Use of optical mapping to sort uropathogenic *Escherichia coli* strains into distinct subgroups

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Optical maps were generated for 33 uropathogenic *Escherichia coli* (UPEC) isolates. For individual genomes, the *Nco*I restriction fragments aligned into a unique chromosome map for each individual isolate, which was then compared with the *in silico* restriction maps of all of the sequenced *E. coli* and *Shigella* strains. All of the UPEC isolates clustered separately from the *Shigella* strains as well as the laboratory and enterohaemorrhagic *E. coli* strains. Moreover, the individual strains appeared to cluster into distinct subgroups based on the dendrogram analyses. Phylogenetic grouping of these 33 strains showed that 32/33 were the B2 subgroup and 1/33 was subgroup A. To further characterize the similarities and differences among the 33 isolates, pathogenicity island (PAI), haemolysin and virulence gene comparisons were performed. A strong correlation was observed between individual subgroups and virulence factor genes as well as haemolysis activity. Furthermore, there was considerable conservation of sequenced-strain PAIs in the specific subgroups. Strains with different antibiotic-resistance patterns also appeared to sort into separate subgroups. Thus, the optical maps distinguished the UPEC strains from other *E. coli* strains and further subdivided the strains into distinct subgroups. This optical mapping procedure holds promise as an alternative way to subgroup all *E. coli* strains, including those involved in infections outside of the intestinal tract and epidemic strains with distinct patterns of antibiotic resistance.

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INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) are the number one cause of urinary tract infections (UTIs), responsible for up to 90 % of the seven million cases that occur each year in the USA (Foxman, 2002; Hooton & Stamm, 1997). Approximately 50–60 % of all women will have a UTI during their lifetime (Foxman, 2002; Kunin, 1994). In the USA, a staggering \$1.6 billion per year in healthcare costs is associated with UPEC infections (Foxman *et al.*, 2000; Hooton & Stamm, 1997). Many people suffer from multiple UTIs that are typically caused by the strain originally isolated in the first UTI (Foxman *et al.*, 1995). To initiate and sustain these UTIs, the UPEC use various virulence factors. Specific virulence factors and O:K:H serotypes are present more often in cystitis-causing isolates

than in isolates found as normal flora in the intestinal tract of humans (Johnson, 2003). Multiple virulence factor genes are found on genetic elements termed pathogenicity islands (PAIs; Blum *et al.*, 1994; Hacker *et al.*, 1990), mobile genetic elements inserted adjacent to tRNA genes. In UPEC, five PAIs have been identified and their role in UPEC pathogenesis has been demonstrated (Hacker *et al.*, 1997).

Besides comparisons of PAI prevalence, molecular epidemiological comparisons (Guttman & Dykhuizen, 1994; Kanamaru *et al.*, 2006; Zhang *et al.*, 2000) and genotypic-phenotypic statistical analysis of many *E. coli* isolates (Johnson, 2003) have been performed to achieve a grouping scheme for UPEC strains. Phylogenetic grouping of the *E. coli* strains has been another popular way to group *E. coli* strains. Four phylogenetic groups have been characterized: A, B1, B2 and D (Ochman & Selander, 1984). Extraintestinal *E. coli* responsible for UTIs belong chiefly to phylogenetic group B2 and less so to group D, whereas commensal *E. coli* (faecal) typically fall into groups

Abbreviations: EHEC, enterohaemorrhagic *Escherichia coli*; MLST, multilocus sequence typing; PAI, pathogenicity island; UPEC, uropathogenic *Escherichia coli*; UPGMA, unweighted pair group method with arithmetic mean; UTI, urinary tract infection.

A and B1 (Picard *et al.*, 1999). Clermont *et al.* (2000) have devised a multiplex PCR for three genetic markers (*chuA*, *yjaA* and TSPE4.12) to separate *E. coli* strains into these four basic phylogenetic groups. The multiplex PCR analysis has provided a first step in separating *E. coli* strains, but most of the UPEC strains fall into only one subgroup using this technique. Both ribotyping and multilocus sequence typing (MLST) have been used to further separate the UPEC strains (Bingen-Bidois *et al.*, 2002; Clermont *et al.* 2001; Tartof *et al.*, 2005), but these techniques are complex methods of grouping UPEC strains.

In addition to phylogenetic grouping, pathotyping is another way to separate strains of a species, and is based on a particular set of virulence factors shared by some strains that assist in the pathogenesis process (Marrs *et al.*, 2005). Although the *E. coli* that cause diarrhoea in humans have been separated into subgroups or pathotypes (Nataro & Kaper, 1998), the same has not been done for UPEC, even though this group of *E. coli* strains has been shown to be quite diverse (Zhang *et al.*, 2000). Some attempts have been made to place the UPEC strains into pathotypes (Foxman *et al.*, 1995; Marrs *et al.*, 2005), but critical genetic differences that may define specific pathotypes remain to be elucidated. As a consequence of these genetic limitations, several *E. coli* genomes have been sequenced to uncover which set of genes may be contributing to UPEC pathogenesis. Because of the limitations outlined above, a clear UPEC pathotype subgrouping scheme has remained elusive.

In this study, we have used an optical mapping scheme to sort UPEC strains into distinct subgroups based on similarities in their restriction fragment patterns across the entire genome. This is believed to be the first time that this technology has been applied to group any bacteria. We have shown that UPEC strains are distinct from both enterohaemorrhagic and laboratory strains of *E. coli*. In addition, we demonstrate that there are two major UPEC subgroups. Furthermore, we show that strain separation by optical mapping correlates with haemolysis, the presence of PAIs, the distribution of several virulence factor genes, and antibiotic resistance.

METHODS

Bacterial strains. All of the *E. coli* strains are listed in Table 1. *E. coli* strains NU149 and NU14 were obtained from women who had a UTI, and have been previously characterized (Hultgren *et al.*, 1986; Schaeffer *et al.*, 1987). Strain 536 (Hacker *et al.*, 1983) was obtained from Jörg Hacker, Universität Würzburg. *E. coli* CFT073 (Mobley *et al.*, 1990) was a kind gift from Rod Welch, University of Wisconsin-Madison. Strain UTI89 (Mulvey *et al.*, 2001) was received from Scott Hultgren, Washington University. The W3110 (K-12) strain was taken from the University of Wisconsin-La Crosse culture collection. ATCC 25922 was obtained from the American Type Culture Collection. The 16 GLMC strains were collected in a consecutive manner from patients with UTIs at Gundersen Lutheran Medical Center, WI. They were all biochemically identified as *E. coli*. All 13 of the CV strains were obtained from Converge and were isolated from patients with UTIs in a consecutive manner around Boston, MA.

Table 1. Bacterial strains used in this study

Strain	Source or reference
<i>E. coli</i> strains	
GLMC 9	Gundersen Lutheran Medical Center
GLMC 10	Gundersen Lutheran Medical Center
GLMC 15	Gundersen Lutheran Medical Center
GLMC 21	Gundersen Lutheran Medical Center
GLMC 100	Gundersen Lutheran Medical Center
GLMC 101	Gundersen Lutheran Medical Center
GLMC 103	Gundersen Lutheran Medical Center
GLMC 106	Gundersen Lutheran Medical Center
GLMC 503	Gundersen Lutheran Medical Center
GLMC 505	Gundersen Lutheran Medical Center
GLMC 506	Gundersen Lutheran Medical Center
GLMC 508	Gundersen Lutheran Medical Center
GLMC 509	Gundersen Lutheran Medical Center
GLMC 513	Gundersen Lutheran Medical Center
GLMC 514	Gundersen Lutheran Medical Center
GLMC 515	Gundersen Lutheran Medical Center
CV1117292	Converge
CV1117298	Converge
CV1117326	Converge
CV1117621	Converge
CV1118322	Converge
CV1118891	Converge
CV1119429	Converge
CV1119511	Converge
CV1119857	Converge
CV1121147	Converge
CV1123039	Converge
CV1125109	Converge
CV1131494	Converge
ATCC 25922	ATCC
ATCC 8739	ATCC
W3110 (K-12)	University of Wisconsin-La Crosse collection
DH10B (K-12)	Durfee <i>et al.</i> (2008)
UTI89	Scott Hultgren (Chen <i>et al.</i> , 2006b)
CFT073	Rod Welch (Welch <i>et al.</i> , 2002)
536	Jörg Hacker (Brzuszkiewicz <i>et al.</i> , 2006)
NU14	Hultgren <i>et al.</i> (1986)
NU149	Schaeffer <i>et al.</i> (1987)
APEC 01	Johnson <i>et al.</i> (2007)
Sakai	Hayashi <i>et al.</i> (2001)
EDL933	Perna <i>et al.</i> (2001)
EC4115	GenBank
E24377A	Rasko <i>et al.</i> (2008)
SMS-3-5	Fricke <i>et al.</i> (2008)
HS	Rasko <i>et al.</i> (2008)
SE11	Oshima <i>et al.</i> (2008)
<i>S. boydii</i> strains	
CDC 3083-94	GenBank
Sb227	Yang <i>et al.</i> (2005)
<i>S. dysenteriae</i> strain	
Sd197	Yang <i>et al.</i> (2005)
<i>S. flexneri</i> strains	
2457T	Wei <i>et al.</i> (2003)
301	Jin <i>et al.</i> (2002)
8401	Nie <i>et al.</i> (2006)

Table 1. cont.

Strain	Source or reference
<i>S. sonnei</i> strain	
Ss046	Yang <i>et al.</i> (2005)

Haemolysis. All of the *E. coli* strains were tested for their ability to lyse sheep red blood cells by plating on sheep blood agar plates (Becton Dickinson) and incubating for 24 h at 37 °C. All of the clinical isolates were tested for haemolysis using bacteria that were freshly isolated from infected urine. A zone of haemolysis around the bacterial colonies was considered a positive reaction for haemolysis.

Distribution of virulence factor genes. The UPEC isolates were tested for nine virulence factor genes characteristic of UPEC: *cnf*, *papGII*, *papGIII*, *hlyC*, *sfa*, *fimH*, *usp*, *aer* and *fyuA*. Each PCR was carried out in a 50 µl volume using 50 pmol of each primer, 200 µM of each dNTP, 2.5 U *Taq* polymerase (Promega) and 1.5 µl bacterial lysate. Detection of the *aer*, *cnf*, *sfa* and *hlyC* genes used the primers designed by Yamamoto *et al.* (1995). Amplification of the *papGII* and *papGIII* genes used the method of Johnson & Brown (1996). PCR detection of *fimH* was done with primers used by Tseng *et al.* (2001). Primers for *usp* were as denoted by Takahashi *et al.* (2006). The primer set of Johnson & Stell (2000) were utilized for amplifying *fyuA*. All PCR runs were carried out in a Perkin Elmer 9700 thermocycler under the following conditions: initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 10 s, 55 °C for 20 s and 72 °C for 30 s; and a final denaturation at 72 °C for 7 min. Each PCR run was repeated at least twice.

Optical map production and analysis. Purified chromosomal DNA was deposited onto an optical mapping surface (derivatized glass coverslip), using a microfluidic device. The DNA was encased in a thin layer of acrylamide and incubated with the restriction enzyme *NcoI* in a humidified chamber at 37 °C for 60–120 min. The digested DNA was labelled with fluorescent JOJO-1 (Invitrogen) and the individual molecules were imaged by fluorescence microscopy. Digital images were collected by a fully automated image-acquisition system and image files were processed to create single-molecule optical maps using OpGen software programs (Zhou *et al.*, 2004). Individual molecule restriction maps were overlapped by optical map assembler software. Briefly, this assembler works by comparing single-molecule restriction maps and estimating the probability that two molecules arose from overlapping genomic locations given a description of the likelihood of possible errors resulting from incomplete digestion of the molecule, spurious cuts of the molecule, and errors in the sizing estimates of the resulting fragments. Through repeated overlapping of molecules, the assembler reconstructs the ordered restriction map of the genome. This technique has been previously applied to map other bacterial genomes (Chen *et al.*, 2006a; Kotewicz *et al.*, 2007), as well as mapping enterohaemorrhagic *E. coli* (EHEC) strains (Kotewicz *et al.*, 2007, 2008).

The *Shigella* sequences (*Shigella boydii* CDC 3083-94, GenBank accession no. CP001063; *S. boydii* Sb227, CP000036; *Shigella flexneri* 2a 2457T, AE014073; *S. flexneri* 2a 301, AE005674; *S. flexneri* 5 8401, CP00026; *Shigella sonnei* Ss046, CP000038; *Shigella dysenteriae* Sd197, CP000034) and the sequences for *E. coli* strains HS (GenBank accession no. CP000802), DH10B (K-12) (CP000948), W3110 (K-12) (AP009048), E24377A (CP000800), Sakai (BA000007), EDL933 (AE005174), SMS-3-5 (CP000970), UTI89 (CP000243), APEC 01 (CP000468), CFT073 (CP000247), ATCC 8739 (CP000946) and 536 (CP000247) came from the published NCBI microbial genomes. The maps were then constructed *in silico* by identifying the location of restriction sites in the sequences.

To construct the similarity cluster, each pair of maps was aligned using a dynamic programming algorithm based upon published methods (Myers & Huang, 1992; Pevzner *et al.*, 2001; Waterman *et al.*, 1984). This method finds the optimal alignment of two restriction maps according to a scoring model that incorporates fragment sizing errors, false and missing cuts, and missing small fragments. For a given alignment, the score is proportional to the log of the length of the alignment, penalized by the differences between the two maps, such that longer, better-matching alignments will have higher scores. This method has been used before to compare *E. coli* genomes (Chen *et al.*, 2006a). From these alignments, adding up the lengths of the unmatched regions from both maps and dividing this by the sum of the lengths of both maps in the pair calculated a dissimilarity score for a pair of maps. A matrix of these pairwise scores was used as input to agnes, an agglomerative clustering method implemented in the R statistical package, which creates dendrograms using the unweighted pair group method with arithmetic mean (UPGMA). An analogous clustering method using sequence information has been shown to produce trees that match existing phylogeny data (Henz *et al.*, 2005); however, we make no specific evolutionary claims based upon our trees.

Phylogenetic grouping. We determined the phylogenetic groups of selected isolates in our study using the PCR-based method described in Clermont *et al.* (2000).

MIC determinations. A VITEK machine was used to determine the antibiotic susceptibility of the 32 recent clinical isolates.

RESULTS

Genome-wide optical mapping comparisons of UPEC isolates

The *NcoI* restriction fragment optical maps of 33 UPEC isolates were arrayed and compared with each other as well as with *in silico* maps of sequenced strains of *E. coli* and *Shigella* deposited in GenBank (Fig. 1). Based on the map clustering, *S. sonnei*, *S. flexneri* and *S. boydii* were found in the *E. coli* cluster, whereas *S. dysenteriae* was the most dissimilar of any of the genomes that were mapped. The cluster is very good at highlighting the genomic diversity of these strains, but the exact cut-off for classifying these isolates into distinct groups is not clear. Using a 30% dissimilarity cut-off, all of the *E. coli* isolates fell into five separate subgroups, yet the clinical UPEC isolates fell into two separate major subgroups, one more diverse than the other. The other three subgroups were EHEC, *E. coli* K-12 and other laboratory strains, and an environmental isolate from a heavy metal-contaminated site (Selander *et al.*, 1987). Moreover, GLMC isolates from Wisconsin mapped side by side with the CV isolates from Massachusetts, demonstrating that geographical distribution contributes to only slight genomic variations between different strains. Although the isolates became segregated into subgroups, optical mapping was able to discriminate between each of the individual isolates because no two isolates were identical. Thus, optical mapping was effective at displaying the versatility and flexibility of the *E. coli* genome.

By using a 15% dissimilarity cut-off, all of the *E. coli* isolates separated into 10 subgroups. Three of these

Fig. 1. Genome similarity clustering using UPGMA for the 33 UPEC isolates and the *in silico* maps of sequenced *E. coli* and *Shigella* strains.

subgroups aligned with the three sequenced UPEC strains (CFT073, UTI89 and 536). The GLMC 101 UPEC isolate aligned with no other isolates at this cut-off.

Detailed view of map and sequence similarity among several clinical isolates

Optical mapping of the entire *E. coli* genome allowed segregation into several subgroups. A closer examination of the sequence alignments of part of one of the subgroups showed the similarities and differences between the isolates (Fig. 2a). The previously sequenced strain CFT073 had the largest genome (5.231 Mb) and the CV1117326 isolate had the smallest genome (5.000 Mb). A strain from the mid 1980s, NU149 (Schaeffer *et al.*, 1987), and the ATCC 25922

strain were both contained in the CFT073 subgroup. Within the CFT073 subcategory of strains (CFT073, GLMC 9, ATCC 25922, NU149, GLMC 505, CV1123039), there appeared to be a conservation of the CFT073 PAIs. All of the isolates except GLMC 9 had PAI III (PAI-CFT073-*aspV*), and all of the isolates except NU149 had PAI-CFT073-*serX*, although the GLMC 505 isolate appeared to have the PAI-CFT073-*serX* inserted upstream from its position in the other genomes. An alignment of the subgroup containing strain 536 showed a similar pattern of conservation of PAIs among those isolates (data not shown).

A more detailed comparison was undertaken between two sequenced UPEC strains, UTI89 and CFT073, as well

Fig. 2. (a) Overview of optical maps of *E. coli* strains from the CFT073 subgroup. Comparison of optical maps with the *in silico* map of CFT073. The alignment software highlights restriction fragment differences shown in white between isolates, whereas homologous regions are highlighted in blue. (b) A closer look at three strains (UTI89, CFT073, NU14), with sequence alignments overlaid on top of the maps. The vertical lines represent matching *NcoI* restriction sites. Green rectangles represent some of the PAIs. The insert shows the PAI-CFT073-*serX* in strain CFT073 that is absent in both the UTI89 and the NU14 strains.

as strain NU14, a strain that is phylogenetically close to strain UTI89. Fig. 2(b) shows the alignment of the optical maps of NU14 and UTI89 to CFT073. Coloured regions indicate a match, and the white segments indicate regions where the optical maps could not be aligned. A great degree of similarity exists between the genome of a strain collected in the mid 1980s (NU14)

and one collected in the late 1990s (UTI89). From the inset to Fig. 2(b), a close-up view of part of the genome for all three strains demonstrated the PAI III-CFT073-*aspV* present in CFT073, but absent in both UTI89 and NU14. Moreover, the *NcoI* restriction patterns between NU14 and UTI89 matched well, but did not match strain CFT073.

Genome similarity clustering versus phylogenetic grouping, haemolytic characterization, and distribution of several virulence factor genes

Optical mapping sorted the clinical isolates into subgroups. To add another layer to the characterization of the isolates, each isolate was phylogenetically typed using the method of Clermont *et al.* (2000). Genomic DNAs from the 30 recent clinical isolates, the two clinical isolates from the mid 1980s (NU14 and NU149), as well as the sequenced reference strains were PCR-amplified with primers specific for *chuA*, *yjaA* and *tspE4C2*. Of the 33 clinical isolates, 32/33 (97 %) typed as phylogenetic group B2. One isolate, GLMC 508, was phylogenetically typed as group A (Fig. 3), even though the phenotype and optical mapping data placed it more closely with the UPEC isolates in group B2. The K-12 laboratory strain of *E. coli* (W3110) displayed the A grouping, as described by Clermont *et al.* (2000). Thus, the phylogenetic grouping data did not add much additional information to further separate the strains.

In addition to the phylogenetic grouping of each isolate, haemolysis of sheep blood was also assessed. Twelve out of 33 isolates displayed haemolysis of sheep blood. Moreover, there was a haemolysis pattern for most of the isolates tested. Although strains NU149 and GLMC 9 did not lyse sheep blood, the remaining strains in the CFT073 subgroup demonstrated haemolysis. Both isolates at the top of the dendrogram in Fig. 3 also showed a haemolysis-positive phenotype, as did UTI89 and NU14. The closely related strains GLMC 503 and CV1117621 displayed haemolysis,

and four out of five isolates found as part of the strain 536 subgroup were haemolytic. The stand-alone strain GLMC 101 also showed the ability to lyse sheep blood. The combination of phylogenetic grouping and the ability to lyse sheep blood added additional information to the optical maps of all of the isolates.

As an additional step to correlate the optical mapping with something tangible, each *E. coli* isolate was screened for the presence of several virulence factor genes that included *aer*, *cnf*, *fimH*, *fyuA*, *papGII*, *papGIII*, *hlyC*, *sfa* and *usp*. All of the strains in the one major UPEC subgroup (based on the 30 % dissimilarity cut-off) had identical virulence factor gene distributions, except for the *usp* gene missing in the GLMC 508 isolate and the *aer* and *fyuA* genes absent in strain GLMC 21 (Table 3). No *cnf*, *papG*, *hlyC* or *sfa* gene was detected in any of these strains. However, they all possessed the *fimH* gene, and all but one had the *aer* and *fyuA* genes. Although the distribution of the virulence factor genes in the other major UPEC subgroup (based on a 30 % dissimilarity cut-off) was more diverse, they aligned fairly closely with the subgroups from the 15 % dissimilarity cut-off; UPEC isolates within the CFT073 subgroup were quite similar, except for strain NU149, which lacked the *papGII*, *hlyC* and *sfa* genes, which is probably tied to the loss of the PAI in this strain. The UPEC strains found within the 536 subgroup were also typically conserved. Moreover, all of the strains that exhibited phenotypic haemolysis were positive for the presence of the *hlyC* gene. In addition, strain GLMC 103 also displayed amplification

Fig. 3. Genome similarity clustering using UPGMA for *E. coli* isolates found in subgroups one and two, as well as phylogenetic grouping and haemolysis patterns.

of the *hlyC* gene, which aligned this strain perfectly with the other strains in that subgroup.

Antibiotic-sensitivity patterns of the recent clinical isolates

Since UPEC strains continue to emerge that are resistant to front-line antibacterial drugs, the 30 recent clinical isolates from Wisconsin and Massachusetts were assayed for their antibiotic-sensitivity patterns against the front-line antibiotics used to treat *E. coli* urinary tract and systemic infections, then compared with the optical mapping patterns for these isolates. Eighteen of 30 isolates were resistant to ampicillin. The distribution for resistance to this antibiotic was well dispersed among the isolates; however, all of the isolates that grouped from CV1121147

to GLMC 21 found at the bottom of the dendrogram in Fig. 3 had the same ampicillin-resistance pattern. Four isolates had resistance to piperacillin, and eight demonstrated resistance to ampicillin/sulbactam. All of the strains tested showed susceptibility to piperacillin/tazobactam, cefazolin, cefotetan, ceftriaxone, cefepime, meropenem and nitrofurantoin (Table 2). Thus, no isolate displayed resistance to the cephalosporin class of drugs. Only seven isolates showed resistance to the sulfa drugs. For these drugs, the resistance patterns were spread out among different optical map subgroups. On the other hand, resistance to ciprofloxacin, levofloxacin and gentamicin was conserved among one subgroup from Fig. 3 that included strains CV1131494, CV1118322, CV1117298 and CV1119857. The grouping of fluoroquinolone-resistant isolates suggests that optical mapping could be useful in

Table 2. Antibacterial drug susceptibilities of 30 recent uropathogenic *E. coli* isolates and ATCC strain 25922

<i>E. coli</i> isolate	Antibacterial drug*														
	AM	SAM	PIP	TZP	CZ	CTT	CRO	FEP	MEM	CIP	LVX	SXT	F/M	NN	GM
GLMC 515	S†	S	S	S	S	S	S	S	S	S	—	S	S	—	S
CV1117326	R	R	R	S	S	S	S	S	S	S	S	R	S	S	S
GLMC 9	R	R	R	S	S	S	S	S	S	S	—	S	—	—	S
ATCC 25922	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 505	S	S	S	S	S	S	S	S	S	S	—	S	S	—	S
CV1123039	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 15	R	R	I	S	S	S	S	S	S	S	—	S	S	—	S
CV1118891	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CV1119511	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 506	R	I	S	S	S	S	S	S	S	S	—	S	S	—	S
GLMC 513	S	S	S	S	S	S	S	S	S	S	—	S	S	—	S
GLMC 509	R	S	R	S	I	S	S	S	S	S	—	S	S	—	S
CV1125109	R	R	R	S	S	S	S	S	S	S	S	R	S	S	S
GLMC 503	S	S	S	S	S	S	S	S	S	S	—	S	S	—	S
CV1117621	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 514	S	S	S	S	S	S	S	S	S	S	—	S	S	—	S
GLMC 103	S	S	S	S	S	S	S	S	S	S	—	R	S	—	S
CV1121147	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S
CV1117292	R	I	I	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 101	R	R	S	S	S	S	S	S	S	S	—	S	S	—	S
GLMC 106	R	I	I	S	S	S	S	S	S	S	—	S	S	—	S
CV1119429	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
GLMC 100	R	R	S	S	S	S	S	S	S	S	—	S	S	—	S
CV1131494	R	I	S	S	S	S	S	S	S	R	R	S	S	I	R
CV1118322	R	I	S	S	S	S	S	S	S	R	R	S	S	I	R
CV1117298	R	I	R	S	S	S	S	S	S	R	R	R	S	S	S
GLMC 508	R	R	I	S	R	S	S	S	S	R	—	R	S	—	S
CV1119857	R	R	I	S	R	S	S	S	S	R	S	S	S	S	R
GLMC 10	R	I	S	S	S	S	S	S	S	S	—	R	S	—	S
GLMC 21	R	R	I	S	S	S	S	S	S	S	—	S	S	—	S

*The following antibacterial drugs were used: AM, ampicillin; SAM, ampicillin/sulbactam; PIP, piperacillin; TZP, piperacillin/tazobactam; CZ, cefazolin; CTT, cefotetan; CRO, ceftriaxone; FEP, cefepime; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfa; F/M, nitrofurantoin; NN, tobramycin; GM, gentamicin.

†S, Susceptible; I, intermediate; R, resistant; —, not determined.

Table 3. Virulence factor gene distribution among uropathogenic *E. coli* isolates

+, Present; –, absent.

<i>E. coli</i> isolate	Virulence factor gene								
	<i>cnf</i>	<i>papGII</i>	<i>papGIII</i>	<i>hlyC</i>	<i>sfa</i>	<i>fimH</i>	<i>usp</i>	<i>aer</i>	<i>fyuA</i>
GLMC 515	+	–	+	+	+	+	+	–	+
CV1117326	+	–	+	+	+	+	+	–	+
CFT073	–	+	–	+	+	+	+	+	+
GLMC 9	–	+	–	–	+	+	+	+	+
ATCC 25922	–	+	–	+	+	+	+	+	+
NU149	–	–	–	–	–	+	+	+	+
GLMC 505	+	–	+	+	+	+	+	–	+
CV1123039	+	–	–	+	+	+	+	–	+
UTI89	+	–	+	+	+	+	+	–	+
NU14	+	–	–	+	+	+	+	–	+
GLMC 15	–	+	–	–	–	+	+	–	+
CV1118891	–	+	–	–	+	+	+	–	+
CV1119511	–	+	–	–	–	+	+	–	+
GLMC 506	–	+	–	–	–	+	+	–	+
GLMC 513	–	–	–	–	+	+	+	–	+
GLMC 509	+	+	–	+	+	+	+	–	+
CV1125109	+	+	+	+	+	+	+	–	+
GLMC 503	+	–	+	+	+	+	+	–	+
CV1117621	+	–	+	+	+	+	+	–	+
GLMC 514	+	–	+	+	+	+	+	–	+
GLMC 103	+	–	+	+	–	+	+	+	+
536	–	–	+	+	+	+	+	–	+
CV1121147	–	–	–	–	+	+	+	–	+
CV1117292	–	–	–	–	–	+	+	–	+
GLMC 101	–	+	–	+	–	+	+	+	+
GLMC 106	–	–	–	–	–	+	+	+	+
CV1119429	–	–	–	–	–	+	–	+	+
GLMC 100	–	–	–	–	–	+	+	+	+
CV1131494	–	–	–	–	–	+	+	+	+
CV1118322	–	–	–	–	–	+	+	+	+
CV1117298	–	–	–	–	–	+	+	+	+
GLMC 508	–	–	–	–	–	+	–	+	+
CV1119857	–	–	–	–	–	+	+	+	+
GLMC 10	–	–	–	–	–	+	+	+	+
GLMC 21	–	–	–	–	–	+	+	–	–
W3110 (K-12)	–	–	–	–	–	+	+	–	–

gauging outbreak strains of some antibiotic-resistant UPEC.

DISCUSSION

Separating UPEC strains is contingent on the phylogenetic background, the acquisition of antibiotic resistance, and the presence or absence of virulence factor genes. In the last 4 years, the genomes of three UPEC strains have been sequenced (Brzuszkiewicz *et al.*, 2006; Chen *et al.*, 2006b; Welch *et al.*, 2002). Each of these sequencing endeavours has collectively added a tremendous amount of information about the genetic makeup of UPEC strains, but has done little to sort *E. coli* into distinct subgroups because of

the lack of comparative analysis. Genome sequencing of more *E. coli* strains would close this gap, but optical mapping is a more rapid and cost-effective technique that could provide the necessary information to assess the prevalence of virulence factor genes and changes that have led to antibiotic resistance, as well as help group the UPEC into distinct subgroups.

In this study, we have optically mapped 33 clinical isolates and compared their maps with *in silico* maps of sequenced strains of *E. coli*, representing what is believed to be the first study in which optical mapping has served as the basis for clustering bacterial species as shown in Fig. 1. From our analysis, the *E. coli* isolates divided into five subgroups using UPGMA alignments and a cut-off of 30 % dissim-

ilarity. All of the sequenced UPEC strains (536, CFT073, UTI89) fell into the first subgroup, which also held most of the recent UPEC isolates and was the more diverse of the two UPEC subgroups. Our optical mapping scheme placed the APEC 01 strain in the same subgroup as the UTI89 strain. Both strains have similar MLST patterns (Johnson *et al.*, 2007). It is possible that some minimal set of virulence factors is found in the genomes of these strains that allows them to initiate infections outside of the intestinal tract. Within the second subgroup, the seven isolates exhibited less diversity at the genetic level than the first subgroup. However, none of the isolates were identical. Past studies have not shown the separation of UPEC strains into major subgroups that we have demonstrated in this study. Subgroup three had the K-12 laboratory substrains of *E. coli*, W3110 and DH10B. The K-12 strain has lost many of the virulence factors associated with *E. coli* and has been altered to accept foreign DNA more readily. EHEC strains were found in the fourth subgroup. Two groups have successfully used optical mapping to distinguish between individual strains of ETEC H10407 and EHEC O157:H7, but strain clustering was not performed (Chen *et al.*, 2006a; Kotewicz *et al.*, 2007, 2008). The fifth subgroup harboured an environmental strain of *E. coli* isolated from a heavy metal-contaminated site in South Carolina. Surprisingly, the UPEC isolates were more different from the other non-UPEC isolates as compared with the differences between *Shigella* isolates and non-UPEC strains.

The only significant difference between the tree based upon sequence (Henz *et al.*, 2005) and the tree based upon optical maps was the relative position of the *Shigella* strains. A sequence-based tree will focus exclusively upon relative changes in conserved genes, whereas an optical map-based tree examines the overall structure of the genome. Since there are numerous genomic rearrangements between *E. coli* and *Shigella* (Yang *et al.*, 2005), focusing only on the conserved genes will tend to mask many of the differences between these bacteria. On the other hand, optical mapping gives an unbiased look at the whole genome as compared with a sequence-based clustering and will pick up the structural changes in the genome more readily.

A high degree of similarity of UPEC isolates from disparate locations was found across the dataset, as isolates from Boston, Massachusetts, clustered closely with isolates collected in Wisconsin. From this optical mapping dataset, the geographical location did not correlate with the backbone structure of the *E. coli* isolates. Thus, optical mapping shows that the geographical structure appears to account for only a small fraction of the genomic variations of our isolates, which is consistent with an earlier study (Selander *et al.*, 1987).

Because optical mapping shows a genome-wide snapshot of each isolate at a fraction of the cost and time needed to sequence a genome, this technology could potentially be used to sort UPEC isolates into pathotypes. Prior attempts

at pathotyping have been hampered by the limited genetic information available and the laborious process of screening for specific virulence factor genes (Marrs *et al.*, 2005; Foxman *et al.*, 1995). A confounding factor for UPEC is their high mutation rate (Guttman & Dykhuizen, 1994; Denamur *et al.*, 2002), so virulence factor genes may be missed because of the heterogeneity that results from mutations that prevent primer binding to a site. Because of these genetic limitations, several UPEC strains have been sequenced to study their pathogenesis: CFT073 (Mobley *et al.*, 1990; Welch *et al.*, 2002), UTI89 (Mulvey *et al.*, 2001; Chen *et al.*, 2006b) and 536 (Hacker *et al.*, 1983). In this study, we have shown that *E. coli* isolates sort into distinct subgroups with similar haemolytic profiles. Our optical maps also demonstrate that PAIs can be identified among the isolates using optical mapping, and this includes differences in PAI positioning within the genome. Acquisition of virulence factors, including those found on PAIs, is independent of the main backbone structure of *E. coli* (Lloyd *et al.*, 2007, 2009; Pupo *et al.*, 1997, 2000), and the optical mapping illustrates that fact. The sorting of the *E. coli* isolates by optical mapping into a few distinct groups offers some hope that the technique will assist in the pathotyping of UPEC strains. In this regard, the screening for virulence factor genes among the 33 UPEC isolates also showed strong correlations with the subgrouping represented by the optical mapping analysis. An examination of virulence factor gene distribution has been applied before to UPEC strains (Bidet *et al.*, 2005; Bingen-Bidois *et al.*, 2002; Johnson & Stell, 2000; Sabate *et al.*, 2006; Takahashi *et al.*, 2006). These studies have shown a correlation between specific virulence factor genes and PAIs. We have also demonstrated this same correlation when comparing the data from Fig. 2 with the data provided in Table 3. Subgroups of UPEC sorted by the optical mapping technology also sorted along similar lines based on the presence or absence of nine virulence factor genes. Although the UPEC isolates were quite diverse, an even more striking observation was that the one major subgroup based on a 30% dissimilarity cut-off was very uniform for the absence of most of these key virulence factor genes. Thus, optical mapping separated the UPEC isolates along similar lines to those that would be associated with their pathotype.

Besides an assessment of PAIs and other virulence factor-related genetic elements, this study also points to the utility of using optical mapping for tracking antibiotic-resistance patterns among UPEC strains. The emergence and spread of antibiotic-resistant *E. coli* strains is a serious health concern, particularly with respect to resistance to front-line drugs such as fluoroquinolones. Although plasmid-based resistance genes are one way to disseminate the resistance genes, more common are transposon or prophage insertion and the integration of the mobile genetic elements known as integrons, which have site-specific recombination systems built in (Rowe-Magnus *et al.*, 2002). Several groups have tried to correlate the phylogenetic background

with antibiotic resistance, with mixed results (Barl *et al.*, 2008; Bruant *et al.*, 2006; Graziani *et al.*, 2009; Piatti *et al.*, 2008; Rijavec *et al.*, 2006; Yu *et al.*, 2004). The most successful techniques used DNA microarrays (Barl *et al.*, 2008; Bruant *et al.*, 2006; Yu *et al.*, 2004), but they are limited to the known genetic sequences available. In this study, all of the ciprofloxacin-resistant strains clustered together. A recent study has also shown that ciprofloxacin-resistant strains possess fewer virulence factor genes than ciprofloxacin-sensitive strains (Graziani *et al.*, 2009). The potential utility of optical mapping has been demonstrated for tracking antibiotic-resistant outbreak strains.

Although optical mapping has shown a lot of commonality among the isolates, the technique was able to identify each individual isolate. The relative ease with which *E. coli* is able to gain and lose genetic elements, such as prophages and PAIs, means that each strain can be tagged as unique. Because optical mapping can track the large-scale changes mentioned above, it is capable of both clustering *E. coli* strains into subgroups based on greater commonality, as shown in this study, and distinguishing between different isolates, as described elsewhere (Kotewicz *et al.*, 2007, 2008). Thus, we have shown for the first time that optical mapping is a viable way to subgroup bacteria and that it could be used as an alternative methodology to ribotyping or MLST analyses. In fact, Kotewicz *et al.* (2007) used optical mapping to track mobile genetic elements. Overall, we see optical mapping as a powerful tool able to analyse whole-genome structure for a fraction of the cost and time needed for whole-genome sequencing.

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