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Adaptation of *Escherichia coli* Traversing From the Faecal Environment to the Urinary Tract

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Abstract

The majority of extraintestinal pathogenic *Escherichia coli* (ExPEC) causing urinary tract infections (UTI) are found in the patient's own gut flora, but only limited knowledge is available on the potential adaptation that may occur in the bacteria for them to traverse the perineum and successfully infect the urinary tract. Here, matching faecal and UTI isolates from 42 patients were compared pairwise using in-depth whole-genome sequencing to investigate whether genetic changes were evident for successful colonization in these two different environments. The identified non-synonymous mutations (0-12 substitutions in each pair) were primarily associated to genes encoding virulence factors and nutrient metabolism; and indications of parallel evolution were observed in genes encoding the major phase-variable protein antigen 43, a toxin/antitoxin locus and haemolysin B. No differences in virulence potential were observed in a mouse UTI model for five matching faecal and UTI isolates with or without mutations in antigen 43 and haemolysin B. Variations in plasmid content were observed in only four of the 42 pairs. Although, we observed mutations in known UTI virulence genes for a few pairs, the majority showed no detectable differences in mutations or mobilome changes when compared to their faecal counterpart. The results show that UPECs are successful in colonizing both the bladder and gut without adaptation.

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Conflict of interest
None to declare

Keywords

urinary tract infection; whole genome sequencing; evolution; faecal flora; single-nucleotide polymorphisms; mutations

Introduction

Symptomatic urinary tract infections (UTIs) are particularly common among women and one in three women have, at the age of 24, been diagnosed with UTI (Foxman, 2003). Extraintestinal pathogenic *Escherichia coli* (ExPEC) are the major cause of UTI, being responsible for up to 80% of all community-acquired UTIs and more than 30% of all nosocomial UTIs (Bouza et al., 2001; Kahlmeter, 2000).

The infecting urinary *E. coli* is in the majority of the cases found in the patient's own gut flora, indicating a faecal-perineal-urethral route of transmission to the bladder (Yamamoto et al., 1997). Comparisons between Uropathogenic and commensal *E. coli*, which do not normally cause infections, have identified associations between the presence of certain genes and ecotypes, but also revealed that isolates from the same infection type exhibit large genomic flexibility (Chattopadhyay et al., 2009). Generally, UPEC are associated with phylogenetic group B2, and to a smaller extent D, and are found to carry more virulence factors than commensal isolates which generally are associated with phylogenetic groups A and B1 (Johnson and Russo, 2002; Moreno et al., 2006; Picard et al., 1999; Zhang et al., 2002). Recent studies have, however, shown that healthy women who have never had a UTI carry a large proportion of B2 in their gut flora (Nielsen et al., 2014a), and that these isolates are able to cause infection in a mouse UTI model (Nielsen et al., 2014b).

Successful colonization in two diverse environments, such as the gut and the urinary tract could require highly specific gene repertoires or gene variants and possibly involve adaptive mutations and acquisition of genetic elements when migrating between environments (Orr and Smith, 1998). Acquisition of mobile genetic elements can affect the fitness of the bacteria, which can lead to selection of more fit sub-lineages (Nielsen et al., 2012; Subbiah et al., 2011). Similarly, point mutations can result in significant adaptive effects and provide rapid adaptation to specific niches (Chattopadhyay et al., 2009; Weissman et al., 2007). The genes in the core genome have generally not been considered significant for the adaptive evolution of the pathogen. However, it has been found that more than 1/3 of the core genes in *E. coli* are targeted by repeated substitutions in hot-spot positions driven by positive selection (Chattopadhyay et al., 2009), including genes encoding DNA metabolism, nutrient acquisition, cell surface structure and metal-binding (Chattopadhyay et al., 2009; Chen et al., 2009). Mutations that increase fitness in the urinary tract are likely to be selected in the urinary tract (Chen et al., 2009) and increased fitness in the urinary tract has been correlated to lower fitness in the faecal habitat and it has therefore been proposed that isolates colonizing the urinary tract run into an evolutionary dead-end (source-sink) (Chattopadhyay et al., 2007; Sokurenko et al., 2006). Recently, Chen *et al.* (Chen et al., 2013) whole-genome sequenced matching UTI and faecal isolates from two patients and identified that the same strain was present in both urine and faecal samples in all samplings of one of the patients,

when studying paired urine and faecal isolates over time at successive UTIs. For the other patient, different faecal and urinary strains were identified, perhaps indicating that adaptation to the urinary tract is not an evolutionary dead-end (Chen et al., 2013).

As gene acquisitions and the occurrence of mutations that provide adaptive traits are variable in nature and limited between faecal and the urinary tract, larger strain collections are needed to detect these. Understanding the adaptation of the *E. coli* genome and genes under selection are important key components to understand the mechanisms of UTI pathogenesis. In this genome-based approach we analyzed a large collection of paired faecal and urinary isolates to identify potential adaptation of the UTI *E. coli* compared to the faecal counterpart. In total, 42 *E. coli* pairs from faeces and urine were characterized with respect to point mutations, phage and plasmid content in order to identify adaptive traits.

Methods

Study Participants

The study was approved by The Danish National Committee on Biomedical Research Ethics and the Danish Data Protection Agency. The study included adult participants only, and informed written consent was obtained for all participants prior to participation.

Isolates in this study have been described in detail by Nielsen *et al.* (Nielsen et al., 2014a). Briefly, we recruited otherwise healthy women with UTI from their own general practitioner in Zealand, Denmark. All participants delivered a urine sample and a faecal swab on the day of inclusion. Inclusion criteria: UTI symptoms, and having 10^4 *E. coli*/mL and leucocytes in the urine sample.

Bacterial Isolates

From the collection of faecal and urinary isolates we analysed the genomes of 45 faecal and urinary pairs with matching RAPD profile as described by Nielsen *et al.* (Nielsen et al., 2014a). Classification of the isolates into the four phylogroups (A, B1, B2, D) and non-typeables (NT) was performed using the multiplex PCR of Clermont *et al.* (Clermont et al., 2000), with an additional PCR for *ibeA*, as recommended by Gordon *et al.* (Gordon et al., 2008), as previously described (Nielsen et al., 2014a).

Sequencing, Assembly and Annotation

Total DNA was purified using DNeasy Blood and Tissue kit (Qiagen), following manufacturer's instructions. All isolates were subject to whole genome sequencing on HiSeq 2000 (Illumina). The UTI isolates were sequenced using both 180 bp fragment libraries and 3 kb mate-pair libraries, as previously described (Grad et al., 2012). Forty-five faecal isolates with similar RAPD and identical phylogroup to the UPEC isolate of the same patient were sequenced using 180 bp fragment libraries produced as previously described (Grad et al., 2012).

The assemblies were performed using ALLPATHS-LG and gene contents were determined for all genomes using Prodigal (Grad et al., 2012). Briefly, annotations were based on BLAST hits against the Swiss-Prot protein database (70% identity and 70% query

coverage), TIGRfam, HMMER, and Pfam. The gene content was grouped into orthologous clusters across the complete set of genomes using Reciprocal best hit BLAST. Core genes were defined as at least one copy in the genomes of all included isolates, auxiliary genes were defined as missing from the genome of at least one isolate.

Phylogenetics and Typing

Phylogenetic reconstruction was performed using FastTree (Price et al., 2009) on phylogenetic informative sites in 3083 single copy core genes of all 90 isolates. This was identified by Reciprocal best hit BLAST and single linkage clustering. MLST types and serotypes were determined using MLST 1.8 (Larsen et al., 2012) and SeroTypeFinder 1.1 (Joensen et al., 2015), respectively (<http://www.genomicepidemiology.org/>).

Mutations

Mutations were identified by pairwise single nucleotide polymorphism (SNP) calls for each of the 45 RAPD pairs. The UTI isolates were chosen as reference as their genomes were assembled using a combination of paired-end and mate-pair libraries, producing a higher quality draft genome with respect to sequencing depth and scaffold counts. We aligned reads of the faecal isolates against the matching UTI genome using BWA (Li and Durbin, 2009) with identification of SNPs using GATK (McKenna et al., 2010), with the following criteria: >90% agreement among reads, 5 unambiguously mapped reads, 50% mapping ambiguity. Raw variant call format (VCF) files were annotated using VCFannotator (<http://vcfannotator.sourceforge.net>). Insertions/deletions were ignored. SNPs in or adjacent to structural phage proteins and transposases were ignored due to the potential presence of multiple copies across the genome. Mutations were verified by manual inspection of the *de novo* assembly of the UTI genome itself and against the genome of its paired faecal isolate.

Mobilome analyses

Phage content of each isolate was determined applying the PHAST database (Zhou et al., 2011) with a cut-off value of 60% similarity. The sequences of all phages were identified in the concatenated genomes of both urinary and faecal isolates, and Illumina reads of the matching isolate were mapped towards these regions to confirm loss/gain between matching isolates.

A plasmid BLAST database that included all complete *E. coli* plasmids (N=287) found at NCBI (database created March 2014) was used to identify possible plasmids in the sequenced isolates. A BLAST search was performed with each plasmid against the 90 concatenated genomes with >80% identity as cut-off value. The results were verified by individual reference mapping of the respective paired-end reads against the plasmid sequence for both isolates in a pair. The isolates were considered to carry identical plasmids when the reference mappings were indistinguishable.

in vivo Virulence

The experiments were permitted by the Animal Experiments Inspectorate under the Danish Ministry of Food, Agriculture and Fisheries. The *in vivo* virulence was investigated in a mouse UTI model (Hvidberg et al., 2000; Jakobsen et al., 2011) with five faecal/UTI pairs:

Pair #1, #3, #11, #18 and #39. Briefly, mouse bladders were emptied prior to transurethral inoculation with 50 µl of bacterial suspension (10^9 CFU/ml). Each *E. coli* isolate was inoculated into eight outbred female albino OF1 mice (28 to 32 g; Charles River Laboratories). For the faecal and UTI isolate of pair #39 it was only possible to inoculate 6 and 7 mice, respectively. Urine was collected 72 hrs. post inoculation and mice were euthanized by cervical dislocation, followed by removal of the bladder and kidneys. Bacterial counts were determined for urine, bladder, and kidneys. Urine samples were processed on the same day, whereas kidneys and bladders were stored in 0.9% saline solution at -80°C until processed. The detection limit was 25 CFU/ml.

Results

Samples and Matching Pairs

Forty-five matching UTI and faecal *E. coli* pairs from 45 patients were identified and compared by RAPD PCR as previously described (Nielsen et al., 2014a). The isolates were subsequently whole genome sequenced. Phylogenetic analyses, based on 3083 single copy core genes revealed that 42 of these were identical pairs, and three pairs differed significantly in their core content (marked with red, orange and yellow squares, respectively; Fig 1). Analyses revealed that the non-matching UTI isolates and their expected faecal counterparts differed by 28,599 (pair #26), 38,351 (pair #45) and 100,716 (pair #6) SNPs, respectively. These were excluded from further analyses. The phylogenetic comparison of the pairs indicated that RAPD PCR was successful in pairing the faecal and UTI isolates (error rate of 6.7%). The 42 matching pairs belonged to phylotypes A (n=3), B1 (n=1), B2 (n=26), D (n=11) and NT (n=1) respectively. Serotypes and MLST types were determined (Supplementary table S2).

Number of Mutations between Pairs

For the remaining 42 pairs, we identified a total of 409 coding (n=265) and non-coding (n=144) mutations with a range of 1-31 (0-8 non-coding and 1-25 coding) between the pairs (Table 1, supplementary figure S1). Overall, 107 non-synonymous (NSY) mutations, with a range of 0-12 in each pair, were identified (complete list of NSY in supplementary table S3). A substantial part of these were positioned in hypothetical proteins (n=42) to which no function had been assigned (Table S3). Eight pairs had no NSY mutations.

The analyses identified 256 intergenic mutations across 11 pairs in the *fim* switch (excluded from the number of non-coding mutations in Table 1), responsible for regulation of type 1 fimbrial expression by inversion of a DNA fragment flanked by inverted repeats (Gally et al., 1996, 1994). The orientation of this fragment determines the transcription of the fimbrial genes, hence fimbriation, in an on/off manner. It is assumed that these mutations are not true polymorphisms, but due to the inverted repeats, hence, the exclusion.

As isolates of phylotype B2 and D are the most common phylotypes in UTI, one could hypothesize that isolates of these phylotypes were more readily prepared to the urinary environment. This hypothesis assumed that the mutations were selective rather than random. However, we observed no correlation between phylotype of the isolates and number of

mutations in the individual pairs; the three pairs with the highest and lowest number of mutations belonged to phylotype B2 and D (data not shown).

Genes with Non-synonymous Mutations

In the 42 pairs, we identified 24 NSY mutations in UTI and virulence-associated genes (Table 2). Three of the 42 pairs had variations in antigen 43 (ag43) (a total of four mutations), and were positioned in the region encoding the α -domain (n=3) or β -domain (n=1) associated to aggregation and membrane-association, respectively. Additionally, we identified five mutations in total across three pairs of isolates in the *yee*-locus controlling cell growth and death under stress conditions (Masuda et al., 2012); two isolates had one mutation in toxin *yeeV*, and one isolate had three mutations in antitoxin *yeeU* (Table 2). Two pairs each had one mutation in *hlyB* responsible for haemolysin transport out of the cell (Cross et al., 1990; Hughes et al., 1983) (Table 2). Finally, UTI-faecal pair #3 was observed to have three NSY mutations in the *sfaH* gene encoding an S-fimbrial subunit.

FimH has been described to have UTI specific adaptive mutations (Hommais et al., 2003; Sokurenko et al., 1998); however, we did not identify any such mutations in the current study.

Mutations in metabolism genes were also observed among the 42 pairs (Table 3). These were mainly observed in genes encoding enzymes relevant for nutritional acquisition. With respect to the local environment surrounding the bacteria, we observed one NSY mutation in cold-shock protein, CspE, as well as a premature stop codon in the gene encoding a protein involved in acid resistance (YdeP) (Table 3). The sequence downstream of the truncated *ydeP* belongs to IS element 1 (*insA* and *insB*) (Escoubas et al., 1994), indicating insertion of a mobile element as cause of gene disruption.

Phages

The phage content was studied for the 42 paired isolates, in order to identify any changes between the matching isolates. Overall, we found identical phage content between the pairs, with only minor differences in three phages between the pairs (pair #5, 12, and 21). Pair #5 had an additional gene (host factor gene) in the urine isolate compared to the faecal isolate (1788 bp). Pair #12 had two additional open reading frames (hypothetical) in a single phage in the urine isolate, compared to the faecal isolate (1170 bp). Finally, pair #21 had an additional fragment (phage tail fiber proteins 07) compared to the faecal isolate (3021bp).

Plasmids

Plasmid contents of the 84 isolates were determined. Two UTI isolates had lost a plasmid, and two UTI isolates had gained a plasmid, compared to the faecal clone. We expect that the uptake of plasmids has occurred in the faecal environment, before transversion to the urinary tract. The plasmid lost by pair #1 was a small plasmid with no virulence or antimicrobial resistance genes predicted from the annotation (identical to GenBank ID DQ995352). Pair #40 had lost a plasmid highly similar to plasmid pHUSEC41-1 (GenBank ID HE603110) (only differences in a *rep* gene and a gene encoding a transposase). This plasmid, encoding e.g. aminoglycoside, streptomycin and β -lactam resistance, was characterized in the

hemolytic uretic syndrome outbreak in Germany in 2011 (Künne et al., 2012). The additional plasmid in the UTI isolate from pair #5 was similar to plasmid pUM146 (GenBank ID CP002168.1), which contains putative virulence-associated factors, such as type IV secretion, iron uptake systems, and antimicrobial peptide transport. Finally, the UTI isolate of pair ID #26 had an additional plasmid compared to the faecal isolate, which was highly similar to plasmid sP1400_89 described in *Salmonella enterica* Enteritidis, increasing its invasiveness and promoting growth of the bacteria (Coward *et al.* unpublished, GenBank ID JN796410.1).

***in vivo* Virulence**

In order to evaluate the *in vivo* virulence potential of both urine and faecal isolates we performed mouse UTI model studies of five pairs: UTI and faecal isolates of three pairs with antigen 43 mutations and two pairs with HlyB mutations (Fig. 2a and 2b, respectively). We performed *in vivo* virulence experiments on these isolates, as we found more than one pair with mutations in these genes in addition to the SNPs being positioned in UTI relevant genes (Cross et al., 1990; Ejrnæs et al., 2011; Hughes et al., 1983). The results showed no detectable difference in *in vivo* virulence in pairs when comparing the faecal isolate to the UTI counterpart, except for one pair, #39 (a HlyB mutant), where we observed lower CFU counts in urine for the UTI isolate compared to the faecal isolate (Fig. 2a). There was, however, no difference in CFU levels of bladder and kidneys for pair #39 (Fig. 2a).

Discussion

The present study is the first to compare a large collection of matching faecal and UTI *E. coli* isolates from the same patients by whole genome sequencing. The genomes and resulting scaffolds were of excellent quality due to the mate-pair sequencing of the UTI isolates, enabling us to do in depth genome analyses with a reliable outcome (supplementary table S1). Overall the pairs were highly similar. However, noteworthy in this study, variations, which distinguish the UTI isolate from its corresponding faecal counterpart were identified, including mutations in virulence associated, nutrient- and DNA-metabolism genes as well as uptake of plasmids. These changes could potentially have occurred en route from the faecal and nutrition rich environment to the urinary tract.

We identified NSY mutations in a number of proteins that serve functions in (1) biofilm formation (ag43), which is an important feature of UTI isolates; (2) in SfaH, necessary for the formation of S-fimbria important for adhesion during urinary tract infection (Herías et al., 2001; Kreft et al., 1995; Schmoll et al., 1989); (3) in HlyB, responsible for export of α -haemolysin (a UTI virulence factor); (4) in cold-shock and acid resistance proteins (CspE and YdeP), possibly involved in adapting to environmental change were also found in a few pairs; and finally, (5) in the Yee proteins encoding a toxin / antitoxin pair. All of the genes with mutations in more than one pair are thought important for UTI development: The *yee*-locus has previously been described to be under positive selection in virulent *E. coli* (Brown and Shaw, 2003; McNally et al., 2013; Petersen et al., 2007), α -haemolysin expression has very recently been shown to be involved in UPEC fitness (Nagamatsu et al., 2015) and finally, ag43 has been correlated to UTI virulence (Ejrnæs et al., 2011; Ulett et al., 2007).

The mutation rate of wild type *E. coli* is approximately 10^{-3} per genome per generation (Lee et al., 2012). For some UPEC, this has been shown to be 100-1000 fold greater (Lee et al., 2012), and mutator strains may be correlated to persistent UTI infections (Denamur and Matic, 2006). Based on the number of identified mutations, we do not suspect any hyper-mutators among the 42 pairs. The study included one isolate from the faecal and urinary samples, respectively. Taken into account possible intra-clonal variation, the observed number of mutations between the two environments is likely to be even smaller than represented here. Therefore the range of total mutations in this study demonstrates that movement to the urinary tract in most instances required no or very few mutations based on 42 paired isolates. The observed mutations could be of either adaptive or random nature, although the authors consider it highly unlikely that, more than one pair had mutations in the same gene (as was the case for ag43, *hlyB* and the *yee*-locus) if this gene was not under selection.

ag43 was a hotspot for variation as we identified four mutations in three pairs. ag43 has previously been shown to be correlated to UTI virulence and biofilm production (Anderson et al., 2003; Danese et al., 2000; Ejrnæs et al., 2011; Henderson et al., 1997; Ulett et al., 2007). Three of the variations identified in this study are situated in the α -domain of the molecule; the domain responsible for the actual aggregation (Heras et al., 2013), whereas the last mutation is present in the β -domain that forms the outer membrane protein (Hasman et al., 1999). The crystal structure of ag43 has recently been resolved, revealing details regarding the aggregation (Heras et al., 2013). The aggregation is caused by self-aggregation of α -domains by hydrogen bonds between the molecules. One of the identified mutations is a neighboring amino acid to one of these hydrogen bonds, suggesting that this amino acid change could influence aggregation. The *in vivo* investigation of UTI virulence of ag43 mutants in a mouse UTI model did not show detectable differences in virulence potential despite ag43 mutations. This indicates (i) that genes with these mutations are selected for although they do not directly influence the *in vivo* virulence potential in the used UTI mouse model or (ii) that the difference in virulence was lower than the sensitivity of the model.

As we observed no adaptation in FimH on the route of transmission, as has been described elsewhere (Hommais et al., 2003; Ronald et al., 2008), we expect that such polymorphisms do not occur on the route of transmission to the bladder or in the bladder, but rather in the intestinal tract. Chen *et al.* (Chen et al., 2013) identified fitness variations to be correlated to differences in the carbohydrate and amino acid utilization profiles. The present study on a larger collection of isolates indicates that nutrient acquisition, in some of the same pathways for nutrient acquisition as identified by Chen *et al.* (Chen et al., 2013), could be adaptive and possibly correlated to fitness improvement. This should be investigated further.

We did not identify any major changes in phage content between the matching isolates. The observed differences in phage content could be due to recombination events in all three isolates. We identified changes in plasmid content for four pairs, hence, approximately 10% of the pairs. Plasmids are often large elements and frequently associated with fitness loss in the host strain (Dahlberg and Chao, 2003), but they are at times essential for survival, e.g. under selection pressure of antibiotics (Subbiah et al., 2011). Pair #40 lost a plasmid with antimicrobial resistance, possibly due to lack of antibiotic selection pressure, as the patient

had not received antibiotics within a year of sampling. Alternatively, the patient could have consumed the antimicrobial resistant isolate. The plasmids acquired by two UTI isolates contain putative virulence factors or have been associated to increased virulence in bacteria, and it is therefore possible that the UTI virulence of these isolates were increased compared to the faecal counterpart. The phages and plasmids were not completely assembled, as the genomes were not closed, thus, these results only indicate the level of variation in the mobilome.

Mutations can affect pathogenesis of the isolates. The mutations in this study could have occurred already before the clone colonized the perineum, urethra and bladder, or could also have taken place once the bacterium settled in the bladder. The present study included one isolate from each predicted faecal clone and one UTI isolate, which limits predictions about the within-strain diversity of both the UTI and faecal clone, hence, the observed differences could also be a consequence of simple intra-clonal variation caused by normal genetic drift. The number of mutations identified across the 42 pairs are normally distributed a part from four outliers (20, 22, 23 and 32 mutations, figure S1). As the isolates were randomly picked, the statistics indicate that we would not expect to find any greater variation within faecal clones than what was observed for faecal-UTI pairs in this study.

Whether the observed mutations affect the pathogenesis of the isolates is difficult to demonstrate *in vitro* and *in vivo*. The urine counts of pair #39 were significantly higher for the UTI isolate compared to the faecal counterpart. Urine counts often have a higher variation compared to bladder and kidney counts, due to the often very sparse sample material. As there was no difference in bladder and kidney counts for this pair, it is assumed that the difference observed in urine is a sampling error rather than a true difference.

Numerous genes are involved in e.g. biofilm formation and adhesion and none of the identified ag43 mutations were nonsense mutations leading to e.g. premature stop codons. Overall, the *in vivo* study revealed no differences in virulence. This could be due to the detection limit of such a model, or on the other hand the pathogenesis was not necessarily affected significantly by the mutations, rather just illustrate a genetic drift of the isolate. As the mutations were not nonsense mutations, did not include mutations at identical positions across the pairs indicating adaptation and the mutations investigated in the mouse UTI model showed no detectable difference in virulence, we assume the effect on pathogenesis to be below the sensitivity of *in vitro* and *in vivo* models.

Conclusion

Taken together, the 42 UTI isolates were generally highly similar to the faecal clones with respect to point mutations, phage- and plasmid-content, suggesting that the bacteria were fit to colonize both bladder and gut without major adaptation. However, we find interesting signs of adaptations in a few pairs without detecting different *in vivo* UTI virulence of those investigated in a mouse UTI model. UTI seems to develop due to exposure to an *E. coli* fit for both the faecal and urinary environment as well as due to host specific factors, including individual immune status and host behavioral factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Tree scale: 0.001

Fig 1.
Core content phylogeny (3083 genes) of 45 RAPD pairs. Colored squares indicate non-matching isolates.

Fig. 2.
in vivo virulence of selected pairs with non-synonymous mutations. Left column: Pair ID #3 and #39 with HlyB mutations, CFU count in urine (a), bladder (b) and kidneys (c). Right column: Pair ID #11, #18 and #1, all containing mutations in antigen 43, CFU count in urine (d), bladder (e) and kidneys (f). Closed circles: Faecal isolates, open circles: UTI isolates. The mutations between the isolates did not infect the *in vivo* virulence, apart from #39 where

the faecal isolate caused higher CFU levels with the faeces isolate compared to the urine isolate (a).

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Table 1

Number of mutations in the 42 pairs

Pair ID	Total mutations [*] N=409	Non-coding [†] > N=144	Coding		
			Total N=265	NSY [§] N=107	SYN [‡] N=158
1	20	3	17	8	9
2	22	5	17	7	10
3	10	3	7	4	3
4	1	0	1	0	1
5	9	3	6	3	3
7	3	0	3	0	3
8	5	2	3	0	3
9	15	5	10	3	7
10	10	3	7	1	6
11	31	6	25	12	13
12	6	3	3	1	2
13	13	4	9	5	4
14	5	1	4	1	3
15	7	5	2	0	2
16	15	5	10	3	7
17	11	6	5	3	2
18	15	4	11	3	8
19	7	2	5	0	5
20	10	2	8	2	6
21	6	2	4	2	2
22	10	8	2	1	1
23	9	4	5	3	2
24	7	6	1	0	1
25	9	3	6	3	3
27	8	4	4	3	1
28	23	3	20	5	15
29	6	1	5	0	5
30	7	2	5	2	3
31	5	2	3	2	1
32	6	4	2	1	1
33	9	3	6	5	1
34	9	6	3	2	1
35	7	4	3	1	2
36	7	3	4	2	2
37	5	1	4	4	0
38	10	4	6	3	3
39	8	3	5	2	3
40	14	8	6	2	4

Pair ID	Total mutations [*] N=409	Non-coding [†] N=144	Coding		
			Total N=265	NSY [§] N=107	SYN [‡] N=158
41	8	2	6	1	5
42	9	3	6	4	2
43	6	2	4	3	1
44	6	4	2	0	2

^{*} The total number of mutations was defined as all mutations identified excluding those in SNPs in structural phage proteins, transposases as well as insertions/deletions.

[§]NSY: Non-synonymous mutations

[‡]SYN: synonymous mutations

[†] Mutation analyses identified 256 intergenic mutations within the fim-switch in 11 pairs which were not included in this table.

Table 2

Non-synonymous mutations in genes associated with virulence or UTI.

Pair ID	Gene	Position *	Amino acid Substitution
11	antigen 43	1382:aTc-aCc	Ile-461-Thr
18	antigen 43	2603:cAg-cTg	Gln-868-Leu
1	antigen 43	923:cTg-cAg	Leu-308-Gln
1	antigen 43	974:aTa-aCa	Ile-325-Thr
17	antitoxin YeeU	43:Cac-Gac	His-15-Asp
17	antitoxin YeeU	50:cGc-cAc	Arg-17-His
17	antitoxin YeeU	82:Tcc-Ccc	Ser-28-Pro
25	toxin YeeV	215:cAg-cTg	Gln-72-Leu
23	toxin YeeV	130:Tcc-Gcc	Ser-44-Ala
28	Fe(3+) dicitrate transport system permease fecD	398:cCc-cAc	Pro-133-His
33	filamentous hemagglutinin family domain-containing protein	667:Ata-Gta,	Ile-223-Val
39	flagellar motor switch protein FlhN	268:Gac-Aac	Asp-90-Asn
3	haemolysin activator HlyB	1016:cGg-cTg	Arg-339-Leu
39	haemolysin activator HlyB	277:Gtg-Atg	Val-93-Met
28	protein rhsB	1549:Ttc-Ctc	Phe-517-Leu
28	protein rhsC	1180:Gac-Aac	Asp-394-Asn
21	rhs element Vgr protein	721:Tcc-Acc	Ser-241-Thr
11	regulator PapX protein	308:aCg-aTg	Thr-103-Met
3	S-fimbrial protein subunit SfaH	763:Gca-Aca	Ala-255-Thr
3	S-fimbrial protein subunit SfaH	772:Tct-Gct	Ser-258-Ala
3	S-fimbrial protein subunit SfaH	787:Ata-Gta	Ile-263-Val
33	TonB-dependent heme/hemoglobin receptor family protein	161:gAt-gCt	Asp-54-Ala
11	type 1 fimbriae regulatory protein fimE	48:atA-atG	Ile-16-Met
16	type VI secretion protein	1454:cCc-cAc	Pro-485-His

* Capital letters indicate nucleotide substitution

Table 3

Non-synonymous mutations in genes related to nutrient acquisition and local environment.

Pair ID	Gene	Position *	Amino acid Substitution
1	GTPase	524:gCa-gAa	Ala-175-Glu
1	L-xylulose/3-keto-L-gulonate kinase	65:cAc-cGc	His-22-Arg
2	taurine-binding periplasmic protein	523:Gtc-Atc	Val-175-Ile
5	protein CsiD	718:Gtg-Atg	Val-240-Met
11	cold shock-like protein CspE	179:gGc-gAc	Gly-60-Asp
11	periplasmic AppA protein	125:cGt-cAt	Arg-42-His
11	D-tagatose-1,6-bisphosphate aldolase subunit kbaZ	169:Ggc-Agc	Gly-57-Ser
11	PTS system protein	761:tAt-tGt	Tyr-254-Cys
11	protein ydeP	2239:Tga-Cga	STP-747-Arg
20	dihydrolipoyl dehydrogenase	1124:gAa-gCa	Glu-375-Ala
22	carboxysome structural protein, ethanolamine utilization	271:Aaa-Taa	Lys-91-STP
23	carbamate kinase	328:Cag-Gag	Gln-110-Glu
23	inner membrane protein	1894:Tca-Cca	Ser-632-Pro
27	maltodextrin phosphorylase	791:gTc-gCc	Val-264-Ala
28	Phosphomannomutase	661:Att-Gtt	Ile-221-Val
31	maltose regulon periplasmic protein	653:tCg-tTg	Ser-218-Leu
32	tautomerase ppt	176:gAa-gCa	Glu-59-Ala
33	GTPase	784:Gtt-Att	Val-262-Ile
33	oxygen-insensitive NADPH nitroreductase	214:Taa-Caa	STP-72-Gln
34	Phosphomannomutase	880:Gtt-Att	Val-294-Ile
37	phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive	198:gaA-gaT	Glu-66-Asp
37	carbon storage regulator	34:Ttc-Ctc	Phe-12-Leu
37	carbon storage regulator	32:aCc-aTc	Thr-11-Ile
37	undecaprenyl-phosphate galactose phosphotransferase WbaP	238:Gaa-Taa	Glu-80-STP
40	Ecotin	56:tTg-tGg	Leu-19-Trp
42	serine/threonine-protein phosphatase 1	126:gaT-gaA	Asp-42-Glu
42	inorganic phosphate transporter 1	1265:cAg-cCg	Gln-422-Pro
43	ribosomal RNA large subunit methyltransferase L	664:Tgt-Cgt	Cys-222-Arg

STP: Stop codon

* Capital letters indicate the codon position of the mutation