

SUPPLEMENTARY MATERIAL

TABLE S1. *In silico* BLAST analysis for the phylogroup specific probes used

Probe	Strains ^a
p _{TspE4.C2} B1	O104:H4, W, IAI1, 55989, SE11, E24377A, KO11, B7A, E22, B171, E110019, O103:H2, B799, H591, TA271, H220, T426, TA141, B088, B574, H420, T408, E1167, H120, H494.
p _{chuA} B2	NA114, O83:H1, UM146, ABU 83972, SE15, LF82, ED1a, IHE3034, S88, O127:H6, APEC O1, 536, UTI89, CFT073, F11, TA104, H001, TA103, B671, M504, H296, H397, TA464, H252, M605, TA435, H588, TA255*, TA206, H305, TA280*, H413, H461, H223, H263, H378, B108, R527, H660.
p _{chuA} D	O55:H7, O7:K1, 042, UMN026, IAI39, SMS-3-5, O157:H7, TA143, FVEC 1412, B706, B354, TA024, FVEC 1302, TA124, M056, M114, TA249, H299, B185, TA054, FVEC 1465, M646, PUTI1459, M718, E1492, B093, TA447, B367, E101.

^aAll strains, except those indicated with a star, were correctly assigned *in silico* by the real-time PCR probes in accordance with the triplex PCR method (1). The TA255 and TA280 strains exhibit a *chuA* gene of phylogroup F but belong to the phylogroup D.

TABLE S2. Efficiencies and reproducibility of the assays

Probe	Slope of the standard curve	Intra-assay reproducibility (% CV ^a of Ct ^b)
p _{TspE4.C2} B1	-3.32	1.00
p _{chuA} B2	-3.42	0.87
p _{chuA} D	-3.46	0.57
p _{yjaA} A1/B2	-3.36	0.86

^aCoefficient of variation.

^bCycle threshold.

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TABLE S3. Distribution of *E. coli* phylogroups in the feces of 100 healthy subjects of Ile-de-France region and comparison between real-time assay and multiplex PCR assays

Subject	Real-time PCR assay	Triplex PCR assay ^a major clone	Triplex PCR assay ^a 20 to 30 clones	Correlation with major clone/20-30 clones ^c
1	99% A, 1% B2	A		1/ND
2	50% A, 50% B2	A		1/ND
3	100 % B2	B2		1/ND
4	63% A, 24 % B2, 13% D	A		1/ND
5	80 % B1, 20 % A	B1	95% B1, 5% A (20 clones)	1/1
6	63% B2, 37% A	A	50% B2, 50% A (20 clones)	0/1
7	82% A, 17 % B1, 1% B2	A		1/ND
8	66% B2, 34% A	D ^b (F)		1/ND
9	58% B2, 42% A	B2		1/ND
10	72% B2, 28%A	B2		1/ND
11	100% D	D	100% D (20 clones)	1/1
12	99 % A, 0.1% B2	A	93% A, 7 % B2 (30 clones)	1/1
13	90% A, 8.5% D, 1.5% B1	A		1/ND
14	100 % A	A		1/ND
15	60 % A, 40 % B1	A	70% B1, 30% A (20 clones)	1/1
16	100 % B2	D ^b (F)		1/ND
17	70 % A, 30% B2	A		1/ND
18	71 % A, 29% B2	A		1/ND
19	80 % A, 20% B2	B2		0/ND
20	57% B2, 30% A, 10% B1, 3% D	B2	73% A, 14% B1, 10% B2, 3% D (30 clones)	1/1
21	100 % B2	B2		1/ND
22	100% B2	B2		1/ND
23	78% B2, 22% A	B2		1/ND
24	70% A, 24% B1, 3% B2, 3% D	D	38% D, 31 % B1, 31 % A (30 clones)	0/0
25	80% B2, 20 % A	D ^b (F)		1/ND
26	100 % B2	B2		1/ND
27	95% B2, 5% D	B2		1/ND
28	98 % B2, 2% B1	B2		1/ND
29	100 % B1	B1	100% B1 (30 clones)	1/1
30	40 % B1, 31% A, 29% B2	A		1/ND
31	100 % B2	B2		1/ND
32	62% A, 32% B2, 3% D, 3% B1	B2		0/ND
33	75% B2, 24 % A, 1% B1	A		0/ND
34	92% A, 8% B2	B2		0/ND
35	99 % B1, 1% A	B1		1/ND
36	66 % A, 26 % D, 8 % B2	D ^b (F)		0/ND
37	100 % B2	B2		1/ND
38	99% B2, 1% D	B2		1/ND
39	98 % A, 2% B2	A		1/ND
40	82% A, 18% B1	B1		0/ND
41	85% D, 15% A	D	100% D1 (30 clones)	1/0
42	71% A, 26% B2, 3% B1	B2	57 % A, 40% B2, 3% B1 (30 clones)	0/1
43	70 % B1, 20% A, 2% D, 2% B2	D		0/ND
44	97% A, 2% B2, 1% B1	A	100% A (30 clones)	1/0
45	95% B1, 4% A, 0.1% B2	B1	100% B1 (30 clones)	1/0
46	99% A, 0.1% B1	A	100% A (30 clones)	1/0
47	98% B2, 2% D	B2		1/ND
48	100 % B2	B2		1/ND
49	100 % B2	B2		1/ND
50	75% A, 15 % B1, 9 % D, 1% B2	A		1/ND
51	45% B1, 35 % B2, 9% D, 11 % A	B2		1/ND
52	95% A, 3% B1, 2% B2	A		1/ND
53	100 % B1	B1		1/ND

54	100% A	A		1/ND
55	80% B2, 20%A	B2		1/ND
56	65% B2, 35%A	B2		1/ND
57	82% D, 18 % A	D		1/ND
58	100% B2	B2		1/ND
59	70% B2, 30% A	B2		1/ND
60	80% D, 18 % A, 1 % B1, 1% B2	D ^b (F)	70% F ^b , 15% A, 10% B1, 5% B2 (20 clones)	1/1
61	67% A, 32% B2, 1% D	A		1/ND
62	86% A, 11% B1, 2% B2, 1% D	A	80 % A, 20% B1 (30 clones)	1/0
63	65% D, 35% B1	D ^b (F)		1/ND
64	73% D, 17% B2, 10% A	D	43% D, 28.5% B2, 28.5% A (28 clones)	1/1
65	87% D, 13% A	D		1/ND
66	85% B2, 15% A	B2		1/ND
67	74% B1, 15 % A, 11% D	B1		1/ND
68	100% D	D ^b (F)		1/ND
69	92% A, 8% D	A		1/ND
70	60 % B2, 40% B1	B1		0/ND
71	68% A, 32% B1	A		1/ND
72	99% A, 1% B2	A		1/ND
73	100% A	A		1/ND
74	65% A, 25% D, 10% B2	A	57% A, 35 % D, 10 % B2 (30 clones)	1/1
75	92% D, 3% B2, 5% A	D		1/ND
76	86% A, 14% D	D		0/ND
77	82% B2, 18% A	D ^b (F)	77% F ^b , 23% B2 (30 clones)	1/0
78	80% B1, 17% A, 3% B2	A		0/ND
79	75% B2, 25% A	B2		1/ND
80	98% B1, 2% A	B1	97% B1, 3% A (30 clones)	1/1
81	80% B2, 17% A, 3% D	B2		1/ND
82	80% B2, 20% A	D ^b (F)		1/ND
83	100% A	A		1/ND
84	90% B1, 10% A	B1	100% B1 (30 clones)	1/0
85	42% B2, 58% A	B2		1/ND
86	74% A, 24% B1, 2% D	B1		0/ND
87	57% A, 43% B2	A		1/ND
88	100% B2	B2		1/ND
89	71% D, 29% A	D		1/ND
90	53% B1, 47% A	B1		1/ND
91	91% B2, 9% A	B2		1/ND
92	100% B2	B2		1/ND
93	100% B2	B2		1/ND
94	59% A, 36% B2, 5% B1	D ^b (F)		0/ND
95	95% A, 4% D, 1% B1	A		1/ND
96	92% A, 8% B2	A		1/ND
97	55% A, 40 % B1, 5% B2	B1		0/ND
98	55% D, 44% A, 1% B2	D		1/ND
99	0	0		1/ND
100	0	0		1/ND

^aThe results are given in four main phylogroups (A, B1, B2, D), as in (1). However, the new quadriplex PCR method was performed on all the strains to (i) detect the presence of clades and (ii) identify F phylogroup (see ^b).

^bThe determination of the F phylogroup was performed as in (2).

^c1, correlation; 0, absence of correlation; ND, not determined.

TABLE S4. Proportions of *E. coli* phylogroups in the fresh feces and corresponding swabs of eight healthy control subjects using real-time PCR

Fresh feces	A	B1	B2	D
Subject 1	99%	0%	1%	0%
Subject 2	72%	1%	3%	24%
Subject 3	0%	5%	95%	0%
Subject 4	40%	0%	60%	0%
Subject 5	30%	60%	10%	0%
Subject 6	54%	1%	5%	40%
Subject 7	97%	0%	1%	0%
Subject 8	72%	1%	5%	22%

Fecal swabs ^a	A	B1	B2	D
Subject 1	98%	0%	2%	0%
Subject 2	67%	0%	0%	33%
Subject 3	0%	4%	96%	0%
Subject 4	25%	0%	75%	0%
Subject 5	46%	31%	21%	2%
Subject 6	45%	1%	2%	52%
Subject 7	99%	0%	1%	0%
Subject 8	79%	1%	1%	19%

^aThe fecal swabs were obtained as described in the ‘materials and methods’ section.

REFERENCES

1. **Clermont O, Bonacorsi S, Bingen E.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* **66**:4555–4558.
2. **Clermont O, Christenson JK, Denamur E, Gordon DM.** 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Rep.* **5**:58–65.