Comparative Genomics of Three Hybrid-Pathogen Multidrug-Resistant Escherichia coli Strains Isolated from Healthy Donors’ Feces

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Abstract: The present study shows the genomic characterization of three pathogenic Escherichia coli hybrid strains. All strains were previously characterized as diarrheagenic pathotypes (DEC), obtained from feces. The three sequenced strains have genes that encode adhesins (fimH and iha) and iron uptake systems (iucC and iutA). Antibiotic resistance genes were also found for fluoroquinolone and aminoglycoside families in the three strains. The presence of genomic islands (GIs) in the sequenced study strains presented 100% identity (Ec-25.2) and 99% identity (Ec-36.1) with previously reported Extraintestinal Pathogenic E. coli (ExPEC) strains. The Ec-36.4 strain shared a 99% identity with GI from the Enterotoxigenic E. coli (ETEC) pathotype of the diarrheagenic E. coli strain. Ec-25.2 belongs to ST69 and harbors a FimH27 variant, while Ec-36.1 and Ec-36.4 belong to ST4238 and share a FimH54 variant. Four incompatibility groups associated with conjugative plasmids were identified (IncFIB, IncF11, IncI1, and IncB/O/K/Z), as well as Insertion Sequences and MITEs elements.

Keywords: Escherichia coli; hybrid pathogens; diarrheagenic pathotype; genome; healthy donors

1. Introduction

Escherichia coli is a Gram-negative rod from Enterobacterales and one of the commensal gut species. However, several clones have acquired different virulence factors (VF) that enhance their abilities to trigger a wide spectrum of diseases, such as diarrheal illness or extraintestinal ones (such as urinary tract infections, neonatal meningitis, and bloodstream infections) [1].

The E. coli pathobionts associated with gut infections are classically known as diarrheagenic pathotypes (DEC) [2]. Within DEC, there are six well-known pathotypes: enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteraggregative (EAEC), enteroinvasive (EIEC), and diffusely adherent E. coli (DAEC) [3]. The strains belonging to these pathotypes are classified by their interaction with the enterocyte, their epidemiology, serology, and virulence properties [4].

On the other hand, the extraintestinal infections associated with E. coli are caused by extraintestinal pathogenic E. coli strains (ExPEC). The diseases associated with these strains are sepsis and bacteremia (caused by sepsis-associated E. coli, SEPEC), neonatal...
meningitis (caused by NMEC), and urinary tract infections (caused by uropathogenic *E. coli*, UPEC) [5].

In recent years, various *E. coli* hybrid pathotypes have been described [6]: the “hetero-pathogenic” strains are those that harbor VF characteristics of two or more DEC pathotypes (properly enteropathogens), and the “hybrid-pathogenic” strains are those that show VF from DEC and from ExPEC also [6].

The conflict arises when these virulence factors are common in different *E. coli* pathogenic strains, which can cause a severe disease expanding their sites of colonization, along with other adaptative features. They can also harbor similar resistance genes present in mobile genetic elements such as plasmids, facilitating their spread and making the disease more difficult to treat. Many studies related to the occurrence of these hybrids have been reported. The most reported cases of “hybrid-pathogenic” have been ExPEC/EAEC and ExPEC/EPEC, because it is proposed that the homology between the different genes coding for the fimbriae, which allow adhesion to the epithelium [6–9], as well as the presence of different toxins of the ETEC pathotype in samples of patients with UTI (ExPEC) [10], contributes to these associations.

The best-documented example of “hetero-pathogenic” was a severe acute gastroenteritis outbreak (EAEC) and hemolytic uremic syndrome (EHEC) [11]. Another common pathotype reported more recently in clinical samples from countries such as Sweden and South Korea has been EHEC/ETEC [12,13].

Previously, our research group reported the presence of hetero-pathogenic *E. coli* strains isolated from donors’ feces. The classification was based on the presence of DEC genetic determinants [14]. In the present work, we report the comparative genomics analysis of three hetero-pathogenic genomes—one of them being a triple hybrid.

2. Materials and Methods

2.1. Strains and Genome Sequencing

From a collection of 40 *E. coli* strains isolated from the feces of healthy donors obtained in Sonora, Mexico, we have chosen to sequence three previously identified strains using PCR. These strains are characterized by the presence of the genes *bfpA* (bundle-forming Pilus), *LT* (heat-labile toxin), and *daaE* (fimbrial protein). They are classified as hetero-pathogenic strains, specifically Ec-25.2 (aEPEC/ETEC), Ec-36.1 (aEPEC/ETEC/DAEC), and Ec-36.4 (aEPEC/ETEC). Notably, Ec-36.1 and Ec-36.4 are clones obtained from the same donor sample [14]. The strains were inoculated in 5 mL of Luria–Bertani (LB) broth for genomic DNA extraction and grown overnight at 37 °C. Genomic DNA was extracted with the Wizard® Genomic DNA extraction kit (Promega Corporation, Madison, WI, USA) following the manufacturer’s directions. The DNA concentration was determined with a Quantus® fluorometer (Promega Corporation, Madison, WI, USA) and the QuantiFluor® dsDNA System (Promega Corporation, USA). The total genomic DNA was sequenced on an Illumina NovaSeq 6000 sequencer (Iowa City, IA, USA) producing 2 × 151 bp paired end reads with an 80× depth at SeqCenter (Pittsburgh, PA, USA) [15].

2.2. Assembly and Annotation

Assemblies of the draft genomes were completed using SPAdes (v3.15.4) [16] and annotated using RAST [17] and the NCBI Prokaryotic Genome Annotation Pipeline [18]. All the open reading frames were blasted against *E. coli* ETEC H10407 (accession number FN649414) as the reference genome and selected based on a relatedness prediction by NCBI BLAST; this is the pathotype shared by the three sequenced strains. The assembly characteristics are summarized in Supplementary Materials Table S1.
2.3. Bioinformatic Analysis

The genomic islands (GI) in the assemblies were determined with the IslandViewer4 tool [19], using three independent methods for island prediction (IslandPick, IslandPath-DIMOB, and SIGI-HMM), and *E. coli* ETEC H10407 was used as control strain. Then, the predicted GIs were searched in BLAST for previously reported genomic islands. The Proksee online tool was used to generate circular maps and sequence comparisons through average nucleotide identity (ANI) (accessed 7 May 2024 at https://proksee.ca/) [20,21].

Several services of the Center for Genomic Epidemiology were used with default settings unless otherwise noted: SeroTypeFinder [22] (for serotype prediction); *fimH* variants were determined by database matching in FimTyper [23]; the presence of antimicrobial resistance genes was analyzed by ResFinder [24–28] and the CARD database [29]; and likewise, the virulence genes (VirulenceFinder) [28,30,31]. Mobile genetic elements, such as plasmids and insertion sequences, were identified with MobileElementFinder [30] and PlasmidFinder [31]. The multiple locus sequence typing was determined with MLST 2.0 [32–37]. Finally, to infer the phylogenetic relationship, we completed the calling and filtering of single nucleotide polymorphisms (SNPs) with CSI Phylogeny (v1.4) using default settings [38] and the iTol [39] platform for generating the images. Different genomes were used to infer the phylogenetic relationship, including some belonging to DEC as well as ExPEC pathotypes (Supplementary Materials Table S2).

3. Results and Discussion

3.1. General Features of the Hybrid Strains

The Ec-25.2 strain belongs to phylogroup A and Ec-36.1 and Ec-36.4 to phylogroup B2. The genomic features are summarized in Supplementary Materials Table S1. The Ec-25.2 genome presented a 100% identity with the genomes UMN026 and 118UI, which are classified as ExPEC and were recovered from urine samples (accession number CU928163.2 and CP032515.1, respectively). Genomic islands were predicted using BLAST against publicly available genomes of *E. coli*. Most of the genomic islands found for the three sequenced strains correspond to genomic islands of phage origin and mobile genetic elements such as plasmids and insertion sequences (Figure 1).

In the same way, the Ec-36.1 assembly showed a 99.97% identity with the genome KE58 (accession number CP141075.1) recovered from a urine sample in Dallas, Texas of a female patient with recurrent urinary tract infections. This finding is interesting because Sonora (where the samples were isolated) has a border with the United States; these relationships in the identity of the genomes between strains may be due to the high migration that exists, causing patients who are carriers of *E. coli* to transmit the bacteria in different regions. Another strain with 99.97% identity was ETEC6329F (accession number CP122609.1), documented as ETEC, similar to our isolate. On the other hand, the Ec-36.4 genome kept a 99.97% identity with 184/2aE (accession number CP072858.1), a strain isolated in Brazil from the feces of a traveler returning from sub-Saharan Africa (Supplementary Materials Figure S1).

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The in silico sequence-type analysis showed that Ec25.2 belonged to ST69; this ST has been previously reported in clinical strains associated with urinary and blood infections [40]. However, Matsui et al., 2020 showed a wide distribution of ST69 among strains recovered from the feces of healthy donors and patients with urinary tract infections [41]. On the other hand, both Ec-36.1 and Ec-36.4 belonged to ST4238, first reported in 2014 in a strain isolated from a child with diarrhea and identified as ETEC in Colombia [42]. Interestingly, when the in silico serotype was performed, we observed that the three genomes were serotyped as H4, similar to the ETEC Colombian strain, suggesting a regional distribution of *E. coli* strains belonging to ST4238 and associated with ETEC in America (Supplementary Materials Figure S2).
Figure 1. Map of the genomic islands (GIs) found in the analyzed genomes. GIs found in the sequenced strains (Supplementary Materials Table S1). Ec-25.2 has more GIs of phage origin and does not show the GI46 corresponding to mobile genetic elements compared to the other two strains (Ec-36.1 and 36.4). The strains Ec-36.1 and Ec-36.4 share mainly GIs of phage origin and mobile genetic elements. GIs are highlighted based on their origin or function: Phages in blue; mobile genetic elements, green; virulence GIs, pink; related to adhesion as fimbriae, orange; toxin–antitoxin systems, yellow; antibiotic resistance GIs.

3.2. Resistance and Virulence Features

Ec-25.2 harbors $fimH27$, which has been described in isolates from human urine and blood [43] (Table 1). In a previous study, Barrios-Villa et al., in 2020, reported the $fimH27$ allele in ExPEC strains belonging to the AIEC pathotype, as well as in EIEC and K12 genomes [44]. The $fimH54$ allele found in Ec-36.1 and Ec-36.4 has been previously reported in strains isolated from urine samples and from vegetables in Portugal [45,46]. The $fimH54$
Table 1. Resistance genes, virulence, and MGEs in the genomes of sequenced hybrid strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ec-25.2</th>
<th>Ec-36.1</th>
<th>Ec-36.4</th>
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<tbody>
<tr>
<td>fmm1 variant</td>
<td>fmm1D7</td>
<td>fmm1H54</td>
<td>fmm1H54</td>
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<tr>
<td>Resistance genes</td>
<td></td>
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<tr>
<td>Efflux pumps</td>
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<tr>
<td>Disinfectant</td>
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<tr>
<td>MGE</td>
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<tr>
<td>Virulence genes</td>
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</tbody>
</table>

MGE: Mobile genetic element; chua: Outer membrane hemin receptor; cia: Colicin; eilA: Salmonella HIA homolog; fmm1: Type 1 fimbriae; fyuA: Yersiniaibactin siderophore receptor; gad: Glutamate decarboxylase; hblE: Avian E. coli haemolysin; ireA: Siderophore receptor; iha: Adherence protein; irp2: High molecular weight protein 2 non-ribosomal peptide synthetase; luxC: Aerobactine synthetase; luxD: Aerobactine receptor; kpsMII: Capsule polysaccharide export inner-membrane protein; kpsMII: Polysaccharide acid transport; Group 2 capsule; lpfA: Long polar fimbriae; ompF: Outer membrane protease (protein protease 7); papC: Major pilin subunit F6: papC: Outer membrane usher P fimbriae; sat: Serine protease autotransporters of enterobacteriaceae (SPATE); sha: Plasmid-encoded enteroxin; shib: Homologs of the Shigella flexneri SH-2 Pathogenicity island gene shaA; sigA: Serine protease autotransporters of enterobacteriaceae (SPATE); iutA: Iron transport protein; terC: Tellurium ion resistance protein; traF: Positive regulator of conjugal transfer operon; shi: Outer membrane protein complement resistance; shiB: Outer membrane usher P fimbriae; shiC: Outer membrane usher P fimbriae; shiD: Outer membrane usher P fimbriae; sigA: Repressor; sigB: AraC-family regulator that promotes mdfl expression to confer multidrug resistance; mdfl: Membrane fusion protein of the mdfl multidrug efflux complex; kdpD: Two-component regulatory system in Escherichia coli. role in potassium transport and homeostasis; cpxA: Membrane-localized sensor kinase that is activated by envelope stress; marA: Transcriptional activator of genes involved in the multiple antibiotic resistance; msbA: Member of the MDR-ABC transporter group, transports lipids; msrA: Methionine sulfoxide reductase A; mphC: Macrolide phosphotransferases; NF: Not found.
The Ec-25.2 genome showed the presence of genetic resistance determinants to fluoroquinolone, aminoglycosides, sulfonamides, carbapenems, and cephalosporines. Ec-36.1 and Ec-36.4 genomes presented genes associated with resistance to fluoroquinolones, macrolides, aminoglycosides, cephalosporins, tetracyclines, nitroimidazole, and phenicol; it is important to note that both strains were recovered from the same sample. It was found that all three genomes show mechanisms of antibiotic resistance, including reduced antibiotic permeability, altered antibiotic fate, and a suggested antibiotic efflux pump which is also involved in other functions such as detoxification and permeability modification (Table 1).

On the other hand, the Virulence Finder tool revealed the presence of genes involved in iron uptake, fimbriae, non-fimbrial adhesins, and toxins involved in E. coli pathogenicity. The common virulence genes for the three strains were fimH (Type 1 fimbriae), iucC (aerobactin synthetase), iutA (ferric aerobactin receptor), iha (adherence protein), traT (outer membrane protein involved in complement resistance) and hlyE (Avian E. coli haemolysin), but also presented homologous genes present in other genera such as eilA (hilA homolog from Salmonella) and shiB (homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA), which can represent an important horizontal gene transfer mechanism among enterobacteria coexisting in the host, causing more severe signs and symptoms, complicating the disease (Table 1).

3.3. Mobilizable Genetic Elements (MGEs)

Based on replicon typing, Plasmid Finder showed four plasmid incompatibility groups in the Ec-25.2 genome [Col(pHAD28), IncFIB, IncF11, IncI1-l]. On the other hand, plasmids with Col(pHAD28) have been previously reported in Salmonella strains obtained from dairy farm samples in Mexico, as well as from poultry in Nigeria [50,51]. These plasmids have been reported in strains of Klebsiella pneumoniae, Cronobacter sakazakii, and E. coli carrying resistance genes to aminoglycosides [52,53]. On the other hand, the plasmids IncFIB, IncF11, and IncI1-l are the most common in E. coli; these plasmids are conjugative and usually harbor resistance and virulence genes [54]. In addition, it has been reported that plasmid IncB/O/K/Z might be found in strains of both clinical and food origin in the Enterobacteriaceae family, as reported by Balbuena-Alonso et al., 2022, and carries resistance genes to azithromycin in strains of K. pneumoniae, which agrees with our results, suggesting that this plasmid is distributed within the Enterobacteriaceae family [55,56].

Other MGEs, such as transposons, integrons, and insertion sequences (IS), can collect or move genes within the host genome and jump across genomes, molding and coevolving with chromosomes [57]. IS are small mobile elements (~0.7 to ~2.5 kbp) and are found in most bacterial genomes, they are the simplest type of bacterial transposable element and generally contain a gene necessary for its transposition. Insertions inside or between genes have the potential to create a mutation, alter promoter function, also create hotspots for genome recombination events, or even induce positive regulation of neighboring genes [58]. In our study, we found IS629 inside the Ec-25.2 genome, a member of the IS3 family whose mobility mechanism is believed to be a replicative transposition (“copy and paste”). This IS contains genes associated with VF as adhesins and fimbriae (iha, pap,C, and papA). IS629 has been reported in verotoxin-producing E. coli (VTEC) serotype O157:H7 and is considered the main cause of severe gastrointestinal infections [59]. Additionally, Ec-25.2 also harbors ISKpn26, with the yehABCD fimbrial operon, this IS has been reported in K. pneumoniae and is mostly associated with IncFII and IncFIB plasmids [60]. ISEc45 (VF as iucC, sat, and iutA) and ISEc46 (VF as irp2 and fyuA) were also found. These findings show that despite being commensal bacteria, they have an important virulence and resistance background that makes them potentially pathogenic.

The ISEc18 belongs to the IS481 family, found in the genomes Ec-36.1 and Ec-36.4, and has been reported in plasmids encoding for the LT (heat-labile enterotoxin) and ST (heat-stable enterotoxin) enterotoxin characteristic of the ETEC pathotype; this finding is
consistent with previous characterization of these hybrid strains [61]. In our study, the *afaD* gene (encoding for a fimbrial adhesin) was observed close to *ISEc18*.

Other mobile genetic elements found in our genomes were the miniature inverted-repeat transposable elements (MITEs). The first prokaryotic MITE was discovered in *Neisseria gonorrhoeae* and *Neisseria meningitidis* [62]. MITEs are a group of non-autonomous class II transposons abundant in eukaryotic genomes, mainly in plants, and are structurally characterized by their relatively small size (generally 50–500 bp long), high copy number, tendency to integrate into AT-rich intergenic regions of the genome, a lack of coding capacity, and are often found close to or within genes where they may affect gene expression [63–66]. It is suggested that these elements have influenced the evolution of individual genomes and genes [65]. The MITEcEc1 was found in the three genomes sequenced and this MITE has also been reported in other bacteria, such as *Salmonella* [66].

### 3.4. Phylogeny

A phylogenetic tree based on UPGMA (unweighted pair group method using arithmetic averages) was constructed according to the SNPs found for each strain, the SNPs variant calling, and phylogeny showed that the Ec-36.1 and Ec-36.4 genomes are part of a clade next to ETEC (Figure 2). This is an expected finding since these strains were characterized by Méndez-Moreno et al. as hybrid pathogens showing genetic determinants associated with ETEC [14]. The Ec25.2 genome belongs to a clade closely related to APEC (Avian Pathogenic *E. coli*), corresponding to the ExPEC pathotype, but also related to EPEC, which is one of the pathotypes with which it was previously associated (Figure 2) [14].

![Phylogenetic tree](image)

Figure 2. UPGMA SNP-based phylogenetic tree. Graphic representation of the SNPs variant calling of the genomes Ec-25.2, Ec-36.1, and Ec-36.4 and compared against those obtained from the reference genomes (Supplementary Materials Table S3). Squares at branch tips represent the *fmH* variant; colored strips indicate the ST (sequence type) to which the genome belongs; the multiple chart bar represents the number of MGEs (Mobile Genetic Elements). EPEC (enteropathogenic *E. coli* E2348/69), ETEC (enterotoxigenic *E. coli* H10407), EHEC (enterohemorrhagic *E. coli* 10942), EAEC (enteroaggregative *E. coli* SAMEA7457016), EIEC (enteroinvasive *E. coli* 53638), and DAEC (diffusely adherent *E. coli* SK1144), UPEC (uropathogenic *E. coli* CFT073), APEC (Avian Pathogenic *E. coli* 102026), AIEC (adherent-invasive *E. coli* LF82), NMEC (neonatal meningitis *E. coli* NMEC O18) and *E. coli* K12 (commensal).
Bioinformatic analysis suggested that the three analyzed genomes belong to hybrid pathotypes. The Ec-25.2 genome, previously reported as (aEPEC/DEC), includes virulence factors defining ExPEC (UPEC), as well as the presence of GI with a BLAST 100% identity from UPEC genomes. On the other hand, the phylogeny showed that genome assemblies of Ec-36.1 (aEPEC/ETEC/DAEC) and Ec-36.4 (aEPEC/ETEC) are grouped in a clade including genomes belonging to diarrheagenic pathotypes. Interestingly, BLAST analysis showed 99% identity between the genomes of Ec-36.1 and Ec-36.4 with those of strains isolated from feces classified as ETEC, in agreement with the classification by Méndez-Moreno et al. in 2022 [14]. These results suggest that these strains must be considered as heteropathogenic-hybrid E. coli.

The strains Ec-36.1 and Ec-36.4 were isolated from the same patient, which makes it logical that they share virulence and resistance characteristics, as well as the presence of markers of the diarrheagenic pathotypes aEPEC/ETEC; however, the strain Ec-36.1 has the daaE adhesin gene corresponding to the DAEC pathotype, which may have been acquired during the horizontal gene transfer.

This work contributes to understanding the genetic diversity and adaptability of hybrid-pathogenic E. coli strains. The findings highlight the potential public health risks posed by these strains, particularly in regions with high migration rates. By identifying key resistance and virulence determinants, the study underscores the necessity for continuous monitoring and development of effective treatment protocols to manage infections caused by such multidrug-resistant pathogens. Moreover, this comparative genomics approach provides a valuable framework for future research on the evolution and spread of pathogenic E. coli strains. The data generated can inform public health policies and help devise strategies to mitigate the spread of these bacteria. Overall, this report contributes significantly to the field of microbiology and epidemiology understanding the dynamics of multidrug-resistant E. coli in human populations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15030095/s1, Figure S1: Maps constructed in Proksee, showing the comparison between the genomes of the present study with reference genomes previously reported in NCBI, Figure S2: Regional distribution of E. coli Sequence Types (STs) and Serotypes in America; Table S1: General Features of Sequenced Strains of pathogenic-hybrid E. coli, Table S2: Genomes used as control for phylogenetic analysis, Table S3: Representative Genes of The Different Genomic Islands Found in the Sequenced Strains of pathogenic-hybrid E. coli (IslandViewer) [67–90].

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