

SUPPLEMENTARY MATERIALS

Table S1. Prevalence of *cnf1*, *hek*, and *hlyA* among lower urinary tract UPEC and the ECOR set by multiplex PCR.

| Gene | UPEC (n=163) Prevalence (%) | ECOR (n=72) Prevalence (%) | Significance (Fisher's Exact) |
|-----------------|--------------------------------|-------------------------------|----------------------------------|
| <i>cnf1</i> | 75 (46) | 6 (8.3) | $P < 0.0001$ |
| <i>hek</i> | 78 (47.9) | 12 (16.7) | $P < 0.0001$ |
| <i>hlyA</i> | 72 (44.2) | 10 (13.9) | $P < 0.0001$ |
| All three genes | 70 (42.9) | 6 (8.3) | $P < 0.0001$ |

Table S2. Leucine codon usage for *fim* recombinases and *fim* operon genes of UPEC strain UTI89

| Genes | Leucine Codon Count (% of Total Leucines) | | | | | | Total Leucines |
|-------------|---|------------|------------|------------|----------------|------------|-------------------|
| | <u>CUC</u> | <u>CUG</u> | <u>CUA</u> | <u>CUU</u> | <u>UUG</u> | <u>UUA</u> | |
| <i>fimB</i> | 1 (4.5) | 3 (13) | 1 (4.5) | 6 (27) | 5 (23) | 6 (27) | 22 |
| <i>fimX</i> | 3 (13) | 4 (17) | 1 (4.3) | 8 (35) | 2 (9) | 5 (22) | 23 |
| <i>fimE</i> | 2 (11) | 5 (28) | 0 (0) | 6 (33) | 1 (5.6) | 4 (22) | 18 |
| <i>fimA</i> | 0 (0) | 11 (65) | 1 (5.9) | 0 (0) | 2 (12) | 3 (18) | 17 |
| <i>fimI</i> | 1 (5.6) | 6 (33) | 3 (17) | 2 (11) | 2 (11) | 4 (22) | 18 |
| <i>fimC</i> | 1 (3.8) | 7 (27) | 1 (3.8) | 4 (15) | 8 (31) | 5 (19) | 26 |
| <i>fimD</i> | 16 (19) | 25 (30) | 3 (3.6) | 8 (9.5) | 10 (12) | 22 (26) | 84 |
| <i>fimF</i> | 0 (0) | 7 (28) | 7 (28) | 7 (28) | 4 (16) | 0 (0) | 25 |
| <i>fimG</i> | 2 (14) | 0 (0) | 1 (7.1) | 3 (21) | 5 (36) | 3 (21) | 14 |
| <i>fimH</i> | 1 (4.5) | 8 (35) | 0 (0) | 5 (23) | 3 (14) | 5 (23) | 22 |

Table S3. Bacterial Strains and Plasmids.

| Strain | Genotype | Reference |
|-------------------|--|-----------------------|
| UTI89 | <i>Wt</i> | (Mulvey et al., 2001) |
| GGA1 | Spontaneous PAI II _{UTI89} deletion mutant: UTI89 <i>leuX</i> PAI II _{UTI89} ⁻ , initial <i>fimS</i> orientation phase ON | This study |
| TJH1 | Spontaneous PAI II _{UTI89} deletion mutant: UTI89 <i>leuX</i> PAI II _{UTI89} ⁻ , phase OFF | This study |
| TJH1 Comp | Restoration of <i>wt leuX</i> at native locus in TJH1: UTI89 <i>leuX</i> ⁺ , PAI II _{UTI89} ⁻ , phase OFF | This study |
| TJH1Δ <i>fimB</i> | Complete deletion of <i>fimB</i> in TJH1, phase OFF | This study |
| TJH1Δ <i>fimX</i> | Complete deletion of <i>fimX</i> in TJH1, phase OFF | This study |

| | | |
|--|--|-----------------------|
| TJH1 Δ <i>fimXB</i> | Complete deletion of <i>fimB</i> in TJH1 Δ <i>fimX</i> , phase OFF | This study |
| TJH2 | UTI89 Δ PAI II _{UTI89} : <i>leuX</i> ⁺ , PAI II _{UTI89} ⁻ | This study |
| UTI89 Δ <i>leuX</i> | Complete deletion of <i>leuX</i> , PAI II _{UTI89} ⁺ , phase OFF | This study |
| UTI89 <i>leuX</i> Δ G ₈₀ | 1 nt deletion in <i>leuX</i> : <i>leuX</i> ⁻ , PAI II _{UTI89} ⁺ , phase OFF | This study |
| UTI89 <i>leuX</i> Δ GC ₈₀₋₈₁ | 2 nt deletion in <i>leuX</i> : <i>leuX</i> ⁻ , PAI II _{UTI89} ⁺ , phase OFF | This study |
| UTI89 _{OFF} Δ <i>fimBE</i> | Δ <i>fimB</i> Δ <i>fimE</i> ; phase OFF | This study |
| UTI89 _{ON} Δ <i>fimBE</i> | Δ <i>fimB</i> Δ <i>fimE</i> ; phase ON | This study |
| UTI89 Δ <i>fimB</i> | Δ <i>fimB</i> , phase OFF | This study |
| UTI89 Δ <i>fimX</i> | Δ <i>fimX</i> , phase OFF | This study |
| UTI89 <i>att</i> _λ :PSSH10-1 | <i>spc</i> ^R | (Wright et al., 2005) |
| Plasmids | Relevant Genes-Sequences | Reference |
| pBAD33 | <i>araC</i> , P _{ara} : Cm ^R | |
| pTRC99a | <i>lacIq</i> ; P _{lac} : Amp ^R | |
| pBAD- <i>fimX</i> | <i>araC</i> ; P _{ara} -6xHN- <i>fimX</i> in pBAD33 | This study |
| pTRC- <i>fimB</i> | <i>lacIq</i> ; P _{lac} -HAT- <i>fimB</i> in pTRC-99a | This study |
| pCR-BLUNT | Kan ^R | Invitrogen |
| p <i>fimB-E</i> | <i>fimB</i> , <i>fimE</i> in pCR-BLUNT | This study |
| pCR- <i>fimX</i> | <i>fimX</i> in pCR-BLUNT with -1170 of 5' UTR | This study |
| pBAD- <i>leuX</i> | <i>leuX</i> in pBAD33 with -144 of 5' UTR | This study |

Table S4: Primer Sequences

| Primer | Sequence | Reference |
|---|---|------------|
| Δ <i>leuX</i> KO #1 | GGTGGCGTGCGACAGGTATAATCCACAACGTTTTCCGCAT ACCTCTTCAGGTGTAGGCTGGAGCTGCTTC | This study |
| Δ <i>leuX</i> G ₈₀ KO KD4 #1 | TGATTCAAAATCAACCGTAGAAATACGTGCCGGTTCGAGT CCGGCCTTCGCACCAAAAGTGTGTAGGCTGGAGCTGCTTC | This study |
| <i>leuX</i> G ₈₀ KO KD4 #2 | AGGTAGATAAATACTGAATTTTAGGCATAAAAAAAGACCT CAGTTGAGGTCTATTTACATATGGGAATTAGCCATGGTCC | This study |
| <i>leuX</i> GC ₈₀₋₈₁ KO KD4 #1 | TGATTCAAAATCAACCGTAGAAATACGTGCCGGTTCGAGT CCGGCCTTCG ACCAAAAGTGTGTAGGCTGGAGCTGCTTC | This study |
| TJH1 COMP #1 | CAGTTGATTCAAAATCAACCGTAGAAATACGTGCCGGTTC GAGTCCGGCCTTCGGCACCACACCGGTGTAGGCTGGAGCT GCTTC | This study |
| TJH1 COMP #2 | GCACGGATATGGGTGATTTTCAGACACAAAAAAGCCGCTC TTGAGCGACTCGATTTGCATAATGGGAATTAGCCATGGTC C | This study |
| <i>fimBE</i> KO #1 | GGCAGGAATAATCGCTAGGGACCTAAGCATTAGCATGATA ATAGCGTGTAGGCTGGAGCTGCTTC | This study |
| <i>fimBE</i> KO #2 | GTCTTGATTTTATTTGTTTTTTAACTTTATTATCAATTAGTT AAAATGGGAATTAGCCATGGTCC | This study |

| | | |
|------------------------------------|--|------------|
| fimB KO #2 | TATCGTAAGAATAATGTAGTTTTTAACACCCATCCCTGGTATCTCAACTATCATGGGAATTAGCCATGGTCC | This study |
| fimX KO #1 | GATAATGAGATTACATCGATAACGTTCTGATTGCAGGCATACTTATCTGGGTGTAGGCTGGAGCTGCTTC | This study |
| fimX KO #2, | CGAGCAGCATTACTGGCTGTGTATCTGACAGTATGCTGAA TATTTTCGATGATGGGAATTAGCCATGGTCC | This study |
| PHASE 1 | CCGTAACGCAGACTCATCCTC | This study |
| PHASE 2 | GACAGAACAACAATTGCCAG | This study |
| fim #14 | TGCTATCGATTCCAGGAAATACACAGTCTG | This study |
| fimX FLANK #1 | TAACGACCAAAAAAGTAAAGAACACCTTGC | This study |
| fimX FLANK #2 | GTAGGAATCTGACATTTTGAATCAGAAGGTA CTG | This study |
| fimX PCR/PROBE #1 | ACA ACTGTACACGGTGGCGTATGAC | This study |
| fimX PCR/PROBE #2 | ACAGGCATGACGTAACATATGAGGA | This study |
| PAI II _{UTI89} ko F | TTAACTCTCTCAAGGTCAACTGATATCAACGTACATCTACC AACACATGTATTCCGGGGATCCGTCGACC | This study |
| PAI II _{UTI89} ko R | TTAACTCTCTCAAGGTCAACTGATATCAACGTACATCTACC AACACATGTATTCCGGGGATCCGTCGACC | This study |
| PAI II _{UTI89} 5' FLANK F | TCCACAACGTTTTTCCGCATA | This study |
| PAI II _{UTI89} 5' INT R | GCGTATTCCCTCCCTGTTGG | This study |
| PAI II _{UTI89} 3' FLANK R | CGGATATGGGTGATTTTCAGACA | This study |
| 16s RT L | CAGCCACACTGGAACTGAGA | This study |
| 16s RT R | GTTAGCCGGTGCTTCTTCTG | This study |
| leuX RT L | GCCGAAGTGCGGAAATCGGTAGACGCA | This study |
| leuX RT R | GGCCGGACTCGAACC GG CACGTA | This study |
| cdiA 4661F | GCTATGCCACAGGCTCTCTGGA | This study |
| cdiA 5298R | CCCGAGGCGGTCAAGAAAATA | This study |
| hek 117F | AGCCGGTGCCTCTGTGATGT | This study |
| hek 584R | CCAAGGCTCCATGCGAAGTT | This study |
| cnf1 1541F | TTCTCTGGACTCGAGGTGGTGG | This study |
| cnf1 1847R | CCCCAGCCGTATGATAAGAGG | This study |
| hlyA 114F | AGCAGAGCAAACCCGCAATG | This study |
| hlyA 314R | CGTTCGGTGAGGCCAATGAGT | This study |

References

- Mulvey, M.A., Schilling, J.D. and Hultgren, S.J. (2001) Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun*, **69**, 4572-4579.
- Wright, K.J., Seed, P.C. and Hultgren, S.J. (2005) Uropathogenic *Escherichia coli* Flagella Aid in Efficient Urinary Tract Colonization. *Infect Immun*, **73**, 7657-7668.