

1 **SUPPLEMENTAL MATERIAL. TABLE S1.** PCR primers used in this study to detect VF-
 2 encoding genes

Pathovar	Gene	Primers (5' to 3')	pb ^a	Reference
First set of screened VF-encoding genes				
STEC (EHEC)	<i>stx1</i>	GAATGCAAAGACGTATGTAGATTG ATCTATCCCTCTGACATCAACTGC	150	(1)
	<i>stx2</i>	ATCCTATTCCCGGGAGTTACG GCGTCATCGTATAACACAGGAGC	584	(2)
EHEC EPEC	<i>eae</i>	GAACGGCAGAGGTTAATCTG CAATGAAGACGTTATAGCCC	203	(3)
EPEC	<i>bfpA</i>	AATGGTGCTTGCCTGCTGC GCCGCTTTATCCAACCTGGTA	330	(4)
ETEC	LT	GGCGACAGATTATACCGTGC GCGAGAGGAACACAAACCGG	581	(5)
	STa	TCCGTCAAACAACATGACGG ATAACATCCAGCACAGGCAG	244	(6)
	STb	GCCTATGCATCTACACAATC TGAGAAATGGACAATGTCCG	279	(6)
ETEC EAEC	<i>astA</i>	GCCATCAACACAGTATATCC GAGTGACGGCTTGTAGTCC	106	(7)
NTEC	<i>cnf</i>	TTATATAGTCGTCAAGATGGA CACTAAGCTTACAATATTGA	760	(8)
Second set of screened VF-encoding genes				
Various pathovars	<i>ehxA</i>	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAACGCT	534	(9)
	<i>subAB</i>	GCAGATAAAATACCCTTCACTTG ATCACCACTCCACTCAGCC	232	(10)
	<i>papC</i>	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	328	(11)
	<i>cdtB</i>	GCCGCCACCTGCCTTAC GCGCATCCGCCACTACTGAAA	321	(12)
	<i>espP</i>	AAACAGCAGGCACTTGAACG GGAGTCGTCACTCAGTAGAT	1832	(13)
	<i>toxB</i>	ATACCTACCTGCTCTGGATTGA TTCTTACCTGATCTGATGCAGC	302	(14)
	<i>katP</i>	TTTAAAACGCTGGGATTGCTC CTCCTGAGAGGGCGTCAGTTC	1174	(15)
EAEC	<i>aggR</i>	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	(7)

3 ^aamplicon size

4 SUPPLEMENTAL MATERIAL. TABLE S2: VFs and CTX-M genes of 133 *E. coli* strains positive for at least *stx1*, *stx2*, *eae*, *asta*, *STa* or *cnf* genes

Adhesin	Phylogroup	Serotype	VFs genes											ESBL			
					Toxins							Intimin	p-O157				
			<i>papC</i>	<i>αH</i>	<i>cnf</i>	<i>stx1</i>	<i>stx2</i>	<i>STa</i>	<i>asta</i>	<i>cdtB</i>	<i>subAB</i>	<i>eae</i>	<i>espP</i>	<i>toxB</i>	<i>katP</i>		
None ^b	B1	O26:H11	-	-	-	-	-	-	-	-	-	eae β	-	-	-	no	1
None	B1	neg	-	+	-	-	-	-	-	-	-	eae β	-	+	+	no	1
None	B1	neg	-	-	+	-	-	-	-	+	-	eae θ	+	-	+	CTX-M-1	1
None	B1	O26:H11	-	+	-	stx1a	-	-	-	-	-	eae β	+	+	+	no	1
None	B1	neg	-	+	-	stx1a	-	-	-	-	-	eae θ	-	-	-	no	1
None	B1	neg	-	+	-	stx1a	stx2d	-	-	-	-	eae β	+	+	+	no	1
None	B1	O26:H11	-	+	-	stx1a	stx2a	-	-	-	-	eae β	-	-	+	no	1
None	B1	neg	-	+	-	-	stx2a	-	-	+	+	-	+	-	-	no	1
None	B2	neg	+	-	+	-	stx2b	-	-	-	-	-	-	-	-	no	1
None	A	ND	-	-	-	-	-	+	-	-	-	-	+	+	-	no	1
None	A	ND	-	-	+	-	-	+	-	-	-	-	-	-	-	no	1
None	A	neg	-	+	-	-	-	-	-	-	-	eae ND	-	-	-	no	1
None	C	ND	-	-	-	-	-	-	+	-	-	-	-	-	+	no	1
None	E	ND	-	-	-	-	-	-	+	-	-	-	-	-	-	no	1
None	C=2, E=1	ND	-	-	-	-	-	-	+	-	-	-	-	-	-	no	3
None	B1	ND	-	-	-	-	-	-	+	+	-	-	-	-	+	no	1
None	B1=3	ND	-	-	-	-	-	-	+	-	-	-	-	-	+	no	3
None	B1	ND	-	-	-	-	-	-	+	-	-	-	-	-	+	no	1
None	D	ND	-	-	-	-	-	-	+	-	-	-	-	-	-	no	1
None	C=2	ND	-	-	+	-	-	-	-	-	-	-	-	-	-	no	2
None	B1	ND	-	-	+	-	-	-	-	-	-	-	-	-	+	no	1
None	D	ND	-	-	+	-	-	-	-	-	-	-	-	-	-	CTX-M-9	1
																48,2	

F5 + F41	A	ND	-	-	-	-	-	+ - - -	-	+ - -	no	14	
F5 + F41	A	ND	-	-	-	-	-	+ - - -	-	- - -	no	1	
F5 + F41	A	ND	-	-	+	-	-	+ - - -	-	+ - -	no	1	
F5 + F41	A	ND	+	-	-	-	-	+ - - -	-	- - -	no	1	
F5 + F41	A	ND	-	-	-	-	-	+ - - -	-	- - +	no	2	
F5 + F41	A	ND	-	-	-	-	-	+ + - -	-	- - +	CTX-M-9	1	
F5 + F41	A	ND	-	-	-	-	-	+ - - -	-	+ - +	CTX-M-1	1	
F5 + F41	A	ND	-	-	-	-	-	+ + - -	-	- - -	no	1	
F5 + F41	B1	ND	-	-	-	-	-	+ - - -	-	- - -	no	1	
F5 + F41	B1	ND	-	-	-	-	-	+ + - -	-	- - -	CTX-M-1	1	
F5 + F41	C	ND	-	-	-	-	-	+ - - -	-	+ - -	no	1	
F5 + F41	B1	ND	-	-	+	-	-	+ - - -	-	- - -	no	1	100,0
F17Ac	A	ND	-	-	+	-	-	- - -	-	- - +	no	1	
F17And	A	ND	-	-	+	-	-	- + -	-	- - +	CTX-M-9	1	
F17Aab	B1	ND	-	-	+	-	-	- - +	-	- - +	no	1	
F17Ab	B1	ND	-	-	+	-	-	- - -	-	- - -	no	1	
F17Ab	B1=3	ND	-	-	+	-	-	- - +	-	- - +	no	3	
F17Abc	B1	ND	-	-	+	-	-	- - +	-	- - -	no	1	
F17And	B1	ND	-	-	+	-	-	- + -	-	- - +	no	1	
F17Aab	B2	ND	-	-	+	-	-	- - -	-	- - -	no	1	
F17Aac	C	ND	-	-	+	-	-	- - -	-	+ - -	no	1	
F17Ab	C	ND	-	-	+	-	-	- + -	-	- - -	no	1	
F17Ac	C	ND	-	-	+	-	-	- + -	-	- - +	no	1	
F17Ac	C	ND	-	-	+	-	-	- - -	-	- - +	CTX-M-1	1	
F17Aab	D	ND	-	-	+	-	-	- + -	-	- - -	no	1	
F17Aa	F	ND	-	-	+	-	-	+ + -	-	- - -	no	1	
F17Aa	C	ND	-	-	+	-	-	+ - -	-	- - -	no	1	
F17Aa	A	ND	-	-	-	-	-	+ - -	-	+ - -	no	1	
F17Aa	D	ND	-	-	-	-	-	+ - -	-	- - -	no	1	
F17Ac	F	ND	-	-	-	-	-	+ - -	-	- - -	no	1	
F17And	B2	ND	+	-	-	-	-	+ - -	-	+ - -	no	1	
F17Aa	A	ND	-	-	-	-	-	+ - -	-	- - -	no	1	29,7

5 ND, Not determined; (-) PCR negative; (+) PCR positive; H, haemolysin; F17And, untypeable F17A variant.

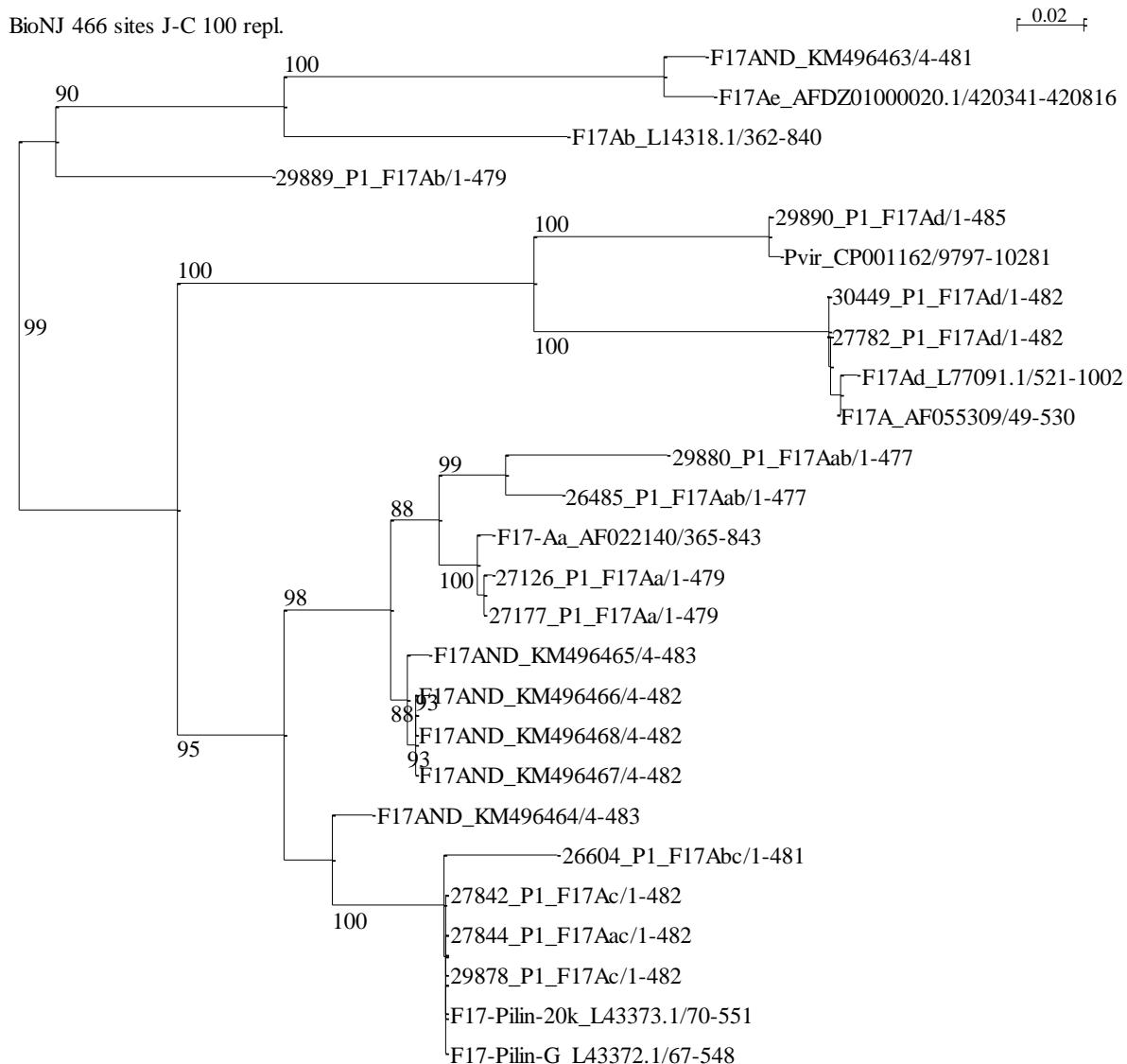
⁶when more than one isolate by lane was illustrated, the number of strains belonging to each phylogroup or CTX-M group was noticed.

7 b“none” group corresponding to strains which did not have any CS31A, F17, F5 or F41 adhesin tested.

8 ^cFrequency expressed by adhesin group

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10 **SUPPLEMENTAL MATERIAL. FIGURE S3:** Dendrogram showing the relationships
11 among the major fimbrial subunit F17A nucleotides sequences



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13 The phylogenetic tree of 25 F17A fimbrial subunit aligned nucleotide sequences was
14 determined by the neighbor-joining distance method BioNJ (16) using the Jukes and Cantor
15 (J-C) -parameter using Seaview4 (17). The tree was labeled with bootstrap numbers based on
16 100 replicate trees, only values above 75 are shown. The analysis involved 18 F17A- derived
17 PCR sequences using P1 primer (18) including F17Aa (n=2), F17Ab (n=1), F17Ac (n=2) and
18 F17Ad (n=3) variants, the combined subtypes F17Aab (n=2), F17Aac (n=1) and F17Abc
19 (n=1) and the untypeable (ND) F17A variants (n=6). The 6 F17AND variant- derived PCR
20 sequences using P1 and P2 primers were deposited in GenBank, the submission ID is:
21 1756755 (GenBank accession numbers KM496463, KM496464, KM496465, KM496466,

22 KM496467 and KM496468). Seven F17A reference nucleotide sequences (GenBank
23 accession numbers CP001162 (12), AF022140 (19), L14318.1 (20), AFDZ01000020.1,
24 L77091.1, AF055309 (21), L43373.1) were included in the analysis. The positions of the
25 targeted aligned nucleotide sequences obtained by using the Clustal Omega (22) from the
26 PCR or reference nucleotide sequences were noticed after the forward slashes.

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28 **SUPPLEMENTAL DATA REFERENCES**

- 29 1. **Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR.** 1990. Differentiation of Shiga
30 toxin and Vero cytotoxin type 1 genes by polymerase chain reaction. *J Infect Dis* **162**:1195-
31 1198.
- 32 2. **Cebula TA, Payne WL, Feng P.** 1995. Simultaneous identification of strains of *Escherichia*
33 *coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation
34 assay-multiplex PCR. *J Clin Microbiol* **33**:248-250.
- 35 3. **Clermont O, Olier M, Hoede C, Diancourt L, Brisse S, Keroudean M, Glodt J, Picard B,**
36 **Oswald E, Denamur E.** 2011. Animal and human pathogenic *Escherichia coli* strains share
37 common genetic backgrounds. *Infection, Genetics and Evolution* **11**:654-662.
- 38 4. **Gunzburg ST, Tornieporth NG, Riley LW.** 1995. Identification of enteropathogenic
39 *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *J Clin Microbiol*
40 **33**:1375-1377.
- 41 5. **Nataro JP, Kaper JB.** 1998. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* **11**:142-201.
- 42 6. **Ojeniyi B, Ahrens P, Meyling A.** 1994. Detection of fimbrial and toxin genes in *Escherichia*
43 *coli* and their prevalence in piglets with diarrhoea. The application of colony hybridization
44 assay, polymerase chain reaction and phenotypic assays. *Zentralbl Veterinarmed B* **41**:49-59.
- 45 7. **Fujioka M, Kasai K, Miura T, Sato T, Otomo Y.** 2009. Rapid diagnostic method for the
46 detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Jpn J Infect Dis* **62**:476-480.
- 47 8. **Van Bost S, Jacquemin E, Oswald E, Mainil J.** 2003. Multiplex PCRs for identification of
48 necrotoxigenic *Escherichia coli*. *J. Clin. Microbiol.* **41**:4480-4482.
- 49 9. **Paton AW, Paton JC.** 1998. Detection and characterization of Shiga toxigenic *Escherichia*
50 *coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA,
51 rfbO111, and rfbO157. *J Clin Microbiol* **36**:598-602.
- 52 10. **Slanec T, Fruth A, Creuzburg K, Schmidt H.** 2009. Molecular analysis of virulence profiles
53 and Shiga toxin genes in food-borne Shiga toxin-producing *Escherichia coli*. *Appl Environ*
54 *Microbiol* **75**:6187-6197.
- 55 11. **Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O.** 1995. Detection of
56 urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS*
57 *Immunol Med Microbiol* **12**:85-90.
- 58 12. **Johnson TJ, DebRoy C, Belton S, Williams ML, Lawrence M, Nolan LK, Thorsness JL.**
59 2010. Pyrosequencing of the Vir plasmid of necrotoxigenic *Escherichia coli*. *Vet Microbiol*
60 **144**:100-109.
- 61 13. **Leyton DL, Sloan J, Hill RE, Doughty S, Hartland EL.** 2003. Transfer region of pO113
62 from enterohemorrhagic *Escherichia coli*: similarity with R64 and identification of a novel
63 plasmid-encoded autotransporter, EpeA. *Infect Immun* **71**:6307-6319.
- 64 14. **Tarr PI.** 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of
65 human infection. *Clin Infect Dis* **20**:1-8.
- 66 15. **Gerrish RS, Lee JE, Reed J, Williams J, Farrell LD, Spiegel KM, Sheridan PP, Shields
67 MS.** 2007. PCR versus hybridization for detecting virulence genes of enterohemorrhagic
68 *Escherichia coli*. *Emerg Infect Dis* **13**:1253-1255.
- 69 16. **Gascuel O.** 1997. BIONJ: an improved version of the NJ algorithm based on a simple model
70 of sequence data. *Molecular biology and evolution* **14**:685-695.
- 71 17. **Gouy M, Guindon S, Gascuel O.** 2010. SeaView version 4: A multiplatform graphical user
72 interface for sequence alignment and phylogenetic tree building. *Molecular biology and*
73 *evolution* **27**:221-224.
- 74 18. **Bertin Y, Martin C, Oswald E, Girardeau JP.** 1996. Rapid and specific detection of F17-
75 related pilin and adhesin genes in diarrheic and septicemic *Escherichia coli* strains by
76 multiplex PCR. *J Clin Microbiol* **34**:2921-2928.
- 77 19. **Lintermans P, Pohl P, Deboeck F, Bertels A, Schlicker C, Vandekerckhove J, Van
78 Damme J, Van Montagu M, De Greve H.** 1988. Isolation and nucleotide sequence of the
79 F17-A gene encoding the structural protein of the F17 fimbriae in bovine enterotoxigenic
80 *Escherichia coli*. *Infect Immun* **56**:1475-1484.

- 81 20. **el Mazouari K, Oswald E, Hernalsteens JP, Lintermans P, De Greve H.** 1994. F17-like
82 fimbriae from an invasive *Escherichia coli* strain producing cytotoxic necrotizing factor type 2
83 toxin. *Infect Immun* **62**:2633-2638.
- 84 21. **Cid D, Sanz R, Marin I, de Greve H, Ruiz-Santa-Quiteria JA, Amils R, de la Fuente R.**
85 1999. Characterization of nonenterotoxigenic *Escherichia coli* strains producing F17 fimbriae
86 isolated from diarrheic lambs and goat kids. *J Clin Microbiol* **37**:1370-1375.
- 87 22. **McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R.**
88 2013. Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res* **41**:W597-600.
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- 90