate *E. coli* strains of the urinary and vaginal tract with virulence factors and determine their correlation with antibiotic resistance.

2. Methods

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2.1. Isolation and identification of bacteria

A total of 102 *Escherichia coli* isolated from urogenital tract of National Public Health Laboratory patients as part of routine institution procedure in the division of Bacteriology between June 1st and December 30 2022. This constituted the biological material of our study. All patients were women. After macroscopic and microscopic examinations, the samples were cultured on the usual entero bacteriaceae media chosen according to the Gram stain. Identification was carried out after transplanting on nutrient agar using conventional microbiology methods and using the Api® 20 E gallery (Biomérieux®).

2.2. Detection of genes encoding pap, sfa/foc and afa adhesins

DNA extraction

The genomic DNA of the strains was obtained using the NucleoSpin Microbial DNA KIT kit (Macherey-NAGEL, Germany) and according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) gene amplification technique has been used for the detection of virulence genes. Multiplex PCR was performed to simultaneously determine the presence of the pap, sfa / foc, and afa adhesion genes. The amplification reaction was performed in a final volume of 50 µL containing: Tris-HCl 10 mM pH 8.3, KCl 50 mM, MgCl2 3 mM, 200 µM of each deoxyribonucleotide triphosphate (dATP, dGTP, dCTP and dTTP), 20 pmol of each primer, 1.5 Taq polymerase units (Perkin-Elmer, Norwalk, Connecticut), and 5 µL of extracted DNA. Analysis of the amplification products was carried out by determining the size of the product after electrophoresis with 1.5% agarose gel. The size of the amplicons is an important criterion in determining the specific bands. After the amplification products with the

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DNA of the strain studied, the size of the fragment obtained was compared to that of the control strain. The thermal cycler (GeneAmp 9700 PCR system 9700; Perkin-Elmer) was programmed as follows: an initial denaturation step of 5 min at 94 ° C followed by a cyclic step repeated 30 times, including a denaturation phase of 30 sec at 94°C, a primer fixation phase of 30 sec at 65°C, and an elongation phase of 1 min at 72°C, finally a final extension stage of 5 min at 72 ° C.

The amplification products were separated according to their size on ethidium bromide-containing agarose gel and visualised on a UV transilluminator.

Table 1: Nucleotide sequences of primers used for multiplex PCR

Genes	Primer	Sequence (5'-3')	Size (bp)
pap	Pap1	GACGGCTGTACTGCAGGGTGTGGCG	328
	Pap2	ATATCCTTTCTGCAGGGATGGAATA	
afa	Afa1	GCTGGGCAGCAAACTGATAACTCTC	750
	Afa2	CATCAAGCTGTTTGTTCGTCCGCGG	
sfa/foc	Sfa/Foc1	CTCCGGAGAACTGGGTGCATCTTAC	410
	Sfa/Foc2	CGGAGGAGTAATTACAAACCTGGCA	

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2.3. Antimicrobial susceptibility testing

The susceptibility of strains with at least one of the virulence factors was tested against 12 antimicrobial agents referring to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM, 2022), using the standard method of Kirby and Bauer, based on the diffusion of antibiotic discs on Mueller-Hinton (HD) agar. The bacterial

inoculum was adjusted to a turbidity of 0.5 McFarland. The antibiotic discs used were as follows: Amoxicillin (20 µg); Amoxicillin + clavulanic acid (20 + 10 µg); Aztreonam (30 µg); Ceftazidime (10 µg), Cefotaxime (5 µg), Imipenem (10 µg); Ticarcillin (75 µg); Fosfomycin (200 µg); Gentamicin (10 µg) and Amikacin (30 µg); Nalidixic acid (30 µg); Ciprofloxacin (5 µg). The diameter of the inhibition zone for each antibiotic disc was measured using the calliper after 18 h of incubation at 35 °C ± 2 °C, and the results were confirmed to be sensitive or resistant. The control strain of *E. coli* ATCC 25922 was used for the confirmation of the results.

2.4. Statistical analysis

Data processing was carried out with Microsoft OfficeTM Excel 2016 and Graph Pad Prism software (version 7.0.0.159, USA). Statistical analysis was done using the ANOVA analysis of variance using the statistical analysis software Statistica 7.1. The results were expressed as proportions. The $P<0.05$ probability values were considered statistically significant.

3. Results

3.1. Bacteriological Data

3.1.1. Distribution of strains according to type of collection

Of the 102 strains of *E. coli* isolated, 75 come from urine samples, that is, an isolation frequency of 73.59%. In terms of vaginal swabs, 27 strains of *E. coli* were isolated, that is, an isolation frequency of 26.41%.

3.2. Molecular Characteristics

Of the 102 strains isolated, 44 strains presented at least one of the three virulence factors sought. This is a carrying frequency of 43.14% (44/102). Of the 44 with virulence factors, 30 strains

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came from urine samples (40% considering the 75 initial strains of urine) and 14 from vaginal exudates (51.85% considering the 27 initial strains of PV).

Regarding strains isolated from urine samples, the detection rate of virulence factors is higher in the female gender, that is, 57% of the strains carrying the virulence factors, while this rate is 43% in the male gender.

3.2.1. Genes encoding pap, sfa/foc, and afa adhesins

The analysis of the gels after electrophoresis shows the presence of bands of 328, 410, and 750 Pb, corresponding to the pap, sfa, and afa genes, respectively (Figure 1).

The sfa/foc gene was detected with predominance (26/44), a rate of 59.10%. The pap gene comes in the second position; it was detected in 20 strains, that is, a detection rate of 45.45%. The afa gene was detected with a frequency of 20.45% in 9 strains (Figure 2). In total, 43.13% of the studied strains amplified together or separately the afa, pap, and sfa genes together or separately.

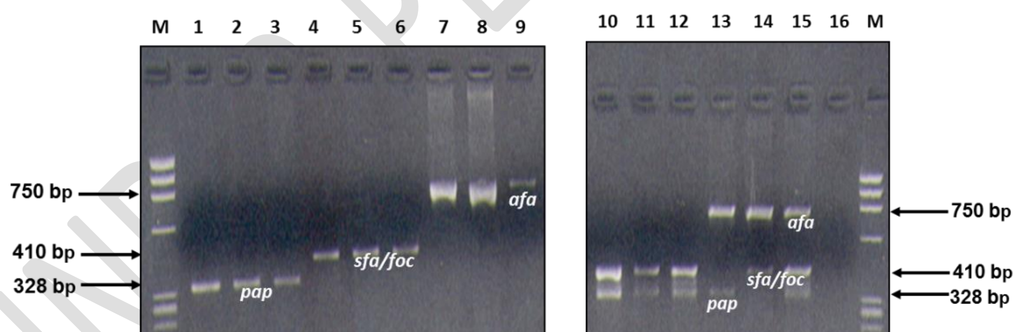

Figure 1: Electrophoretic profile of PCR products of the afa, pap, and sfa genes.

M: molecular weight marker (100 bp DNA ladder, Promega), tracks 1 to 3 = strains carrying the pap operon; tracks 4 to 6 = strains carrying the SFA/FOC operon; tracks 7 to 9 = strains carrying the AFA operon; tracks 10 to 12 = strains carrying both PAP and SFA operons; lane 13 = strain carrying both PAP and AFA operons; lane 14 = strain carrying both SFA/FOC and AFA operons; track 15: Positive control for the PAP, SFA and AFA genes and Track 16: Negative control

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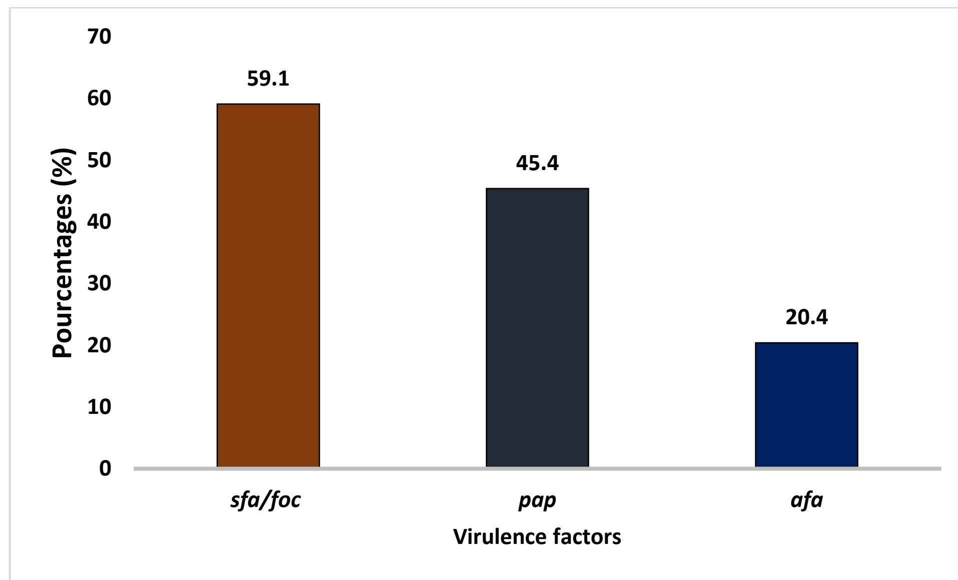

Figure 2: Frequencies of identified virulence factors

3.2.2. Genotypic profiles

Analysis of the genotypic profile shows that one or more types of genes encoding adhesins are detected in *E. coli* strains. Three types of bands with seven genotypic profiles were observed. These genes are detected in isolation, in the case of P1, P2, and P3, or in combination with each other in the rest of the cases. The sfa / foc, afa and pap genotypes were detected in isolation in the strains at rates of 34, 09, and 13,963%, respectively. The sfa/foc-afa-pap genotype has been observed in only one strain. Pairs of the sfa / foc-pap, afa-pap and sfa/foc-afa genes were found in the strains at rates of 20.45, 4.54, and 4.54%, respectively (Table 2).

Table 2: Genotypic profiles of isolated strains

Genotypic profile	Genes	Effective	Frequency (%)
P1	*sfa/foc*	15	34,09
P2	*pap*	9	20,45

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P3	*sfa/foc-pap*	9	20,45
P4	*afa*	6	13,63
P5	*afa-pap*	2	4,54
P6	*sfa/foc-afa*	2	4,54
P7	*sfa/foc-afa-pap*	1	2,27

Based on the type of collection, the different genotypes were distributed as shown in Table 3.

Table 3: Genotypic profiles of strains according to type of collection

Sample	**Genotypic profile**	**Genes**			**No. of strains**
		sfa/foc	***afa***	***Pap***	
Urines	**P1**	+	-	-	10
	P2	-	-	+	07
	P3	+	-	+	06
	P4	-	+	-	04
	P5	-	+	+	02
	P6	+	+	-	02
	Total				**31**
PV	**P1**	+	-	-	05
	P2	-	-	+	02
	P3	+	-	+	03
	P4	-	+	-	02
	P7	+	+	+	01

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	Total	13

Legend : PV, + : presence, - : absence

3.2.3. Antimicrobial susceptibility

The different strains tested showed strong resistance to the Betalactam family, with levels of around 60% to Ceftazidime, Cefotaxime and Imipenem. These levels are around 80% for Aztreonam and Amoxicillin. In aminoglycosides, the resistance rate was 20%. Finally, the strains tested for Fosfomycin, Ciprofloxacin, and Ticarcilin also showed resistance of 20%.

Table 4 : Resistance to antimicrobial profile

Antibiotic	Number of strains (n=44)	Percentage of resistance
AML	39	(88,6%)
AUG	18	(40,9%)
AT	35	(79,5%)
CTX	26	(59%)
CAZ	27	(61,3%)
IMI	30	(68,1%)
TC	9	(20,4%)
FOS	9	(20,4%)
CN	13	(29,5%)
AK	9	(20,4%)
NA	15	(34%)
CIP	9	(20,4%)

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Legend : AML= Amoxicillin ; AUG= Amoxicillin + clavulanic acid ; AT= Aztreonam ; CTX= Cefotaxim ; CAZ=Ceftazidim ; IMI= Imipenem ; TC=Ticarcillin ; FOS= Fosfomycin ; CN=Gentamicin ; AK= Amikacin ; NA=Nalidixic acid ; CIP=Ciprofloxacin.

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Table 5: Relation between antimicrobial resistance and virulence factor genes

	Antibiotic resistance (%)											
Virulence marker	**AML**	**AUG**	**AT**	**CTX**	**CAZ**	**IMI**	**TC**	**FOS**	**CN**	**AK**	**NA**	**CIP**
Pap												
Positive=20	19 (95%)	13 (65%)	20 (100%)	17 (85%)	18 (90%)	19 (95%)	6 (30%)	7 (35%)	11 (55%)	5 (25%)	10 (50%)	6 (30%)
Negative= 24	20 (83,3%)	05 (20,8%)	15 (62,5%)	09 (37,5%)	9 (37,5%)	11 (45,8%)	3 (12,5%)	2 (8,3%)	2 (8,3%)	4 (16,6%)	5 (20,8%)	3(12,5%)
P value	**0,455**	**0,005**	**0,002**	**0,002**	**0,001**	**0,000**	**0,261**	**0,029**	**0,001**	**0,710**	**0,042**	**0,261**
sfa/foc												
Positive=26	25 (96,1%)	16 (61,5%)	25 (96,1%)	19 (73%)	20 (76,9%)	21 (80,7%)	5 (19,2%)	6 (23%)	8 (30,7%)	6 (23%)	9 (34,6%)	4 (15,4%)
Negative=18	14 (77,7%)	2 (11,1%)	10 (55,5%)	7 (38,8%)	7 (38,8%)	9 (50%)	4 (22,2%)	3 (16,6%)	5 (27,7%)	3 (16,6%)	6 (33,3%)	5 (27,7%)
P value	**0,142**	**0,001**	**0,002**	**0,023**	**0,015**	**0,049**	**1,000**	**0,716**	**1,000**	**0,716**	**0,930**	**0,451**
afa												
Positive=6	6 (100%)	5 (83,3%)	6 (100%)	6 (100%)	6 (100%)	5 (83,3%)	4 (66,6%)	5 (83,3%)	5 (83,3%)	4 (66,6%)	4 (66,6%)	5 (83,3%)
Negative=38	33 (86,8%)	13 (34,2%)	29 (76,3%)	20 (52,6%)	21 (55,2%)	25 (65,7%)	5 (13,1%)	4 (10,5%)	8 (21%)	5 (13,1%)	11 (28,9%)	4 (10,5%)

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P value	**1,000**	**0,034**	**0,319**	**0,067**	**0,067**	**0,645**	**0,011**	**0,000**	**0,006**	**0,011**	**0,159**	**0,001**

* A P value less than 0,05 is statistically significant

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4. Discussion

This study is an original work in Congo, we determined the operons encoding adhesion factors and showed the link with antimicrobial resistance.

During a period of six months, 350 urine and 276 vaginal samples were taken at the National Public Health Laboratory in Brazzaville, 102 of which met the classic criteria for urinary and vaginal infections, that is, 16.3% positivity. These samples mainly involved outpatient basis.

Regarding the distribution of strain isolation according to the type of sample, this study shows that the frequency of strain isolation differs depending on the sample, as described in previous surveillance studies. In fact, *E. coli* were more isolated in the urinary tract with a frequency of 73.59% compared to a frequency of 26.41% in the vaginal tract. This observation is consistent with the high frequency of isolation of *E. coli* strains in the literature. In fact, *E. coli* is the enterobacterium most often implicated in lower urinary tract infections [**16**], it remains the most incriminated species in urinary tract infections (UTIs) because it is the most dominant aerobic intestinal germ, while knowing that the main bacterial reservoir in UTIs is the digestive tract and that it has many factors of virulence and uropathogenicity, including flagellum, which gives it the ability to migrate against the current by ascending route, and adhesins, which allow it to adhere to the urinary epithelium and prevent its elimination by bladder emptying [**17**].

In vaginal samples, out of a total of 276 samples analysed, only 27 strains of E. coli were isolated, that is, a frequency of 9.78%. This low frequency of isolation could be explained in part by the fact that the E. coli species is not a germ of the vaginal flora, as the vagina is not a reservoir for E. coli [**18**]. However, proximity between the urethral, vaginal, and anal orifices could explain the isolation of these strains during this study. In fact, the vaginal tract is often colonised by the anal route, and the genital tract then often serves as a relay between the anal reservoir and the urinary meatus [**19**]. Thus, vaginal contamination was found to precede urinary tract infection in two-thirds of cases [**20**]. Furthermore, the isolation of E. coli strains

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in the vaginal tract was high frequency compared to urine. Vaginal carriage of *E. coli* has been correlated with the risk of preterm delivery [**13**].

Among the pathogenicity factors commonly expressed by E. coli strains, extraintestinal (urinary and genital), adhesion to epithelial cells appears to be the most important for the pathogenicity of the bacterium. Specific adhesion is mediated by bacterial proteins called adhesins that may or may not be associated with fimbria. Genes involved in the biosynthesis of adhesins from pathogenic urinary and vaginal strains belong to groups of genes that are phylogenetically independent and organized into operons. 43.14% of the strains in this study presented at least one of the three virulence factors sought. The distribution of virulence factors between strains isolated from vaginal swabs and urine shows that there is a predominance of virulence factors in strains isolated from vaginal swabs, with a rate of 51.85%. This rate is 40% for strains isolated from urine. This high frequency of virulence factors in vaginal strains could be explained by the fact that the vagina is not a reservoir of E. coli. The proliferation of these strains in the vaginal tract then requires the presence of adhesins to allow the strains to adhere to the vaginal cells [**21**].

Strains isolated from urine had a higher virulence factor detection rate in females (57%), while the rate is 43% in males. This predominance of pathogenic bacteria in females could be due to fecal contamination due to the proximity between the urethral, vaginal, and anal orifices. In fact, the urethra and vagina can be contaminated by fecal strains, by poor hygiene of the perianal toilets carried out from the back to the front [**18**].

Our study allowed the simultaneous detection of the afa, pap, and sfa genes, encoding respectively the nonfimbriary adhesins AFA and the fimbriary PAP and SFA, in 43.13% of the strains studied. These genes encode virulence factors involved in Escherichia coli to uroepithelial cells and colonisation of the urinary and vaginal tracts. This detection rate is lower than that reported by Safarpoor in Iran, which found a prevalence of 72.72% for these genes in

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E. coli strains responsible for urinary tract infections [**22**]. This difference could be explained by the fact that our study included a majority of strains from patients with asymptomatic bacteriuria or cystitis, while the study focused on vaginal swab samples collected from fertile and infertile women [**22**]. Also, the isolated urine strains came from patients with cystitis and not pyelonephritis. In fact, strains of E. coli cells responsible for pyelonephritis generally have a higher affinity for uroepithelial cells than those responsible for cystitis or asymptomatic bacteriuria [**5**].

This study identified the sfa/foc gene as the most predominant (59.10%) in patients with symptoms of cystitis and asymptomatic bacteriuria. This result differs from those reported by Safarpoor in Iran, where he identified a predominance of the afa and sfa genes with a rate of 72.72% [**22**], and by Maris in 2016 in Guadeloupe, where the pap operon was predominant [**5**]. This discrepancy is probably explained by the fact that during this study we were dealing with patients with signs of cystitis and patients who had consulted for vaginal infections. The study also found the presence of the pap gene in 45.40% of E. coli strains. This type of fimbria is essential for renal cell adhesion and the development of pyelonephritis [**5**].

The genotypic profile of the strains studied showed different types of genotype. The pap, sfa/foc, afa, pap-sfa/foc, pap, afa, sfa / foc, sfa / foc-afa, pap, afa, sfa / foc-afa, pap genotypes were identified, also the genotype sfa/foc-afa-pap had been identified in a vaginal E coli strain. The presence of such a genotype determines the pathogenicity or virulence status of the strain [**5**]. Previous studies have shown that adhesions can be multiple within the same strain [**23**]. This variability of adhesins, necessary for the recognition of several receptors, seems to be an important factor in the development of *E coli* infection in the urinary tract and vaginal tract. It would help increase the pathogenicity of the strains and could be the basis of ascending vaginal infection.

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Strains of *E. coli* isolates showed resistance to the different families of antibiotics tested. Resistance with an average rate of around 60% was observed in Beta-Lactam in our study. The genus Escherischia is known to produce penicillas that confer resistance to beta-lactam [**24**]. Additionally, the uncontrolled use of antibiotics and the misuse of certain antibiotics in hospital settings may confer acquired resistance to this bacterium, which would explain the differences in the sensitivity of the strains in our study.

Regarding aminoglycosides, the strains showed an average resistance rate of around 25%. This rate is close to that reported by [**25**]. Resistance to aminoglycosides could be mainly due to the inactivation of 'aminoglycoside drugs' following the production of enzymes such as acetylases, adenylases, and phosphorylase transferases [**26**]. Resistance to aminoglycosides could result from 16S rRNA-coding gene *rrs* mutations that interfere with aminoglycoside binding [**27**]. It can also be associated with active efflux mechanisms [**27**].

An average resistance rate of around 27% for the fluoroquinolone family. Lopez has also reported similar results [**28**]. The resistance observed in this study could be attributed to the coexistence of several mechanisms, including efflux, acquisition of resistance gene acquisition, and modification of the target [**29**].

The results presented in Table 5 show a correlation between antibiotic resistance and the expression of virulence factors. Amoxicillin-clavulanic acid was 61.5% and 83.3% resistant in strains positive for the sfa/foc and afa genes, respectively, while resistance was only 11.1% and 34.2% in strains that did not express these genes. Scientifically statistical P-values were obtained, respectively, 0.001 for sfa/foc and 0.034 for afa. Other statistically significant results were obtained with the other antibiotics and also within the Pap gene. These results confirm the heterogeneous distribution between virulence factors and antibiotic resistance observed in UPECs by Miranda-Estrada [**30**].

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Conclusion

The resistance mechanisms that enterobacteria develop in general, as well as the propagation of their resistance genes, now constitute a major public health concern. This work showed an increase in the resistance of *E. coli* compared to antibiotics often prescribed in the hospital setting. Furthermore, these strains express significantly associated virulence genes with antibiotic resistance. The expressed virulence genes could be factors that favour UPEC infection. These facts do not facilitate the therapeutic treatment of patients and constitute a concern.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical committee

No ethical approval is need for the use of these samples

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