

Additional File 1

1 **Table S1.** List of genes, sequences of primers, cycling conditions for PCR assays, and amplicon sizes.

| Assay | Gene | Primer sequence | PCR program ^a | Product size (bp) | Reference |
|--|-------------|--------------------------------|--|-------------------|-----------|
| <i>E. coli</i> confirmation | <i>uspA</i> | F: 5'-CCGATACGCTGCCAATCAGT-3' | 94°C/ 120 s, 70°C/ 60 s, 72°C/ 60 s (30 cycles) | 884 | 1 |
| | | R: 5'-ACGCAGACCGTAGGCCAGAT-3' | | | |
| Phylogenetic typing (Triplex method) | <i>chuA</i> | F: 5'-GACGAACCAACGGTCAGGAT-3' | 94°C/ 30 s, 55°C/ 30 s, 72°C/ 30 s (30 cycles) | 279 | 2 |
| | | R: 5'-TGCCGCCAGTACCAAAGACA-3' | | | |
| | <i>yjaA</i> | F: 5'-TGAAGTGTGAGGAGACGCTG-3' | | 211 | |
| | | R: 5'-ATGGAGAATGCGTTCCTCAAC-3' | | | |
| | TspE4.C2 | F: 5'-GAGTAATGTCGGGGCATTCA-3' | | 152 | |
| | | R: 5'-CGCGCCAACAAAGTATTACG-3' | | | |
| Phylogenetic typing (Quadruplex method) | <i>chuA</i> | F: 5'-ATGGTACCGGACGAACCAAC-3' | 94°C/ 5 s, 57°C/ 20 s (30 cycles) | 288 | 3 |
| | | R: 5'-TGCCGCCAGTACCAAAGACA-3' | | | |
| | <i>yjaA</i> | F: 5'-CAAACGTGAAGTGTGAGGAG-3' | | 211 | |
| | | R: 5'-AATGCGTTCCTCAACCTGTG-3' | | | |
| | TspE4.C2 | F: 5'-CACTATTCGTAAGGTCATCC-3' | | 152 | |

| | | | | | | |
|------------------|-----------------------------------|-----------------------------------|---|-----|---|--|
| | | R: 5'-AGTTTATCGCTGCGGGTCGC-3' | | 400 | | |
| | <i>arpA</i> (Group F) | F: 5'-AACGCTATTCGCCAGCTTGC-3' | | | | |
| | | R: 5'-TCTCCCCATACCGTACGCTA-3' | | 301 | | |
| | <i>arpA</i> (Group E) | F: 5'-GATTCCATCTTGTCAAAATATGCC-3' | | | | |
| | | R: 5'-GAAAAGAAAAAGAATTCCCAAGAG-3' | 94°C/ 5 s, 59°C/ 20 s (30 cycles) | 219 | | |
| | <i>trpA</i> (Group C) | F: 5'-AGTTTTATGCCCAGTGCGAG-3' | | | | |
| | | R: 5'-TCTGCGCCGGTCACGCCC-3' | 94°C/ 5 s, 57°C/ 20 s (30 cycles) | 489 | | |
| | <i>trpA</i> (Internal control) | F: 5'-CGGCGATAAAGACATCTTCAC-3' | | | | |
| | R: 5'-GCAACGCGGCCTGGCGGAAG-3' | | | | | |
| DEC ^b | <i>eae</i> | F: 5'-GACCCGGCACAAGCATAAGC-3' | 95°C/ 30 s, 54°C/ 90 s, 72°C/ 90 s (30 cycles) | 384 | 4 | |
| | | R: 5'-CCACCTGCAGCAACAAGAGG-3' | | | | |
| | <i>stx1</i> | F: 5'-ATAAATCGCCATTCGTTGACTAC-3' | 95°C/ 30 s, 52°C/ 60 s, 72°C/ 60 s (35 cycles) | 180 | | |
| | | R: 5'-AGAACGCCCACTGAGATCATC-3' | | | | |
| | <i>stx2</i> | F: 5'-GGCACTGTCTGAAACTGCTCC-3' | | 255 | | |
| | | R: 5'-TCGCCAGTTATCTGACATTCTG-3' | | | | |

| | | | | | |
|--------------------|-------------|-------------------------------------|---|------|---|
| | <i>ltA</i> | F: 5'-GGCGACAGATTATACCGTGC-3' | 94°C/ 60 s, | 696 | |
| | | R: 5'-CCGAATTCTGTTATATATGTC-3' | 50°C/ 60 s, | | |
| | <i>st1A</i> | F: 5'-TCTGTATTATCTTTCCCCTC-3' | 72°C/ 120 s | 186 | |
| | | R: 5'-ATAACATCCAGCACAGGC-3' | (35 cycles) | | |
| | <i>ipaH</i> | F: 5'-GTTCTTGACCGCCTTCCGATACCGTC-3' | 94°C/ 40 s, | 620 | |
| | | R: 5'-GCCGGTCAGCCACCCTCTGAGAGTAC-3' | 60°C/ 60 s, 72°C/ 60 s (35 cycles) | | |
| | <i>bfpA</i> | F: 5'-ATTGAATCTGCAATGGTGC-3' | 95°C/ 40 s, | 461 | |
| | | R: 5'-ATAGCAGTCGATTTAGCAGCC-3' | 55°C/ 40 s, 72°C/ 40 s (30 cycles) | | |
| | <i>aggA</i> | F: 5'-ATGCATTACTTTGGGTTTAG-3' | 94°C/ 60 s, | 414 | |
| | | R: 5'-TCAACCTTGACACTTGCC-3' | 50°C/ 60 s, 72°C/ 120 s (35 cycles) | | |
| ExPEC ^c | <i>vat</i> | F: 5'-TCAGGACACGTTCAAGGCATTCACT-3' | 94°C/ 30 s, | 1100 | 5 |
| | | R: 5'-GGCCAGAACATTTGCTCCCTTGTT-3' | 63°C/ 90 s, | | |
| | <i>fyuA</i> | F: 5'-GTAAACAATCTTCCCGCTCGGCAT-3' | 72°C/ 90 s | 850 | |

| | | | | | |
|--|---------|-----------------------------------|--|-----|---|
| | | R: 5'-TGACGATTAACGAACCGGAAGGGA-3' | (30 cycles) | | |
| | chuA | F: 5'-CTGAAACCATGACCGTTACG-3' | | 652 | |
| | | R: 5'-TTGTAGTAACGCACTAAACC-3' | | | |
| | yfcV | F: 5'-ACATGGAGACCACGTTCCACC-3' | | 292 | |
| | | R: 5'-GTAATCTGGAATGTGGTCAGG-3' | | | |
| | kpsM K1 | F: 5'-TAGCAAACGTTCTATTGGTGC-3' | 94°C/ 30 s, 63°C/ 30 s, 68°C/ 3 min (25 cycles) | 153 | 6 |
| | | R: 5'-CATCCAGACGATAAGCATGAGCA-3' | | | |
| | kpsM II | F: 5'-GCGCATTTGCTGATACTGTTG-3' | 94°C/ 30 s, 58°C/ 30 s, 72°C/ 3 min (25 cycles) | 577 | 7 |
| | | R: 5'-AGGTAGTTCAGACTCACACCT-3' | | | |

2 ^aBefore starting the PCR cycle, DNA was first denatured at 95°C/15 min. After completion

3 of the cycle, there was a final primer extension at 72°C/8 min.

4 ^bDiarrheagenic *E. coli*

5 ^cExtra-intestinal pathogenic *E. coli*

References in Table 1

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50 **Table S2.** Sample collection dates and locations, and phylogenetic groupings of *E. coli* by
 51 triplex and quadruplex PCR assays.

| Date of sample collection | Location (no. of confirmed <i>E. coli</i>) | Phylogenetic grouping by triplex PCR (no. of isolates) | Phylogenetic grouping by quadruplex PCR (no. of isolates) |
|---------------------------|---|--|---|
| May 2018 | J ^a (1) Z ^b (2) | B1 (2) D (1) | B1 (2) D (1) |
| June 2018 | J (7) Z (2) H ^c (5) | A (6) B1 (3) D (5) | A (6) B1 (3) D (4) E (1) |
| July 2018 | J (1) Z (3) H (2) | A (3) B1 (1) B2 (1) D (1) | A (3) B1 (1) B2 (1) D (1) |
| August 2018 | J (6) Z (6) H (7) | A (9) B1 (5) D (2) | A (9) B1 (5) D (1) E (1) |
| September 2018 | J (2) Z (1) H (9) | A (8) B1 (3) B2 (1) | A (7) B1 (3) B2 (1) C (1) |
| October 2018 | J (4) Z (1) H (4) | A (4) B1 (3) D (2) | A (4) B1 (3) D (1) F (1) |

| | | | |
|------------------|--------------------------|------------------------------------|--|
| November 2018 | J (4) Z (1) H (4) | A (7) B1 (2) | A (7) B1 (2) |
| December 2018 | J (3) Z (4) H (3) | A (3) B1 (3) D (4) | A (3) B1 (3) D (2) F (2) |
| January 2019 | J (4) Z (5) H (5) | A (3) B1 (3) B2 (2) D (6) | A (3) B1 (3) B2 (2) D (3) F (1) E (2) |
| February 2019 | J (4) Z (2) H (2) | A (3) B1 (3) D (2) | A (3) B1 (3) F (2) |
| March 2019 | J (3) Z (6) H (3) | A (6) B1 (1) B2 (4) D (1) | A (6) B1 (1) B2 (4) D (1) |
| April 2019 | J (7) Z (7) H (10) | A (16) B1 (5) D (3) | A (14) B1 (5) D (1) F (2) C (2) |

52 ^aJabriya.

53 ^bZahraa.

54 ^cHateen.

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56 **Table S3.** Distribution of ExPEC according to location and date of collection of samples.

| Month/Year | No. of ExPEC isolates | Location | | |
|----------------|-----------------------|----------|--------|--------|
| | | Jabriya | Zahraa | Hateen |
| May/2018 | 1 | 0 | 1 | 0 |
| June/2018 | 7 | 2 | 1 | 4 |
| July/2018 | 2 | 0 | 1 | 1 |
| August/2018 | 8 | 1 | 2 | 5 |
| September/2018 | 2 | 0 | 0 | 2 |
| October/2018 | 4 | 1 | 0 | 3 |
| November/2018 | 3 | 2 | 1 | 0 |
| December/2018 | 5 | 2 | 2 | 1 |
| January/2019 | 7 | 2 | 3 | 2 |
| February/2019 | 4 | 0 | 2 | 2 |
| March/2019 | 7 | 2 | 3 | 2 |
| April/2019 | 7 | 2 | 0 | 5 |

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