

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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