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Complete sequence of pEC14_114, a highly conserved IncFIB/FIIA plasmid associated with uropathogenic *Escherichia coli* cystitis strains

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are known to cause important diseases of humans and animals, and they have been shown to carry a variety of plasmids associated with increased virulence and decreased antimicrobial susceptibility. Here, the completed DNA sequence of a human uropathogenic *E. coli* (UPEC; O6:H31 isolate) plasmid, pEC14_114, was determined. The plasmid was 114,222 bp in length and was highly similar to plasmid sequences or draft contiguous sequences from three other human cystitis-associated UPEC isolates. pEC14_114 contained 141 coding regions, including a number of genes associated with mobile genetic elements, F-type transfer, plasmid maintenance and stability, colicin immunity, and plasmid replication. This plasmid also possessed a "genetic load" region containing genes with predicted similarity to iron acquisition systems and virulence factors. The prevalence of pEC14-associated genes was determined for a collection of 1456 *E. coli* isolates, including those from food products, humans, dogs, cats, pigs, chickens, and turkeys. pEC14_114-associated genes were found significantly more often (16–35%) among human UPEC and neonatal meningitis-associated isolates than among food- and animal-source isolates (0–8%). Overall, this plasmid represents a novel IncFIB/FIIA plasmid type associated with human ExPEC belonging to the B2 phylogenetic group. The overall role of this plasmid, if any, in human ExPEC infections remains to be determined.

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains have been implicated in a variety of diseases of humans, including urinary tract infection (UTI), pneumonia, neonatal meningitis, and septicemia (Johnson, 1991). Additionally, this pathotype has been associated with important diseases of production and domestic animals, including colibacillosis in poultry, bovine septicemia and hemorrhagic pneumonia, and UTI in canine and feline hosts (Breitschwerdt et al., 2005; DebRoy et al., 2008; De Rycke et al., 1999; Handt et al., 2003; Sura et al., 2007; Smith et al., 2007). Because of the vast diversity of ExPEC, subpathotypes have been described specific to each disease type, including uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), necrotoxigenic *E. coli* (NTEC), and avian pathogenic *E. coli* (APEC), all of which are considered to be ExPEC because they carry some common virulence factors that are distinct from other diarrheagenic *E. coli* (Kaper et al., 2004; Kaper, 2005). Of the ExPEC-associated diseases, UTIs are the most commonly recognized infection. During the establishment of UTI, UPEC are thought to colonize the urogenital tract, form biofilms, and subsequently infect other urothelial cells. UPEC then have the ability to lead to recurrent or persistent UTI, or

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to disseminate to the bladder and kidneys to cause cystitis and pyelonephritis, respectively (Johnson, 1991; Johnson and Russo, 2002). They may ultimately invade the bloodstream to cause septicemia.

UPEC carry a diverse array of virulence genes enabling them to cause disease, encoding traits such as adhesins, toxins, siderophores, capsule, iron-utilization factors, surface coatings, outer membrane proteins, and lipopolysaccharides (Johnson et al., 2008; Ewers et al., 2007; Donnenberg and Welch, 2005; Johnson and Russo, 2005; Rodriguez-Siek et al., 2005). The genomes of several human UPEC have been sequenced, including those isolated from cases of uncomplicated UTI (strain IAI 39) (Touchon et al., 2009), cystitis (strains UTI89, F11, and UMN026) (Touchon et al., 2009; Chen et al., 2006), and pyelonephritis (strains CFT073 and 536) (Brzuszkiewicz et al., 2006; Welch et al., 2002). Historically, it has been postulated that UPEC virulence factors are primarily found within pathogenicity-associated islands (PAIs) on the bacterial chromosome (Dobrindt, 2005). Indeed, the archetypic UPEC genomes have been characterized by their chromosomal PAI possession (Brzuszkiewicz et al., 2006; Welch et al., 2002; Dobrindt et al., 2002; Guyer et al., 1998; Rasko et al., 2001). A role for plasmid-encoded virulence in UPEC is not well established, despite the fact that ExPEC strains are known to carry large plasmids (Johnson et al., 2007, 2008; Ewers et al., 2007; Rodriguez-Siek et al., 2005; Zhao et al., 2009; Sorsa et al., 2003). In contrast, several plasmids associated with APEC and NTEC virulence have been described, such as the ColV and ColBM plasmids of APEC and the Vir plasmid of NTEC-2 (De Rycke et al., 1999; Ewers et al., 2007; Mellata et al., 2009; Tivendale et al., 2009; Johnson et al., 2006a,b; Rodriguez-Siek et al., 2005; DebRoy and Maddox, 2001).

Recent genome sequencing efforts involving three cystitis-associated UPEC strains (Touchon et al., 2009; Chen et al., 2006; Rasko et al., 2008) identified a conserved IncFIB/FIIA plasmid as a component of their genome that includes a large number of uncharacterized coding regions. Population-based approaches have proven effective at identifying traits that are highly prevalent among ExPEC and, as such, potentially implicated in ExPEC pathogenesis (Johnson, 1991; Johnson et al., 2005, 2008; Ewers et al., 2007; Johnson and Russo, 2005). Here, we describe the completed sequence of a plasmid from a UPEC strain and utilize comparative genomics and gene prevalence in an effort to understand the conservation and distribution of this plasmid type among ExPEC populations.

2. Materials and methods

2.1. Bacterial strains and characterization

UPEC strain EC14 (O6:H31; ECRC# 89.0590), isolated from a human UTI case, was used for plasmid isolation. This strain was grown in Luria–Bertani broth medium (LB broth, Difco Laboratories, Detroit, MI) overnight at 37 °C with shaking. For determining prevalence of plasmid-associated genes among ExPEC strains, a total of 1456 ExPEC isolates were examined. These included *E. coli* isolated from UTI from humans (n = 300), dogs (n = 139), and cats (n = 90); neonatal meningitis from humans (n = 91); diarrhea in humans (n = 206); porcine diarrhea (n = 206); retail food products (n = 94); and colibacillosis from chickens (n = 330). Human UPEC were isolated from the urine of patients or animals experiencing UTI (Rodriguez-Siek et al., 2005), while those from cases of neonatal meningitis were isolated from the cerebrospinal fluid of affected patients (Johnson et al., 2002, 2008). Isolates from avian colibacillosis (APEC) were isolated from organ lesions of infected chickens (Rodriguez-Siek et al., 2005). All isolate collections were obtained from multiple hospitals, veterinary diagnostic laboratories, retail grocers, and commercial poultry operations throughout the United States and Europe.

2.2. Plasmid isolation, sequencing, assembly, and annotation

An isolated colony of UPEC strain EC14 was grown in 500 ml of LB broth overnight at 37 °C with shaking. Plasmid isolation was performed using a Qiagen Plasmid Midi Kit with procedures optimized for BACs and large plasmids (http://www.qiagen.com/) (Kado and Liu, 1981). Ten micrograms of purified plasmid DNA was sequenced at Biomedical Genomic Center at University of Minnesota using the Roche 454 FLX machine (454 Life Sciences, Branford, CT). Sequencing reads were assembled using SeqMan Pro software from DNASTAR (Lasergene, Madison, WI). PCR and bidirectional sequencing of the resulting product was used for amplifying and confirming low coverage areas. Open reading frames (ORFs) in the plasmid sequence were identified using GeneQuest from DNASTAR, followed by manual inspection. Translated ORFs were then compared to known protein sequences using BLAST (Altschul et al., 1997). Those with greater than 90% homology with database protein sequences were considered matches, while ORFs with less than 90% identity to published sequences were classified as hypothetical proteins. The G + C content of individual ORFs was analyzed using GeneQuest and insertion sequences (IS) and repetitive elements were identified using IS finder (http://-is.biotoul.fr/). A circular map was constructed using GenVision (Lasergene, Madison, WI). The completed sequence and annotation of pEC14_114 was deposited in GenBank under Accession No. GQ398086.

2.3. Gene prevalence studies

Strain EC14 was characterized for the presence of 22 virulence-associated genes associated with UPEC using multiplex PCR previously described (Johnson and Stell, 2000). The genes tested were *fimH*, *focG*, *sfaA*, *cnf1*, *fyuA*, *iroN*, *chuA*, *entC*, *iroB*, *sitA*, *marR*, *yhjX*, *emrA*, *glnA*, *traT*, *ompT*, *uspA*, *kII*, *kpsII*, *uidA*, *paI*, and *papG* allele III. Antimicrobial susceptibility testing to ten antimicrobials (ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, spectinomycin, tetracycline, trimethoprim, and sulfamethoxazole) was conducted using BBL Sensi-Disk antimicrobial susceptibility test disk (BD, Franklin Lake, NJ) using standard Kirby-Bauer disk diffusion method (Ferraro, 2002) in accordance with CLSI (formerly NCCLS).