Research Article

Molecular Screening of Virulence Genes in Extraintestinal Pathogenic *Escherichia coli* Isolated from Human Blood Culture in Brazil

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) is one of the main etiological agents of bloodstream infections caused by Gram-negative bacilli. In the present study, 20 *E. coli* isolates from human hemocultures were characterized to identify genetic features associated with virulence (pathogenicity islands markers, phylogenetic group, virulence genes, plasmid profiles, and conjugative plasmids) and these results were compared with commensal isolates. The most prevalent pathogenicity island, in strains from hemoculture, were PAI IV, described by many researchers as a stable island in enterobacteria. Among virulence genes, *iutA* gene was found more frequently and this gene encodes the aerobactin siderophore receptor. According to the phylogenetic classification, group B2 was the most commonly found. Additionally, through plasmid analysis, 14 isolates showed plasmids and 3 of these were shown to be conjugative. Although in stool samples of healthy people the presence of commensal strains is common, human intestinal tract may serve as a reservoir for ExPEC.

1. Introduction

*Escherichia coli* is one of the most common microorganisms of the human intestinal microbiota. However, a small percentage of *E. coli* is capable of causing extraintestinal infections (extraintestinal pathogenic *Escherichia coli*—ExPEC), and these ExPECs are considered some of the main etiological agents of bacteremia caused by Gram-negative bacilli [1, 2]. According to the phylogenetic classification, ExPECs typically belong to group B2 and less commonly to group D, whereas commensal intestinal strains belong to group A or B1 [3]. ExPEC pathogenicity is due to the presence of genes, located on plasmids or chromosomes that encode virulence factors. When present on the chromosome, these genes are typically found in specific regions called pathogenicity islands (PAI). Given that the severity of bacterial infections is often due to the genetic features of the pathogenic agent and that few studies have investigated genetic aspects of ExPEC isolates from bacteremia in Brazil [4, 5], the aim of the present study was to characterize 20 *E. coli* isolates from human hemocultures for genetic features associated with ExPEC. The investigation was based on the screening for PAI associated sequences, determination of phylogenetic group, genotypic identification of the major virulence factors of ExPEC, and plasmid analyses, and these results compared with commensal strains. This information may help us better understand the pathogenesis of these bacteria.

2. Materials and Methods

2.1. Bacterial Isolates. To perform the study, 20 *E. coli* strains isolated from human hemocultures were kindly provided by Professor Marilda C. Vidotto (Brazil) [6] and 51 *E. coli* strains were obtained from stools of 19 healthy Brazilians. The strains were stored in brain heart infusion with 20% glycerol at −20°C. The polymerase chain reaction (PCR) was used for the genetic characterization of PAI associated sequences, presence of virulence genes, and for phylogenetic classification. PCR amplicons were visualized on 1.0% agarose gels stained with GelRed (Biotium). After gel electrophoresis
the images were captured using Image Capture Systems (LI-PixImageHE).

2.2. Detection of PAI Markers. The presence of sequences associated with seven different PAIs, previously characterized in uropathogenic *E. coli* (UPEC), was determined (PAI I_{536}, II_{536}, IV_{536}, CFT073, II_{CFT073}, I_{pap} and II_{pap}) [7] (Table 1).

This PCR contained 1 U Taq DNA polymerase (Invitrogen) in 2x PCR buffer (Invitrogen), 0.2 mM of each dNTP, 2.5 mM MgCl₂, and 20 pmol/μL of each primer (Table 1). The program consisted of 94 °C for 4 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 10 min [7]. The positive control used in the PCR was J96.

2.3. Phylogenetic Classification. Phylogenetic classification showed that the *E. coli* strains belonged to four groups (A, B1, B2, or D) based on the presence of the chuA and yjaA genes and the DNA fragment (TSPE4.C2). This PCR contained 1.25 U Taq DNA polymerase (Invitrogen) in 1x PCR buffer (Invitrogen), 20 pmol of each dNTP, 2.5 mM MgCl₂, and 1 μM of each primer (Table 1). The program of PCR consisted of 94 °C for 4 min, followed by 30 cycles of 94 °C for 5 seg and 54 °C for 10 seg, with a final extension step at 72 °C for 5 min [3].

2.4. Virulence Factors Genes. The pathogenicity of *E. coli* is associated with the presence of virulence factors that can be encoded by chromosomal and plasmid genes, and thus 19 genes encoding virulence factors were investigated. The genes selected were specific for hemolysins (*hly*A and *hly*F), cytotoxic necrotizing factors (*cnf*1 and *cnf*2), colicin V (*cva*C), aerobactin (*iut*A), yersiniabactin (*fyu*A), salmochelin (*iro*N), P-fimbriae (*pap*C and *pap*G), S-fimbrial adhesin (*sfa*A and *sfa*S), afimbrial adhesin (*afa*), serum resistance (*iss* and *tra*T), brain microvascular endothelium invasion (*ibe*l0), K1 capsule (*kps*I and K1), and ompT outer membrane protein (*omp*T) [8–10]. This PCR contained 1.25 U Taq DNA polymerase (Invitrogen) in 1x PCR buffer (Invitrogen), 0.2 mM of each dNTP, 2.5 mM MgCl₂, and 1 μM of each primer (Table 1). The program of PCR consisted of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 10 min.

2.5. Plasmids Profile. To analyze the plasmid profile, the plasmids of wild strains and the plasmids R27 (110 MDa), JPN11 (66 MDa), PSA (23 MD), and pRK (13, 2 MDa) used as markers of molecular mass were extracted by alkaline lysis [11]; the molecular weight of the plasmids was measured (LabImage 1D software) and the ability to transfer was determined.

The strains that harbored plasmids were chosen for mating experiments. The strains were grown in LB (Luria Bertani Broth) until the exponential phase. 1.2 mL of this culture was transferred to a flask containing 0.4 mL of the recipient culture in the stationary phase, *E. coli* KI2-711 [12]. The mixture was incubated at 37 °C for 3 hours. Transconjugants resistant to drugs were selected on MacConkey agar containing inhibitory concentrations of nalidixic acid (resistance present in *E. coli* KI2-711), tetracycline, ampicillin, or kanamycin. The colonies grown on each selective plate were tested for the presence of virulence genes and pathogenicity islands, according to the virulence pattern of the donor strain. The resistance profile, necessary for this test, is shown in Table 2.

3. Results and Discussion

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is one of the leading causes of bloodstream infections (BSI) worldwide. In Brazil, it was observed a high mortality associated with BSI [13]. Despite the importance of *E. coli* bloodstream infections due to their high morbidity and mortality, the pathogenesis is not well known [14, 15] and not studied enough in South America [4, 5].

In this study we screened 20 strains from bacteremia from newborns (10%), children aged between 6 months and 14 years (20%), and adults (70%) and from two Brazilian teaching hospitals for the determination of phylogenetic group, pathogenicity islands (PAI) associated sequences, and important virulence factors responsible for extraintestinal pathogenesis. The results were compared with those obtained with isolates from the stools of healthy humans. The relationship between the presence of PAIs, virulence genes, and the phylogenetic group was analyzed.

Among the 20 isolates from the hemocultures tested, 70% of the isolates displayed PAI associated sequences (total of 22 islands), while in the commensal strains 52.94% of them displayed PAI (total of 45 islands). In agreement with previously published data [7, 16, 17], PAI IV_{536} was the most prevalent in both groups, following the PAI I_{CFT073} and PAI II_{CFT073}, as shown in Tables 3 and 4. PAI IV_{536} has been described by many researchers as a stable island, and it is one of the most commonly found PAIs in enterobacteria [7, 18].

Our results show that the islands present in UPEC, although poorly researched in septicaemic strains, are also found in *E. coli* isolated from hemocultures. This similarity can be associated with the fact that the urinary tract infection is one of the most common infections and bacteraemia is often a complication of this infection. But there are other ways for the presence of bacteria in the blood, such as meningitis and polymicrobial intra-abdominal infections. The presence of bacteria in the bloodstream suggests the ability of these pathogens to survive in an environment with scarce free iron and to resist the bactericidal activity in the blood [4].

It is not clear yet if all *E. coli* from the intestinal tract of healthy people can be considered commensals, as some isolates showed up to five pathogenicity islands. Already it has been reported that ExPEC can asymptptomatically colonize the intestinal tract [7].

In this study, genes related to toxin and hemolysin production were researched and included *cnf*2, *cnf*1, *hly*A, *hly*F, and *cva*C. The *hly*A gene is frequently detected in ExPEC and it was the most prevalent gene in our strains of hemoculture (30%) [19, 20]. *Escherichia coli* hemolysin (*hly*A) is a pore-forming bacterial exotoxin that may contribute to
Table 1: Primers for detection of PAI markers, phylogenetic analysis, virulence genes, and their respective virulence factors.

| Genes | Sequence (5' to 3') | Size of product (bp) | Virulence factors | Reference |
|-------|----------------------|----------------------|-------------------|-----------|
| PAII<sub>336</sub> | TAA TGC CCG AGA TTC ATT GTC AGG ATT TTT CTC AGG GCT' TT | 1.800 | α-Haemolysin, CS12 fimbriae, and F17-like fimbrial adhesin | Sabaté et al., 2006 [7] |
| PAIII<sub>336</sub> | CAT GTC CAA AGC TCG AGC C CTA CGT CAG GCT GGC TTT G | 1.000 | α-Haemolysin and P-related fimbriae | Sabaté et al., 2006 [7] |
| PAIV<sub>336</sub> | AAG GAT TCG CGT TTA CCG GAC TCG TCG GGC AGC TGT TCT TCT | 300 | Yersiniabactin siderophore system | Sabaté et al., 2006 [7] |
| PAII<sub>CFT073</sub> | GGA CAT CCT GTT ACA GCG CGC A TCG CCA CCA ATC ACA GC GAA C | 930 | α-Haemolysin, P-fimbriae, and aerobactin | Sabaté et al., 2006 [7] |
| PAII<sub>CFT073</sub> | ATG GAT GGT GAA TGG CGC AGC AGC ATG TGG ATC TGC | 400 | P-fimbriae and iron-regulated genes | Sabaté et al., 2006 [7] |
| PAI<sub>96</sub> | TCG TGC TCA GGT CCG GAA TTT TGG CAT CCC ACA TTA TCG | 400 | α-Haemolysin and P-fimbriae | Sabaté et al., 2006 [7] |
| PAI<sub>96</sub> | GGA TCC ATG AAA ACA TGG TTA ATG GG GAT ATT TTT GTC GCC ATT GTT TAC C | 2.300 | α-Haemolysin, Prs-fimbriae, and cytotoxic necrotizing factor 1 | Sabaté et al., 2006 [7] |
| chuA | GAC GAA CCA AGC GTC AGG AT TGC CGG CAG TAC CAA AGA CA | 279 | Hemetransport in enterohemorrhagic O157:H7 E. coli | Clermont et al., 2000 [3] |
| yjaA | TGA AGT GTC AGG AGA CGC TG ATG GAG AAT GCG TTC CTC TCA AAC | 211 | Protein of function unknown | Clermont et al., 2000 [3] |
| TSPE4.C2 | GAG TAA TGT CGG GCC ATT CA CGC GCC AAC AAA GTA TTA CG | 152 | Putative DNA fragment (TSPE4.C2) in E. coli | Clermont et al., 2000 [3] |
| kpsII | GCC CAT TTT CTT ATG ATG TTG CAT CCC GAC GAT AAG CAT GAG CA | 272 | Capsule synthesis K1 e K5 | Johnson and Stell, 2000 [8] |
| K1 | TAG CAA AGC TTT TAT TTG TGC CAT CCA GAC GAT AAG CAT GAG CA | 156 | Capsule K1 | Johnson and Stell, 2000 [8] |
| cvaC | CAC ACA CAA AGC GGA GCT GTT CTG CCC GCA GCA TAG TTT TCC TAT | 680 | Colicin V | Johnson and Stell, 2000 [8] |
| iutA | GCC TGG ACA TCA TGG GAA ATG GCT CTG G CGT CGG GAA CGG GTA GAA TCG | 302 | Aerobactin siderophore receptor | Johnson et al., 2008 [9] |
| fyuA | TGA TTA ACC CCG CGA CGG AA CGC AGT AGG CAC GAT CTT GTA | 880 | Yersiniabactin | Johnson and Stell, 2000 [8] |
| papC | GAC GGC TGG ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GCA GGG TGT GGC | 328 | P fimbriae | Le Bouguène et al., 1992 [10] |
| papG | CTG TAA TTA CGG AAG TGA TTT CTG ACT ATC CGG CTC CGG ATA AAC CAT | 1070 | P fimbriae | Johnson and Stell, 2000 [8] |
| Genes | Sequence (5' to 3') | Size of product (bp) | Virulence factors | Reference |
|-------|---------------------|---------------------|------------------|-----------|
| sfaA  | CTC CGG AGA ACT GGG TGC ATC TTA C GGC AGG AGT AAT TAC AAA CCT GGC A | 410 | Sfa fimbriae | Le Bouguéne et al., 1992 [10] |
| sfaS  | GTG GAT ACG ACG ATT ACT GTG CGG CCA GCA TTC CCT GTA TTC | 240 | Sfa fimbriae | Johnson and Stell, 2000 [8] |
| Afa   | GCC AGA GGG CCG GCA ACA GGC CCC GTA ACG CGC CAG CAT CTC | 559 | M fimbriae | Johnson and Stell, 2000 [8] |
| ibe10 | AGG CAG GTG TGC GCC GCG TAC TGG TGC TCC GGC AAA CCA TGC | 170 | Invasion of brain endothelium | Johnson and Stell, 2000 [8] |
| hlyA  | AAC AAG GAT AAG CAC TGT TCT GGC ACC ATA TAA GCG GTC ATT CCC GT C | 1177 | Hemolysin | Johnson and Stell, 2000 [8] |
| cnf1  | AGG ATG GAG TTT CCT ATG CAG GAG CAT TCA GAG TCC TGC CCT CAT TAT T | 498 | Cytotoxic necrotizing factor 1 | Johnson and Stell, 2000 [8] |
| cnf2  | AAT CTA ATT AAA GAG AAC CAT GCT TTT TAT ATC TA | 543 | Cytotoxic necrotizing factor 2 | Blanco et al., 1996 [34] |
| traT  | GGT GTG GTG CGA TGA GCA CAG CAC GTG TCA GCC ATC CCT GAG | 290 | Serum resistance | Johnson and Stell, 2000 [8] |
| iroN  | AAT CCG GCA AAG AGA CGA ACC GCC T GTT CGG GCA ACC CCT TCT TTT ACT TT | 553 | Salmochelin siderophore receptor | Johnson et al., 2008 [9] |
| ompT  | TCA TCC CGG AAG CCT CCC TCA CTA T TAG CTT TGG CAC TTT CCT TCT ATA C | 496 | Episomal outer membrane protease | Johnson et al., 2008 [9] |
| hlyF  | GCC CAC AGT GGT TTA GGG TGC TTA GC GCC GTT TTA GGC ATT CCG ATA CTC AG | 450 | Putative avian hemolysin | Johnson et al., 2008 [9] |
| Iss   | CAG CAA CCC GAA CCA CTT GAT G AGC ATT GCC AGA GCG GCA GAA | 323 | Episomal increased serum survival | Johnson et al., 2008 [9] |
Table 2: Plasmid and resistance profile of strains from hemocultures.

| Strains | Plasmids          | Molecular size of the plasmids (MDa) | Resistance profile                  |
|---------|-------------------|-------------------------------------|------------------------------------|
| 1       | p1a               | 78                                  | ApCbCfCmKnTr                        |
| 2       | p2a, b, c, d      | 113, 112, 86, 82                    | ApCbSmFoKnTr                        |
| 3       | p3a, b, c, d, e   | 112, 99, 78, 58, 29                 | ApCbCfSmFoGnKnSiSuTbTcTr            |
| 4       | p4a               | 59                                  | ApCfFoTc                            |
| 5       | p5a, b            | 99, 71                              | ApCbCfSmFoKnSuTcTr                  |
| 6       | p6a               | 112                                 | ApCbCfSmFoGnKnSiTcTr                |
| 7       | p7a               | 99                                  | ApCbSmFoSu                          |
| 8       | NP                | —                                   | ApFo                               |
| 9       | NP                | —                                   | ApFo                               |
| 10      | p10a              | 82                                  | ApCmFoTc                            |
| 11      | p11a              | 68                                  | ApCbCmSmFo                          |
| 12      | NP                | —                                   | Ap                                  |
| 13      | p13a, b, c, d, e  | 87, 68, 59, 50, 29                  | ApCbCfSmFoGnKnSuTbTcTr              |
| 14      | p14a, b           | 62, 44                              | ApCbSm                               |
| 15      | NP                | —                                   | Ap                                  |
| 16      | NP                | —                                   | Ap                                  |
| 17      | p17a              | 87                                  | ApCbCfSmKnTcTr                       |
| 18      | p18a              | 62                                  | Ap                                  |
| 19      | p19a              | 92                                  | ApCbCmSmKnSuTcTr                     |
| 20      | NP                | —                                   | Ap                                  |

NP: no plasmid; Ap: ampicillin; Cb: carbenicillin; Cf: cephalothin; Cm: chloramphenicol; Sm: streptomycin; Fo: fosfomycin; Gn: gentamicin; Kn: kanamycin; Si: sisomicin; Su: sulphonamide; Tb: tobramycin; Tc: tetracycline; Tr: trimethoprim.

the virulence of bacteria during bloodstream infection and sepsis [20, 21]. The PAIs I536, II536, I_CFT073, I96, and II96 harbor a copy of hlyABCD system encoding α-hemolysin, and in our study, six strains harbor hlyA gene and three also had these PAIs (Table 3). Commensal strains showed a large prevalence of the hlyA gene too, with 52.94% of the strains, and did not show a good correlation with the PAIs, since only 3 of 27 isolates that had the hlyA had the respective PAI. Of the virulence genes encoding adhesins (papC, papG, sfaA, sfaS, and afa), papC and papG were the most prevalent in the strains of hemoculture. These two genes were present in 30% of our strains and were always found together in the isolates, including in commensal strains. These genes are part of the mannose-resistant P-fimbriae operon and have been associated with E. coli isolated from bacteremia [22, 23]. The PAIs I96, I_CFT073, and II_CFT073 harbor genes encoding P-fimbriae. Of the six strains of hemocultures containing the genes papC and papG, three contained corresponding PAIs (Table 3). Meanwhile, all the commensal strains containing the papC and papG genes also have PAI I_CFT073, showing a good correlation between them.

Siderophore production is important for bacterial survival in the bloodstream. The aerobactin siderophore system is an important virulence factor that contributes to bacterial growth in host tissues and fluids where iron availability is limited [24, 25]. The aerobactin receptor (IutA) is commonly associated with extraintestinal E. coli and those isolated from bacteremia [22, 26]. In the present study, iutA was the most commonly found virulence gene, present in 65% of the isolates tested (Table 3), different from commensal which did not show this gene. PAI I_CFT073 contains aerobactin genes, and 5 of the 13 strains containing the iutA gene also contained sequences similar to PAI I_CFT073. Genes corresponding to other iron uptake systems were also identified: iroN, encoding the salmochelin receptor, was present in 55% of isolates; and fyuA, encoding the yersiniabactin receptor [24, 25], was present in 45% of isolates. PAI IV536 contains yersiniabactin encoding genes, and 8 of the 9 isolates exhibiting the fyuA gene contained PAI IV536 related sequences. Thus, there is a good correlation between the presence of this island and the genes encoding yersiniabactin. There was a good correlation between the commensal strains too and of the 24 isolates that contained the fyuA gene, 22 isolates had also PAI IV536.

Of the genes that confer serum resistance (kspsII, K1, traT, and iss), traT, which encodes an outer membrane lipoprotein that contributes to serum resistance [27] was detected in 50% of the isolates.

The invasion determinant encoded by the ibe10 gene was present in 10% of the isolates, whereas the ompT gene, encoding an outer membrane protease, was present in 15% of the isolates.

These results demonstrate that the virulence of septicaemic E. coli is not summarized by the presence of a single virulence factor, since each step in the infection process can be mediated by different virulence factors [14, 28], but the expression of the combination of virulence factors together with the
Table 3: Study of pathogenicity islands, phylogenetic classification, and virulence genes in 20 ExPEC strains from human hemocultures.

| Strains | PC     | Pathogenicity islands | Virulence genes |
|---------|--------|-----------------------|-----------------|
|         | IV536  | LCF073                |                 |
|         | LCF073 |                      |                 |
| 1       | B2     | +                     | +               |
| 2       | B2     | +                     | +               |
| 3       | B2     | +                     | +               |
| 4       | B2     | +                     | +               |
| 5       | A      | +                     | +               |
| 6       | A      | +                     | +               |
| 7       | B2     | +                     | +               |
| 8       | B2     | +                     | +               |
| 9       | B2     | +                     | +               |
| 10      | B1     | +                     | +               |
| 11      | A      | +                     | +               |
| 12      | B1     | +                     | +               |
| 13      | A      | +                     | +               |
| 14      | A      | +                     | +               |
| 15      | A      | +                     | +               |
| 16      | B2     | +                     | +               |
| 17      | D      | +                     | +               |
| 18      | B1     | +                     | +               |
| 19      | D      | +                     | +               |
| 20      | B1     | +                     | +               |

PC: phylogenetic classification; pathogenicity islands: PAI IV536, PAI ICF073, PAI II CF073, and PAI I36; adhesions: papC, papG, sfaA, sfaS, and afa; invasion: ibe10; toxins: cnf2, cnf1, hlyA, hlyF, and cvaC; iron uptake systems: fyuA, iutA, and iroN; serum resistance: kps II, K1, traT, and iss; proteases: ompT.
imbalance between immune defenses and characteristics of the environment determines a multifactorial outcome [29].

Several studies have demonstrated that isolates belonging to phylogenetic group B2 are more commonly extraintestinal pathogenic strains [3, 15, 22]. Our results demonstrated that group B2 (40%) was the most common group among the E. coli strains from hemoculture (Table 4). Strains belonging to group B2 also had the most PAI associated sequences, and 15 of the total 22 PAIs identified were present in this group. In contrast, in the commensal strain the most prevalent group was A (72.54%) and this group had a greater number of PAIs (86.95%) (Table 4). Moreover, seven of eight strains of group B2 had PAIs, against only one of six strains of group A. However, despite reports in the literature that virulence is associated with the presence of multiple virulence factors, the study of genetic factors is important to better understand these important pathogens.

Similarly, the virulence genes are not only associated with the PAIs, because some ExPEC also harbor virulence genes in plasmids [31]. Furthermore, PAI are located in regions of high genetic mobility, which show elements that allow recombination and, consequently, PAI rearrangement, deletion, and/or acquisition of foreign DNA [7].

Given the importance of plasmids as mobile elements in the horizontal gene transfer, the plasmid profile was investigated. In this study, 14 strains showed these plasmids, which ranged from 1 to 5 plasmids per strain, and three plasmids transferred to another E. coli by conjugation, appearing to be conjugative plasmids. The transconjugants (sample 5, 17, and 19) received genes iutA, ompT, hlyF, iroN, traT, and iss, showing the possible presence of these virulence genes in plasmids (Table 5). Thus, the presence of transferable plasmids in E. coli isolated from hemocultures can contribute to the horizontal transfer of virulence genes to nonpathogenic isolates and interestingly the conjugative plasmids 5.2, from strain 5, had iss, iroN, ompT, and hlyF genes, whose genes are generally present in typical APEC plasmids in a conserved plasmidic (CVP) region [32]. These findings support the idea that APEC or E. coli from commercial chicken carcasses has a potential zoonotic risk, as well as serves as a reservoir for virulence genes for ExPEC strains [9, 28, 33].

As ExPEC pathogenicity is due to genetic features such as virulence genes, pathogenicity islands, and plasmids associated with the virulence, the study of genetic factors is important to better understand these important pathogens.

Table 4: Distribution of pathogenicity islands (PAI) according to phylogenetic classification (PC), among ExPEC and commensal strains.

| PC             | PAI I\(_{536}\) | PAI II\(_{536}\) | PAI IV\(_{536}\) | PAI I\(_{96}\) | PAI I\(_{CFT073}\) | PAI II\(_{CFT073}\) | Total of PAIs | Total of strains |
|----------------|----------------|-----------------|-----------------|-------------|-----------------|-----------------|---------------|----------------|
Thus, for the screening and even prevention of bloodstream diseases caused by ExPECs, further research should be conducted on the genetic features associated with the virulence of these pathogens. Although in stool samples of healthy people the presence of commensal strains is common, our results showed that the intestinal microbiota may harbor *E. coli* of phylogenetic group B2. Also *E. coli* with PAs and virulence genes suggest that the intestinal microbiota may act as a reservoir of ExPEC with virulence genetic factors present at the *E. coli* from blood stream infection.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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