Pandemic ST131 *Escherichia coli* presenting the UPEC/EAEC and ExPEC/EAEC hybrid pathotypes recovered from extraintestinal infections in a clinical setting of the Brazilian Amazon region

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BACKGROUND *Escherichia coli* is a commensal organism but may become pathogenic by the acquisition of virulence factors involved with intestinal (IPEC) or extraintestinal (ExPEC) infections. Some strains, known as hybrids, may harbour virulence determinants of both IPEC and ExPEC pathotypes, increasing their virulence potential. Reports of hybrid *E. coli* in Brazil are rare, and the associated lineages were poorly explored.

OBJECTIVES This study characterised ExPEC *E. coli* strains focusing on the occurrence of hybrid pathotypes.

METHODS Fifteen clinical ExPEC strains were submitted to multilocus sequence typing (MLST), susceptibility test, and polymerase chain reaction (PCR) targeting IEC/ExPEC virulence markers.

FINDINGS All strains were multidrug-resistant, and 11 STs were determined among the 15 ExPEC strains, including local/new and pandemic lineages, such as ST69 and ST131. Twelve/15 isolates were classified as hybrids, due to the presence of virulence markers of both Enteroaggregative *E. coli* (EAEC) and ExPEC or UPEC pathotypes. These UPEC/EAEC (n = 10) and ExPEC/EAEC (n = 2) hybrid strains were found among distinct phylogroups and lineages, including new STs. Interestingly, most hybrids belonged to the pandemic ST131 lineage, and this genotype had never been previously reported in the ST131 circulating in Brazil.

MAIN CONCLUSIONS Therefore, this study provides new information on the epidemiological scenario of hybrid *E. coli*, contributing to a better understanding of the occurrence and pathogenic potential of these organisms.

Key words: HyPEC - ExPEC - ST69 - ST13 - virulence marker - pandemic lineage

Escherichia coli inhabits the intestinal tract of humans and other animals as an important member of their microbiota.[1] However, the acquisition by horizontal gene transfer of virulence determinants by certain *E. coli* clones has enabled them to cause not only intestinal but also extraintestinal infections in different hosts. In this way, based on the body site of infection, the host, and the presence of specific virulence markers, these bacteria can be classified into diarrhoeagenic (DEC), mainly featured by the presence of specific virulence factors directly related to diarrhoea, and extraintestinal pathogenic (ExPEC) *E. coli*, defined primarily by their site of isolation.[2] The DEC group encompasses several pathotypes, including enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and Shiga toxin-producing (STEC) *E. coli*. EPEC and EAEC are considered the major pathotypes of diarrhoeagenic *E. coli* causing disease worldwide, including in Brazil. These pathotypes carry genetic determinants involved with disease development and can be used as molecular markers of the pathotype.

The ExPEC group includes the pathotypes neonatal meningitis-associated (NMEC), human sepsis-associated (SEPEC), avian pathogenic (APEC), and uropathogenic (UPEC) *E. coli*, which is the most prevalent pathotype among extraintestinal infections worldwide. UPEC is particularly associated with urinary tract infections (UTIs) and is a leading cause of both community-acquired and healthcare-associated UTIs. Currently, the most frequently reported high-risk ExPEC lineages belonging to the multilocus sequence typing (MLST) ST131, ST69, ST10, ST405, ST38, ST95, ST648, ST73 and ST1193, which are known for their high prevalence in extraintestinal infections worldwide and for their significant role in global spread of multidrug resistance.[3]

Studies *in vivo* revealed that the presence of at least two of five virulence genes (*pap*, *afa/dra*, *sfa*, *kpsMTII*, and *iut/iuc*) could be enough to cause extraintestinal infection in immunocompetent individuals,[4] and that *E. coli* co-harbouring of *chuA*, *fyuA*, *vat*, and *yfcV* virulence genes were able to cause UTIs.[5] Additionally, due to horizontal gene transfer events, organisms may

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emerge as hybrid strains capable of causing intestinal and extraintestinal diseases due to the presence of virulence factors of both ExPEC and DEC pathotypes.[6]

Strains harbouring a blend of virulence markers of different pathotypes have been described worldwide in both high-risk and local lineages, however, most of them correspond to hetero-pathogenic strains (characterised by a combination of virulence markers from DEC pathotypes).[7,8,9] A recent study in South Africa demonstrated the high prevalence of several different DEC hybrid pathotypes, including strains sharing three virulence markers of three different DEC pathotypes, which were recovered from inanimate surfaces and presented a higher resistance profile compared to the classic pathotypes identified.[8] Another report demonstrated the occurrence of different DEC hybrid strains recovered from healthy individuals in Mexico.[9]

In Brazil, few studies have characterised and reported the occurrence of hybrid strains in extraintestinal infection cases harbouring the combination of DEC and ExPEC or UPEC virulence markers, such as UPEC/EAEC, UPEC/aEPEC, ExPEC/STEC, ExPEC/aEPEC.[10-18] However, there is a lack of association concerning the STs of such hybrid strains circulating in the country. Moreover, most of these reports on hybrid strains were restricted to São Paulo, a cosmopolitan city of the Southeast Brazilian region, and until now, all studies reporting *E. coli* infections in the Amazon region were associated only with diarrhoeagenic infections.[19,20,21,22] Therefore, further studies are required to investigate the distribution and potential impact of these strains on the clinical outcomes of affected individuals. For these reasons, the objective of this study was to characterise *E. coli* strains recovered from extraintestinal infections in the Brazilian Amazon region by means of lineage determination (MLST), antibiotic resistance profile, and investigation of DEC and ExPEC virulence markers.

MATERIALS AND METHODS

Clinical data, bacterial strains and antimicrobial susceptibility test - From December 2016 to February, 2018, 15 *E. coli* isolates were recovered from extraintestinal infection cases at the General Hospital of Roraima (GHR), placed in Boa Vista. The strains were isolated from blood (n = 2), urine (n = 7), catheter tip (n = 1), vagina secretion (n = 1), leg secretion (n = 1), soft tissue (n = 1), and operative wound secretion (n = 2). Species identification was performed with the automated VITEK2, and confirmed by sequencing the 16S rRNA and the MLST genes. These strains are part of the Bacterial Culture Collection of the Laboratory of Molecular Genetics of Microrganisms, FIOCRUZ.

Antimicrobial susceptibility testing - The antibiotic susceptibility profile was determined by disc-diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[23] For the following antibiotics: gentamicin, amikacin, kanamycin, tobramycin, neomycin, streptomycin, imipenem, meropenem, ertapenem, cephalothin, cefoxitin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime, cefepime, azithromycin, clarithromycin, erythromycin, ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanic acid, carbenicillin, penicillin, aztreonam, piperacillin/tazobactam, ticarcillin/clavulanic acid, nalidixic acid, ciprofloxacin, norfloxacin, levofloxacin, ofloxacin, colistin sulphate, sulphonamide, trimethoprim, sulfamethoxazole/trimethoprim, chloramphenicol, fosfomycin, nitrofurantoin, tetracycline and minocycline.

Genotyping by multilocus sequence typing - The genetic relationship among the ExPEC strains was established by MLST based on the Achtman MLST scheme (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) (http://mlst.warwick.ac.uk). Clonal complexes (CCs) were considered when sequence types (STs) shared five or more identical alleles taking into account the seven genes considered in the MLST scheme.

Detection of resistance genes by polymerase chain reaction (PCR) and sequencing - The isolates were screened by PCR and sequencing for the presence of the genes frequently associated with β-lactam resistance in *E. coli* such as bla_{SHV}, bla_{GES}, bla_{TEM}, bla_{CTX-M} (class A β-lactamases). The plasmid-mediated fluoroquinolone (FQ) resistance *qnr* genes and the presence of mutations in the quinolone resistance-determining region (QRDR) of *gyrA* was also investigated (Table I).

Molecular characterisation of hybrid strains - The presence of DEC and ExPEC virulence determinants was screened by PCR (Table I). Those strains presenting at least one of the DEC pathotypes virulence markers were considered hybrid strains.[13] The DEC virulence factors screened were *eae* and *bfpB* genes, associated with epithelial adhesion in EPEC; the *aggR* gene, a universal regulator of EAEC virulence involved with the aggregative adhesion fimbriae (AAF) production; the *stx1* and *stx2* genes, related to the Shiga toxins production in STEC; the *estH*, *estP* and *elT*, involved with the production of heat-stable and heat-labile toxins in ETEC; the *invE* gene that encodes a regulatory protein that controls the transcriptional of invasion genes in EIEC pathotype; the *vat*, *fuyA*, *chuA* and *yfcV*, all markers of UPEC; and ExPEC markers *iutA*, *KPSMTII*, *sfaDE*, *papC*, *afaBC*, *iucD*.

Ethics - Only secondary data was used in this study. It was submitted to the Research Ethics Committee (CEP) of the Oswaldo Cruz Institute (IOC) and was approved under the CAAE number 39978114.5.0000.5248.

RESULTS AND DISCUSSION

The phenotypic analysis revealed that all *E. coli* ExPEC strains (n = 15) were classified as multidrug-resistant (MDR) according to resistance classification criteria (Table I).[24] Among the β-lactam antibiotics, all isolates were resistant to at least one of these antibiotics, and such phenotype could be explained by the presence of the bla_{TEM} gene in 14/15 isolates. In fact, bla_{TEM} is frequently associated with plasmids in *E. coli*, and it was the most prevalent gene among ExPEC recovered from UTI cases in São Paulo, Brazil.[25] However, although the bla_{CTX-M} has been frequently associated with ExPEC in Brazil and worldwide,[26] this gene was not found in our sample, as well as the other screened β-lactamase genes bla_{GES} and bla_{SHV}.

TABLE I
Primers used in this study

Primer name	Primer sequence (5' - 3')	Target
Antibiotic resistance genes		
SHV F SHV R	TTCATGGCGTTACCTTTGAC CCGACAGAGTGCGGTATTTA	Class A β-lactamase bla_{SHV} genes
GES F GES R	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGGA	ESBL Class A β-lactamase bla_{GES} genes
TEM F TEM R	GTATCCGCTCATGAGACAATA TCTAAAGTATATATGAGTAAACTTGGTCTG	Class A β-lactamase bla_{TEM} genes
CTX-M F CTX-M R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	ESBL Class A β-lactamase bla_{CTX-M} genes
QnrVC F QnrVC R	AAGTGAACTTCTCACATCAGG ATGATTACCCCTAATTGCTCC	Quinolone resistance *qnrVC* genes
QnrA F QnrA R	GATTTCTCACGCCAGGATTT TCKGAGCCCATCAAGGAAG	Quinolone resistance *qnrA* gene
QnrB F QnrB R	GATCGWGAAAGYCAGAAAGG CCACARYTCRCAYTTTTC	Quinolone resistance *qnrB* gene
QnrC F QnrC R	TGCAGACCTACGAGATGCTT TTCACGCCAGTTAAATCCAC	Quinolone resistance *qnrC* gene
QnrS F QnrS R	ACGTGTTAACTTGCGTGAT GCAATTTTGATACCTGATG	Quinolone resistance *qnrS* gene
GyrA F GyrA R	CGTTGGTGACGTAATCGGTA GTGTTCCATCAGCCCTTCA	QRDR of *gyrA* gene
Virulence genes		
Eae F Eae R	GTGGCGAATACTGGCGAGACT CGACGGTGAAAAAGAATGGGG	EPEC virulence marker
BfpB F BfpB R	CGATAAAACTGATACTGGGCAGC GTGCTTCCCGAACAGTCACT	
AggR F AggR R	CGATACATTAAGACGCCTAAAG TCTGATACATTAAATTCATCTGC	EAEC virulence marker
Stx1 F Stx1 R	CAGTTAATGTGGTGGCGAAGG CACCAGACAATGTAACCGCTG	STEC virulence marker
Stx2 F Stx2 R	ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	
EstH F EstH R	GCTAAACCAGTAGAGTC CACCCGGTACAAGCAGG	ETEC virulence marker
EstP F EstP R	AGTCAGTCAACTGAATCAC ATTTTCTCAGCACCAATAC	
elT F elT R	CACACGGAGCTCCTCAG CAAACTAGTTTTCCATACTG	
invE F invE R	CGATAGATGGCGAGAAATTATATCCCG CGATCAAGAATCCCTAACAGAAGAATCA	EIEC virulence marker
Vat F Vat R	TCAGGACACGTTCAGGCATTCAGT GGCCAGAACATTTGCTCCCTTGTT	UPEC virulence marker
FyuA F FyuA R	GTAAACAATCTTCCCGCTCGGCAT TGACGATTAACGAACCGGAAGGGA	
ChuA F ChuA R	CTGAAACCATGACCGTTACG TTGTAGTAACGCACTAAACC	
yfcV F yfcV R	ACATGGAGACCACGTTCACC GTAATCTGGAATGTGGTCAGG	

Primer name	Primer sequence (5' - 3')	Target
iutA F	GGCTGGACATCATGGGAACTGG	ExPEC virulence marker
iutA R	CGTCGGGAACGGGTAGAATCG	
KpsMTII F	GCGCATTTGCTGATACTGTTG	
KpsMTII R	CATCCAGACGATAAGCATGAGCA	
sfaDE F	CTCCGGAGAACTGGGTGCATCTTAC	
sfaDE R	CGGAGGAGTAATTACAAACCTGGCA	
papC F	GTGGCAGTATGAGTAATGACCGTTA	
papC R	ATATCCTTTCTGCAGGGATGCAATA	
afaBC F	GGCAGAGGGCCGGCAACAGGC	
afaBC R	CCCGTAACGCGCCAGCATCTC	
iucD F	TCCTCATTTTTCCTGGCATC	
iucD R	AGTTTATTTCCGCCGCTTCT	

Moreover, a high prevalence of fluoroquinolone resistance was observed (73%) among the ExPEC strains. Although none of them carried any *qnr* allele, all FQ-resistant isolates presented mutations in the QRDR of *gyrA* that led to the amino acid substitution Ser83Leu and Asp87Asn, known to be involved with FQ resistance emergence,[27] including ExPEC strains recovered from UTI cases in São Paulo.[12]

The MLST analysis revealed a great diversity, in which 11 STs were assigned to the 15 ExPEC strains: ST69, ST131, ST8886, ST1196, ST9403, ST3180, ST12394, and four different new sequence types belonging to CC155 (n = 2), CC14 (n = 1) and CC131 (n = 1), demonstrating the polyclonal nature of these hybrid strains. The most prevalent lineage among our sample was ST131, a pandemic clonal complex of the B2 phylogroup often associated with an arsenal of resistance and virulence genes, contributing to the success of this clone in spreading through clinical settings worldwide. The ST69 is another pandemic lineage belonging to the phylogroup D usually associated with clinics and food/environment contamination in several countries.[27] As found here, Lara and Colleagues have demonstrated the occurrence of ExPEC ST69 presenting the hybrid pathotype UPEC/EAEC in Brazil.[10] However, in spite of ST131 prevalence in Brazil, this is the first report of this lineage presenting a hybrid pathotype (UPEC/EAEC) in that country (Table II).

Considering the *E. coli* virulome, 9/19 virulence factors characterising DEC and ExPEC pathotypes were found in the studied samples in different frequencies and combinations (Table II). The presence of at least one extraintestinal virulence marker was observed in all isolates, corroborating the nature of the infection sites from which they were recovered. The *iucD* gene, which is part of the operon involved with aerobactin siderophore synthesis, was the most prevalent ExPEC marker among the isolates (12/15). This gene can be carried and widespread by mobile genetic elements, such as genomic islands and plasmids such as ColV plasmids, and it is associated with iron uptake systems, facilitating its survival and pathogenicity and playing a crucial role in the bacteria's ability to cause infections and evade the host's immune response.

Among the 15 ExPEC isolates analysed, 12 (80%) had at least one marker used to define ExPEC and DEC pathotypes, being characterised as hybrid strains. The three strains classified as non-hybrids belonged to the pandemic lineages ST131/phylogroup B2 (EC44) and ST69/phylogroup D (EC39) and to the local ST8886 (EC40). Only 13 entries in Enterobase (https://enterobase.warwick.ac.uk/species/index/ecoli) have been found for ST8886, and in most cases, they corresponded to strains recovered from environmental sites in USA in 2015 and only one recovered from an animal source in Canada in 2022. Thus, this study reported the first clinical case belonging to this lineage.

Concerning the hybrid strains, it was verified the presence of UPEC/EAEC (n = 10) and ExPEC/EAEC (n = 2) (when UPEC markers were absent, or the strain had been recovered from a different body site other than urine). The *aggR* gene, considered a diarrhoeagenic factor that defines the EAEC pathotype, was found in all hybrid isolates, indicating that they would have the ability to cause intestinal disorders despite presenting in extraintestinal infections. EAEC is recognised as an important cause of diarrhoea worldwide and can be considered the most common DEC pathotype in *E. coli* in Brazil. However, although ExPEC strains with a hybrid enteroaggregative/uropathogenic (UPEC/EAEC) genotype have been identified worldwide, mainly associated with pandemic ST38 and ST131 lineages,[28] such hybrid pathotype have sporadically emerged as a cause of extraintestinal infections in Brazil.[10-18] Here we demonstrated the high prevalence of UPEC/EAEC hybrid pathotype in distinct ExPEC lineages causing infections in the Amazon region.

Most of UPEC/EAEC hybrid strains belonged to the pandemic and widespread ST131 (one of them was assigned to a new ST of the CC131) (Table II). They were recovered from distinct extraintestinal sites and presented a heterogeneous profile of virulence markers. The unique non-hybrid ST131 strain was the EC44, which was a UPEC recovered from UTI that presented both UPEC and ExPEC virulence markers. On the other hand, local STs (ST9403, ST3180 and ST12394) belonging to different phylogroups (A, B1 and B2) and recovered from distinct body sites, including blood, were also

TABLE II

Clinical and genetic information of the hybrid *Escherichia coli* strains in this study

Strain	Infection site	ST	CC	Phylogroup	Antibiotic resistance profile	Pathotype genetic markers			Hybrid classification
						DEC	EXPEC	UPEC	
EC39	Urine	69	69	D	NEO, CEF, CLA, ERI, CB, AMP, PEN, SXT, SUL, TMP	-	*kpsMTII*	-	-
EC40	Blood	8886	-	B2	NEO, CXM, AZI, CLA, ERI, AMP, PEN, SXT, SUL, TMP	-	-	*fyuA, yfcV*	-
EC41	Leg secretion	131	131	B2	KAN, NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, AMX, CB, PEN, TIM, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP, MIN	*aggR*	*iucD*	*fyuA, yfcV*	UPEC/EAEC
EC42	Urine	131	131	B2	GEN, TOB, KAN, NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, SAM, AMX, AMC, CB, PEN, TIM, ATM, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP, MIN, TET	*aggR*	*iucD*	*yfcV*	UPEC/EAEC
EC43	Catheter tip	131	131	B2	NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, AMX, CB, PEN, TIM, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP, TET	*aggR*	*iutA, kpsMTII, iucD*	*yfcV*	UPEC/EAEC
EC46	Soft tissue	NEW 4	131	B2	GEN, TOB, KAN, NEO, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, SAM, AMX, AMC, CB, PEN, TIM, ATM, CLA, NAL, CIP, NOR, LEV, OFX	*aggR*	*iutA, kpsMTII, papC, iucD*	*fyuA, yfcV*	UPEC/EAEC
EC47	Urine	1196	-	AxB1	KAN, NEO, CEF, AMP, CB, PEN, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX	*aggR*	*iutA*, *afaBC*	-	EXPEC/EAEC
EC48	Vagina secretion	9403	-	A	KAN, NEO, STR, AMP, CB, PEN, AZI	*aggR*	*iutA*, *kpsMTII*, *papC*, *iucD*	*fyuA, yfcV*	UPEC/EAEC
EC44	Urine	1	131	B2	GEN, TOB, KAN, NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, SAM, AMX, AMC, CB, PEN, TIM, ATM, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP, TET	-	*iutA, kpsMTII, papC iucD*	*fyuA, yfcV*	-
EC45	Urine	131	131	B2	TOB, KAN, NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, SAM, AMX, AMC, CB, PEN, TIM, TZP, ATM, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP	*aggR*	*iutA, kpsMTII, afaBC, iucD*	*fyuA, yfcV*	UPEC/EAEC
EC49	Urine	NEW 1	14	B2	KAN, NEO, STR, CEF, CTX, AMP, SAM, AMX, AMC, CB, PEN, TIM, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, SXT, SUL, TMP	*aggR*	*iutA, kpsMTII, papC, afaBC, iucD*	*vat, fyuA, yfcV*	UPEC/EAEC
EC50	-	3180	14	B2	KAN, NEO, STR, CRO, AMP, PEN, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, CHL	*aggR*	*iutA, kpsMTII, iucD*	*yfcV*	UPEC/EAEC
EC51	Blood	NEW 2	155	B1	KAN, NEO, STR, CEF, CRO, AMP, SAM, AMX, CB, PEN, TIM, CLA, ERI, SXT, SUL, TMP, MIN, TET	*aggR*	*iutA, iucD*	*fyuA*	UPEC/EAEC
EC52	Urine	NEW 3	155	B1	AMP, PEN, CLA, NAL, CIP, NOR, LEV, OFX, MIN	*aggR*	*iutA, afaBC, iucD*	-	EXPEC/EAEC
EC53	wound secretion	12394	46	A	AMK, GEN, TOB, KAN, NEO, STR, CEF, FOX, CXM, CAZ, CRO, CTX, FEP, AMP, SAM, AMX, CB, PEN, TIM, ATM, AZI, CLA, ERI, NAL, CIP, NOR, LEV, OFX, TMP, MIN, TET	*aggR*	*iutA, iucD*	*fyuA*	UPEC/EAEC

AMK: amikacin; GEN: gentamicin; TOB: tobramycin; KAN: kanamycin; NEO: neomycin; STR: streptomycin; CEF: cephalothin; FOX: cefoxitin; CXM: cefuroxime; CAZ: ceftazidime; CRO: ceftriaxone; CTX: cefotaxime; FEP: cefepime; AMP: ampicillin; SAM: ampicillin/sulbactam; AMX: amoxicillin; AMC: amoxicillin/clavulanate; CB: carbenicillin; PEN: penicillin; TIM: ticarcillin/clavulanate; TZP: piperacillin/tazobactam; ATM: aztreonam; AZI: azithromycin; CLA: clarithromycin; ERI: erythromycin; NAL: nalidixic acid; CIP: ciprofloxacin; NOR: norfloxacin; LEV: levofloxacin; OFX: ofloxacin; SXT: trimethoprim/sulfamethoxazole; SUL: sulphonamide; TMP: trimethoprim; MIN: minocycline; TET: tetracycline; CHL: chloramphenicol.

classified as UPEC/EAEC hybrid strains. Interestingly, in spite of the diversity of infection sites, most of the ExPEC strains were recovered from urine, and all but one were resistant to the tested fluoroquinolones (Table II), which are the first-choice antibiotics for treating UTIs. These findings probably accounted for treatment failure, compromising the success of clinical outcomes.

Interestingly, despite the UPEC/EAEC EC49 strain being a new local ST of CC14/phylogroup B2, this isolate exhibited the highest number of ExPEC and UPEC virulence markers, besides the EAEC marker *aggR* (Table II). This demonstrates that not only pandemic high-risk clones display an enhanced pathogenicity and that emerging strains may possess a high level of virulence fitness.

The other class of hybrids (ExPEC/EAEC) corresponded to strains (n = 2) that carried ExPEC virulence markers and the *aggR* EAEC marker. The strains belonged to a new ST from CC155/phylogroup B1 (EC52) and to the local ST1196 belonging to phylogroup AxB1 (EC47) (Table II). A recent study in Mozambique demonstrated that a hybrid ExPEC/EAEC strain recovered from blood belonged to the pandemic ST131 lineage and presented a remarkable virulence profile.[29] These findings revealed that ExPEC/EAEC hybrids are associated with distinct lineages. Interestingly, both ExPEC/EAEC strains identified here were recovered from urine although no UPEC virulence marker had been identified. This was also the case of the non-hybrid EC39 strain, belonging to the pandemic ST69/phylogroup D, that had been characterised as a UPEC pathotype due to the site of infection (urine), although all UPEC virulence markers were absent (Table II). These findings suggest that their ability to infect the urinary tract would be related to other mechanisms that were not investigated in this study. In fact, UPEC employs various virulence strategies, such as binding to urinary tract epithelial cells, iron acquisition, toxin production, and evasion of the host's immune system. These virulence factors are commonly encoded by genes found in pathogenicity islands (PAIs), which are often mobilised through horizontal gene transfer and could have been lost from those UPEC strains.[14] Furthermore, the presence of UPEC factors in strains recovered from sites other than urine, such as the non-hybrid EC40 strain (Table II), demonstrates their ability to colonise and survive in the urinary tract, but also that other virulence determinants were involved in the establishment of extraintestinal infections in those cases.

In conclusion, this study contributes to a better understanding of the occurrence and pathogenic potential of hybrid strains of *E. coli*. Besides, the association of pathotype and lineage definition provides new information in the scenario of the global epidemiology of ExPEC hybrid strains.

AUTHORS' CONTRIBUTION

NMSB - Writing original draft, investigation, data curation, methodology; ELF - writing, review & editing, data curation, supervision, conceptualisation; FSF and SMM - data curation; ACPV - writing, review & editing, conceptualisation, funding acquisition. The authors declare that there are no conflicts of interest.

REFERENCES

1. Leimbach A, Hacker J, Dobrindt U. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol. 2013; 358: 3-32.
2. Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. Extraintestinal pathogenic *Escherichia coli*: virulence factors and antibiotic resistance. Pathogens. 2021; 10(11): 1355.
3. Kocsis B, Gulyás D, Szabó D. Emergence and dissemination of extraintestinal pathogenic high-risk international clones of *Escherichia coli*. Life (Basel). 2022; 12(12): 2077.
4. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother. 2003; 47(7): 2161-8.
5. Spurbeck RR, Dinh Jr PC, Walk ST, Stapleton AE, Hooton TM, Nolan LK, et al. *Escherichia coli* isolates that carry vat, fyuA, chuA, and yfcV efficiently colonize the urinary tract. Infect Immun. 2012; 80(12): 4115-22.
6. Braz S, Melchior K, Moreira CG. *Escherichia coli* as a multifaceted pathogenic and versatile bacterium. Front Cell Infect Microbiol. 2020; 10: 1-9.
7. Lee W, Sung S, Ha J, Kim E, An ES, Kim SH, et al. Molecular and genomic analysis of the virulence factors and potential transmission of hybrid enteropathogenic and enterotoxigenic *Escherichia coli* (EPEC/ETEC) strains isolated in South Korea. Int J Mol Sci. 2023; 24(16): 12729.
8. Rakhalaru P, Munzhedzi L, Abia ALK, Kabue JP, Potgieter N, Traore AN. Prevalence and antimicrobial resistance profile of diarrheagenic *Escherichia coli* from fomites in rural households in South Africa. Antibiotics (Basel). 2023; 12(8): 1345.
9. Méndez-Moreno E, Caporal-Hernandez L, Mendez-Pfeiffer PA, Enciso-Martinez Y, López RR, Valencia D, et al. Characterization of diarreaghenic *Escherichia coli* strains isolated from healthy donors, including a triple hybrid strain. Antibiotics (Basel). 2022; 11(7): 833.
10. Lara FBM, Nery DR, Oliveira PM, Araujo ML, Carvalho FRQ, Messias-Silva LCF, et al. Virulence markers and phylogenetic analysis of *Escherichia coli* strains with hybrid EAEC/UPEC genotypes recovered from sporadic cases of extraintestinal infections. Front Microbiol. 2017; 8: 146.
11. Schüroff PA, Abe CM, Silva JW, Coelho CP, Andrade FB, Hernandes RT, et al. Role of aggregate-forming pilus (AFP) in adherence and colonization of both intestinal and urinary tracts. Virulence. 2022; 13(1): 1423-33.
12. Nascimento JAS, Santos FF, Valiatti TB, Santos-Neto JF, Santos ACM, Cayô R, et al. Frequency and diversity of hybrid *Escherichia coli* strains isolated from urinary tract infections. Microorganisms. 2021; 9(4): 693.
13. Nascimento JAS, Santos FF, Santos-Neto JF, Trovão LO, Valiatti TB, Pinaffi IC, et al. Molecular epidemiology and presence of hybrid pathogenic *Escherichia coli* among isolates from community-acquired urinary tract infection. Microorganisms. 2022; 10(2): 302.
14. Tanabe RHS, Dias RCB, Orsi H, de Lira DRP, Vieira MA, Dos Santos LF, et al. Characterization of uropathogenic *Escherichia coli* reveals hybrid isolates of uropathogenic and diarrheagenic (UPEC/DEC) *E. coli*. Microorganisms. 2022; 10(3): 645.
15. Furlan JPR, Ramos MS, Dos Santos LDR, Rosa RS, Stehling EG. Multidrug-resistant Shiga toxin-producing *Escherichia coli* and hybrid pathogenic strains of bovine origin. Vet Res Commun. 2023; 47(4): 1907-13.

16. Munhoz DD, Richards AC, Santos FF, Mulvey MA, Piazza RMF. *E. coli* common pili promote the fitness and virulence of a hybrid aEPEC/ExPEC strain within diverse host environments. Gut Microbes. 2023; 15(1): 2190308.

17. de Lira DRP, Cavalcanti AMF, Pinheiro SRS, Orsi H, Dos Santos LF, Hernandes RT. Identification of a hybrid atypical enteropathogenic and enteroaggregative *Escherichia coli* (aEPEC/EAEC) clone of serotype O3:H2 associated with a diarrheal outbreak in Brazil. Braz J Microbiol. 2021; 52(4): 2075-9.

18. Valiatti TB, Santos FF, Santos ACM, Nascimento JAS, Silva RM, Carvalho E, et al. Genetic and virulence characteristics of a hybrid atypical enteropathogenic and uropathogenic *Escherichia coli* (aEPEC/UPEC) strain. Front Cell Infect Microbiol. 2020; 10: 492.

19. Vicente AC, Teixeira LF, Iniguez-Rojas L, Luna MG, Silva L, Andrade JRC, et al. Outbreaks of cholera-like diarrhoea caused by enterotoxigenic *Escherichia coli* in the Brazilian Amazon Rainforest. Trans R Soc Trop Med Hyg. 2005; 99(9): 669-74.

20. Benevides-Matos N, Pieri FA, Penatti M, Orlandi PP. Adherence and virulence genes of *Escherichia coli* from children diarrhoea in the Brazilian Amazon. Braz J Microbiol. 2015; 46(1): 131-7.

21. Taborda RLM, Silva LAD, Orlandi PP, Batista FS, Rodrigues RS, Matos NB, et al. Characterization of enteroaggregative *Escherichia coli* among diarrheal children in Western Brazilian Amazon. Arq Gastroenterol. 2018; 55(4): 390-6.

22. Rodrigues RS, Lima NCDS, Taborda RLM, Esquerdo RP, Gama AR, Nogueira PA, et al. Antibiotic resistance and biofilm formation in children with enteropathogenic *Escherichia coli* (EPEC) in Brazilian Amazon. J Infect Dev Ctries. 2019; 13(8): 698-705.

23. CLSI - Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing supplement M100. 32nd ed. Wayne: CLSI; 2021.

24. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3): 268-81.

25. Santos-Neto JF, Santos ACM, Nascimento JAS, Trovão LO, Santos FF, Valiatti TB, et al. Virulence profile, antibiotic resistance, and phylogenetic relationships among *Escherichia coli* strains isolated from the feces and urine of hospitalized patients. Pathogens. 2022; 11(12): 1528.

26. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother. 2017; 72(8): 2145-55.

27. Riley LW. Pandemic lineages of extraintestinal pathogenic *Escherichia coli*. Clin Microbiol Infect. 2014; 20(5): 380-90.

28. Roy Chowdhury P, Hastak P, DeMaere M, Wyrsch E, Li D, Elankumaranet P, et al. Phylogenomic analysis of a global collection of *Escherichia coli* ST38: evidence of interspecies and environmental transmission? mSystems. 2023; 8(5): e0123622.

29. Santona A, Sumbana JJ, Fiamma M, Deligios M, Taviani E, Simbine SE, et al. High-risk lineages among extended-spectrum β-lactamase-producing *Escherichia coli* from extraintestinal infections in Maputo Central Hospital, Mozambique. Int J Antimicrob Agents. 2022; 60(4): 106649.