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Uropathogenic *Escherichia coli* are less likely than paired fecal *E. coli* to have CRISPR loci

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ABSTRACT

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are short fragments of DNA that act as an adaptive immune system protecting bacteria against invasion by phages, plasmids or other forms of foreign DNA. Bacteria without a CRISPR locus may more readily adapt to environmental changes by acquiring foreign genetic material. Uropathogenic *Escherichia coli* (UPEC) live in a number of environments suggesting an ability to rapidly adapt to new environments. If UPEC are more adaptive than commensal *E. coli* we would expect that UPEC would have fewer CRISPR loci, and – if loci are present – that they would harbor fewer spacers than CRISPR loci in fecal *E. coli*. We tested this *in vivo* by comparing the number of CRISPR loci and spacers, and sensitivity to antibiotics (resistance is often obtained via plasmids) among 81 pairs of UPEC and fecal *E. coli* isolated from women with urinary tract infection. Each pair included one uropathogen and one commensal (fecal) sample from the same female patient.

Fecal isolates had more repeats ($p = 0.009$) and more unique spacers ($p < 0.0001$) at four CRISPR loci than uropathogens. By contrast, uropathogens were more likely than fecal *E. coli* to be resistant to ampicillin, cefazolin and trimethoprim/sulfamethoxazole. However, no consistent association between CRISPRs and antibiotic resistance was identified. To our knowledge, this is the first study to compare fecal *E. coli* and pathogenic *E. coli* from the same individuals, and to test the association of CRISPR loci with antibiotic resistance. Our results suggest that the absence of CRISPR loci may make UPEC more susceptible to infection by phages or plasmids and allow them to adapt more quickly to various environments.

1. Introduction

Uropathogenic *E. coli* (UPEC) is the most common cause of urinary tract infection (UTI), a condition that affects 12.6% of women and 3% of men each year in the United States (Foxman, 2010). In addition to causing ~80–90% of UTI among patients in the community and ~60% of UTI among patients treated in hospital (Foxman, 2010), as many as 10% of the population at large carry UPEC asymptomatically in their bladder or urethra (Kass, 1956). UPEC have multiple genetic origins (Chaudhuri and Henderson, 2012), and diverse virulence profiles that include adhesins, siderophores, and toxins (Dobrindt et al., 2003; Oelschlaeger et al., 2002), many of which can be obtained via horizontal gene transfer. However, UPEC virulence profiles are not sufficiently distinctive to distinguish them from commensal *E. coli* (Vigil et al., 2011; Marrs et al., 2002; Zhang and Foxman, 2003).

Commensal *E. coli* living within the human gastrointestinal tract are highly adapted to that environment (Tenaillon et al., 2010), but UPEC or UPEC-like *E. coli* live in the gastrointestinal tract, vaginal cavity and periurethral opening (Stamey, 1987) and *E. coli* containing virulence genes associated with UPEC have been isolated from sewage treatment plants and environmental waters (Anastasi et al., 2012), retail meats (Xia et al., 2011), and chickens (Bergeron et al., 2012). The ability to live in multiple environments implies a high degree of adaptability. Bacteria can acquire new genetic material via infection by virus (phage) and plasmids (Casas and Maloy, 2011), but since this is not without cost, *E. coli* have an adaptive immune system that enables sequence-specific immunity against bacteriophage and plasmids. This system is encoded by the clustered regularly interspaced short palindromic repeats (CRISPR) loci (Molloy, 2012). CRISPR and CRISPR-associated sequence (cas) were identified as an immune system against undesirable genetic elements in bacteria and archea (Horvarth and Barrangou, 2010).

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Two CRISPR-Cas systems-Ecoli (Type I–E) and Ypest (Type I–F) have been identified in *E. coli* (Makarova et al., 2011). While the type *E. coli* system has not been shown to confer immunity against phage or plasmid in the lab in wildtype K12 strains (Pougach et al., 2010; Westra et al., 2010), the Ypest system has recently been shown to confer immunity (Almendros et al., 2012).

The CRISPR region consists of several direct repeats separated by spacers (Sorek et al., 2008). The spacers between the repeats contain unique sequences used to prevent infection by specific bacteriophages or plasmids. New spacers are added following infection; however, existing spacers may also be deleted. Previous surveys of different *E. coli* collections have shown extensive heterogeneity of CRISPR/CAS content and spacer identity (Diez-Villasenor et al., 2010; Touchon and Rocha, 2010, Touchon et al., 2011, 2012). There have been two arrays in each of the two CRISPR systems identified so far in *E. coli*: CRISPR1 and -2 in type Ecoli system and CRISPR3 and -4 in the Ypest system (Touchon and Rocha, 2010). These two pairs of arrays were also named as CRISPR2.1 and −2.2/2.3, and CRISPR4.1 and −4.2, respectively (Diez-Villasenor et al., 2010).

Touchon et al. (2011) reported that the CRISPR distribution within the *E. coli* species was not suggestive of immunity-associated diversifying selection. By contrast, several authors have hypothesized that if the presence of a CRISPR system reduces ability of bacteria to acquire new traits via conjugation or infection, then the absence of a CRISPR system would be associated with an increased ability to adapt to different environments (Marraffini, 2010; Palmer and Gilmore, 2010; Bikard et al., 2012). If UPEC are more highly adaptive then commensal *E. coli* we would expect that UPEC would have fewer CRISPR loci, and if CRISPR loci are present, UPEC would harbor fewer spacers than CRISPR loci in fecal *E. coli*. We tested this hypothesis *in vivo*, by comparing paired uropathogenic *E. coli* (UPEC) and fecal *E. coli* isolated from the same individual. Since CRISPR sequences prevent plasmid conjugation (Marraffini and Sontheimer, 2008; Westra et al., 2012) and plasmids often carry antibiotic resistance genes (Lipps, 2008), we also hypothesized that the number of CRISPR loci and spacers would be inversely correlated with antibiotic resistance.

2. Materials and methods

2.1. Study design

We compared 81 matched pairs of fecal (commensal) and UPEC isolates, from the same UTI female patient. We quantified the number of CRISPR loci, repeats and spacers; all isolates were phenotyped for resistance to selected antibiotics.

2.2. Study population

The 81 *E. coli* pairs were randomly selected from an existing collection acquired from 166 women with physician-diagnosed, culture-confirmed UTI, who had visited the University of Michigan Health Service in Ann Arbor, Michigan between September 1996 and April 1999 (Foxman et al., 2002). Demographic and risk factor data, including sex history, were collected using a self-administered questionnaire. Most women selected were aged 20–24 (70.9%) and were Caucasian (76.5%) (Table 1).

2.3. Bacteria identification

Clean-catch midstream urinary specimens and fecal specimens using rayon-tipped swabs were self-collected, to detect UPEC and commensal isolates, respectively (Foxman et al., 2002). Specimens were inoculated on trypticase soy agar with 5% sheep blood and Mac Conkey agar, and incubated for 18–24 h at 37 °C (Foxman et al., 1995, 1997). Three isolates were selected for further testing per plate: the predominant presumptive *E. coli,* a randomly chosen colony from another quadrant on the plate,and any colony with different morphology. *E. coli* was identified by a manual test system (API 20E, Biomeriex-Vitek, Hazelwood, MO) and frozen glycerol stocks were made. To exclude fecal isolates that were identical to that causing the UTI, UPEC and fecal isolates were compared using pulsed-field gel electrophoresis (Foxman et al., 2002). A fecal isolate from the same individual that had a different band pattern from the UPEC was included in this study.

The included isolates were quite variable based on presence and absence of 13 different virulence genes (Foxman et al., 1995): there were 33 different virulence signatures among the 81 UPEC isolates and 25 different signatures among the 81 fecal isolates. Seventy percent of the virulence signatures found among the fecal isolates overlapped with the UPEC isolates.

Table 1
Characteristics of 81 women visiting the University of Michigan Health Service in Ann Arbor, Michigan between Sep. 1996 and Apr.1999 with physician-diagnosed, culture-confirmed urinary tract infection due to *E. coli* from whom study strains were derived.[$]

Characteristic	No.	%
Age (years)		
18–19	9	11.1
20–24	57	70.4
25–29	10	12.3
30–34	2	2.5
⩾35	1	1.2
Race/ethnicity		
African America	3	3.7
Asian	9	11.1
Caucasian	62	76.5
Other	5	6.2

[$] Numbers do not sum to totals because of missing values and percentages do not sum to 100 because of rounding.

2.4. CRISPR amplification and sequencing

Four CRISPR loci (CRISPR1 (C1), CRISPR2 (C2), CRISPR3 (C3) and CRISPR4 (C4)) were amplified by polymerase chain reaction (PCR) and sequenced. We used primers and PCR conditions as described by Touchon et al. (2011) (Table 2). *E. coli* strains K12 and CFT 073 were used as controls.

E. coli was grown, and genomic DNA was extracted and purified using Wizard Genomic DNA Purification Kit (Promega, U.S.A). PCR was performed using Accuprime Supermix II (Invitrogen Corporation, USA) at 1 μmol/L concentrations. PCR conditions included an initial denaturation step for 2 min at 94 °C, followed by 10

Table 2
Primers used to amplify CRISPR loci.[a]

Primer	Sequence (5′–3′)[*]	PCR target
CRISPR1 Fw	GTTATGCGGATAATGCTACC	iap
CRISPR1 Rev	CGTAYYCCGGTRGATTTGGA	cas 2
CRISPR2 Fw	AAATCGTATGAAGTGATGCAT	ygcE
CRISPR2 Rev	GTCGATGCAAACACATAAATA	ygcF
CRISPR3 Fw	GCGCTGGATAAAGAGAAAAAT	clpA
CRISPR3 Rev	GCCCACCATTCACCTGTA	cas1
CRISPR4 Fw	CTGAACAGCGGACTGATTTA	cys4
CRISPR4 Rev	GTACGACCTGAGCAAAG	infA

[*] Y: C or T; R: A or G; M: A or C.
[a] Ref. : Touchon, M. et al, 2011. CRISPR distribution within the *Escherichia coli* species is not suggestive of immunity-associated diversifying selection. J. Bacteriol. 193, 2460–67.