**Figure S1**. Schematic design for experiment two. The experiment was arranged as a two (**A**; ± stress) by two (**B**; ± enterohemorrhagic *Escherichia coli* O157:H7 [EHEC] FRIK-2001) by three (**C**; commensal *E. coli* treatments) factorial design arranged as a completely randomized design with three replicate germ-free or gnotobiotic mice per treatment combination (36 mice total). Commensal *E. coli* strains were cultured communally (CC) or separately (CS), whereas the Control treatment mice were orally administered phosphate buffered saline alone. Stress was incited by the administration of corticosterone via drinking water, and EHEC and commensal *E. coli* strains were administered orally to mice via consumption of Jell-O formulated with the bacteria. See the manuscript text for additional information.
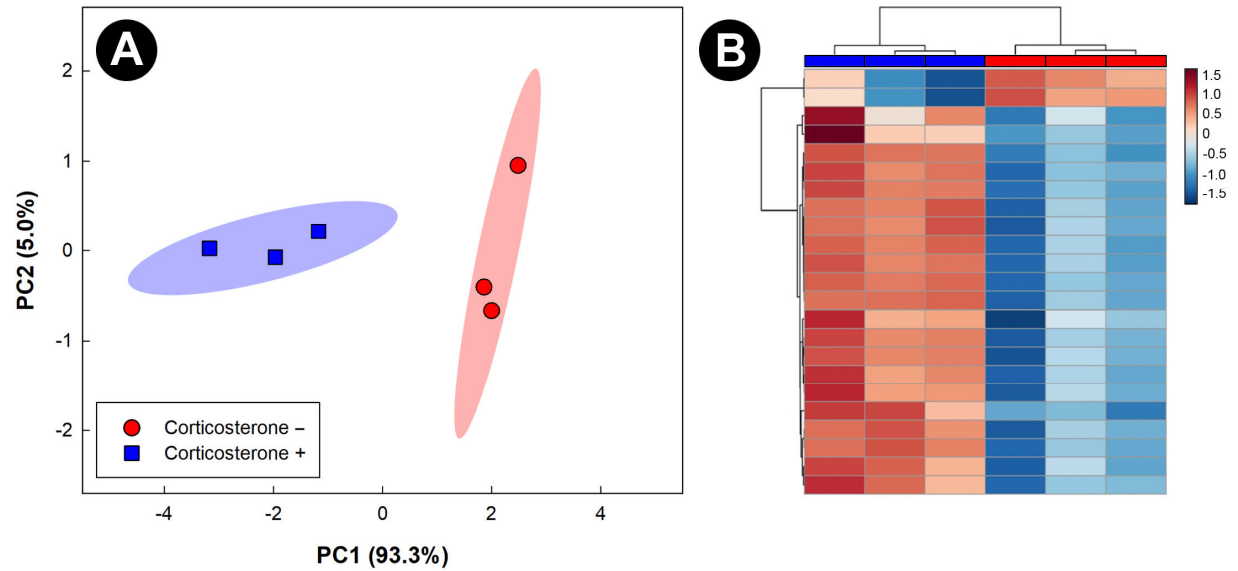
**Figure S2.** Principal component analysis (PCA) score plot and heat map of metabolite bins in livers of mice administered corticosterone in water for 9 days (corticosterone +; blue markers and bars) or mice administered water free of corticosterone (corticosterone –; red markers and bars). (**A**) PCA score plot, where each marker represents one mouse, and the shaded ellipses represent 95% confidence intervals. The x- and y-axes show principal components one and two, respectively, with the number in brackets indicating the percent variance of each component. (**B**) Heat map showing the degree to which metabolite bins were up- or down-regulated (i.e., denoted with red and blue coloration, respectively). The dendrogram associated with the heat map illustrates the results of the unsupervised hierarchical clustering analysis.
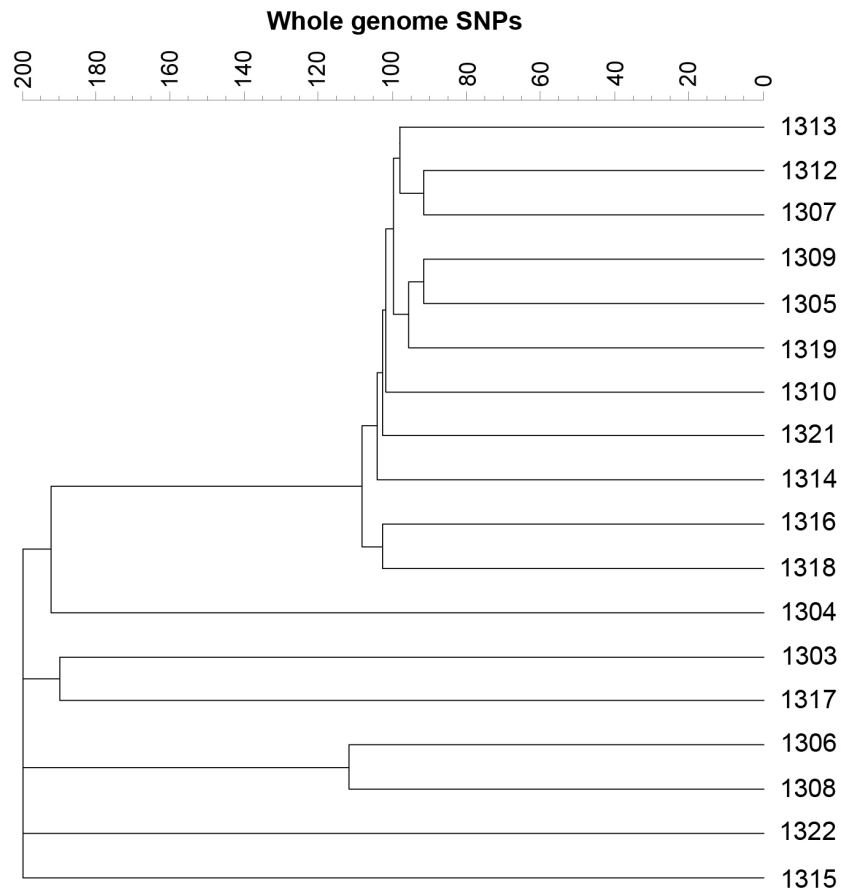
**Whole genome SNPs**



**Figure S3**. Single nucleotide polymorphism dendrogram showing phylogenetic differences among the 18 commensal *Escherichia coli* isolates that were evaluated in gnotobiotic mice.
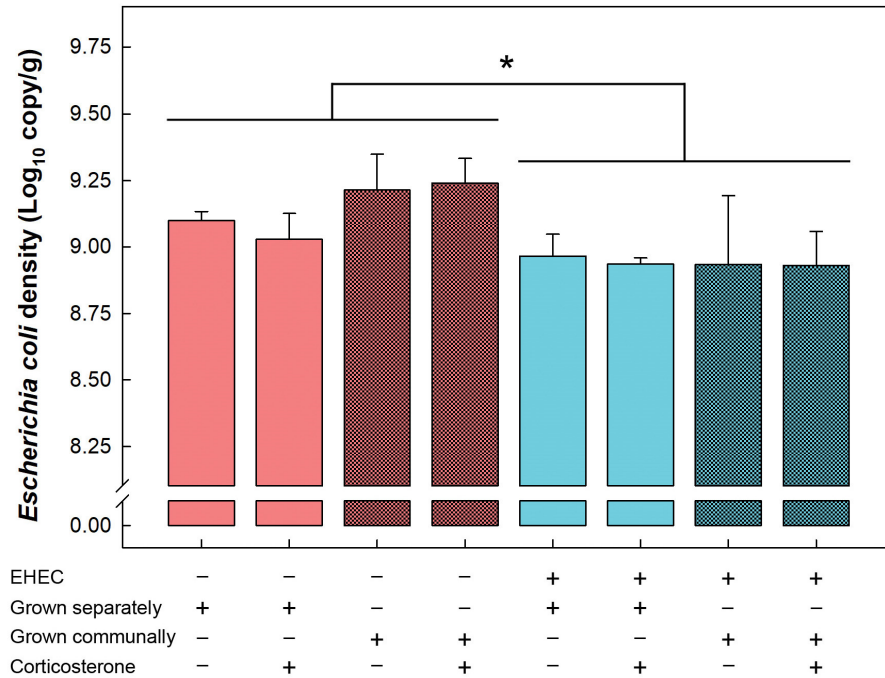
**Figure S4**. Densities of total commensal *Escherichia coli* cells in distal colonic digesta. Treatments are ± enterohemorrhagic *E. coli* O157:H7 (EHEC), ± commensal *E. coli* strains grown communally or separately, and ± corticosterone administered to mice. Densities were determined using quantitative PCR with primers specific for total bacteria (i.e., all commensal *E. coli* strains as well as the EHEC strain, FRIK-2001). Histogram bars indicated with as asterisk differ (*p* = 0.036) (i.e., densities of commensal *E. coli* cells were reduced in mice that were administered EHEC O157:H7 (i.e., FRIK-2001).
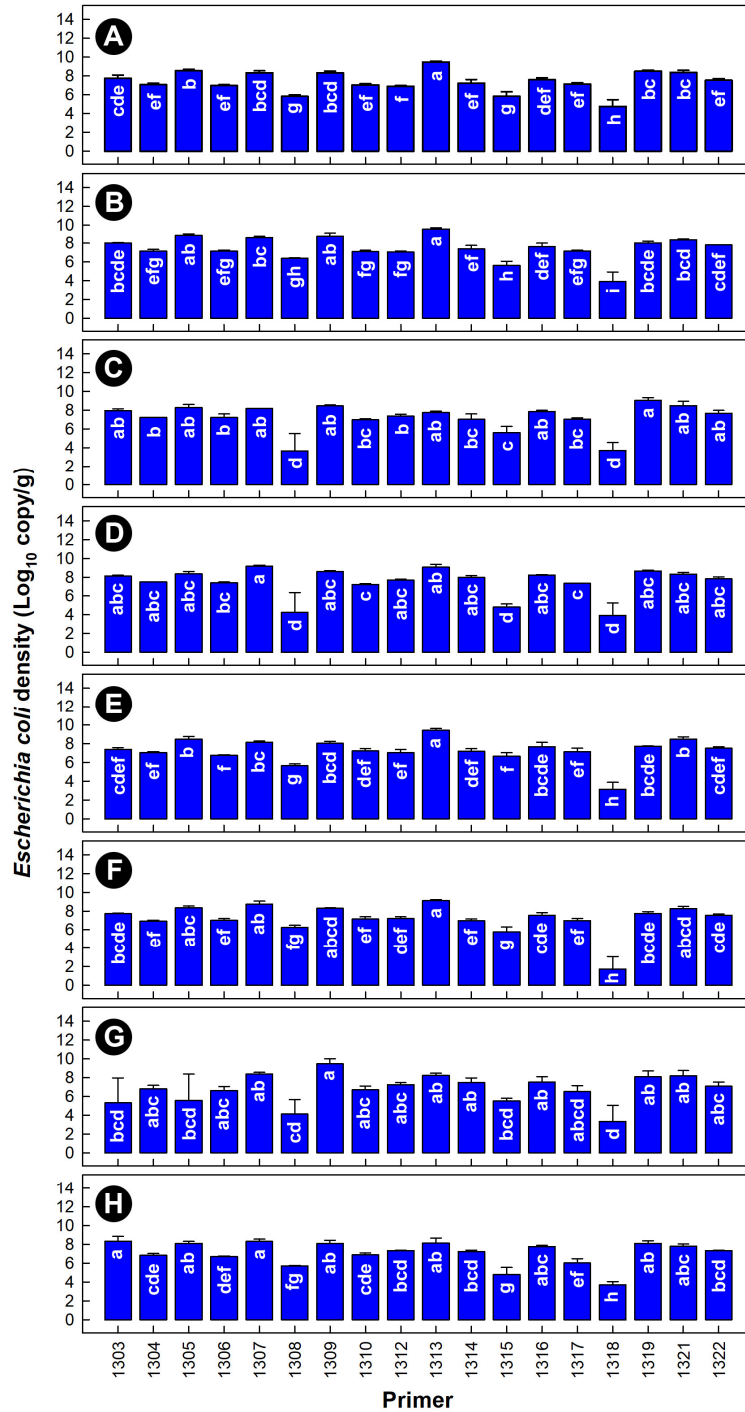
**Figure S5**. Densities of individual commensal *Escherichia coli* strains in distal colonic digesta. (**A**) enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) (–), Cultured Separately (CS), corticosterone (–). (**B**) EHEC (–), CS, corticosterone (+). (**C**) EHEC (–), Cultured Communally (CC), corticosterone (–). (**D**) EHEC (–), CC, corticosterone (+). (**E**) EHEC (+), CS, corticosterone (–). (**F**) EHEC (+), CS, corticosterone (+). (**G**) EHEC (+), CC, corticosterone (–). (**H**) EHEC (+), CC, corticosterone (+). Densities were determined using quantitative PCR with designed primers that were specific for individual commensal *E. coli* strains. Within individual graphs, histogram bars not indicated with the same letter differ ($p \leq 0.050$).

**Figure S6**. Densities of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) in intestinal digesta. (**A**) Ileum. (**B**) Cecum. (**C**) Proximal colon. (**D**) Distal colon. Treatments include mice that were inoculated with EHEC (i.e., EHEC, EHEC + CC, EHEC + CS) or with phosphate buffer alone (i.e., CC, CS, Control). Commensal *E. coli* strains administered to mice were grown communally (i.e., CC) or separately (i.e., CS). EHEC and Control treatment mice were not administered commensal *E. coli*. EHEC densities were determined using quantitative PCR with primers that were specific for FRIK-2001. Histogram bars indicated with asterisks differ (** $p < 0.010$; and *** $p < 0.001$). As there was no impact of corticosterone on densities of EHEC, data were averaged over the corticosterone treatment.

**Table S1.** Primers and annealing temperatures used to quantify enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) strains. See the manuscript text for PCR-specific information.

| Strain | Forward primer | Reverse primer | Annealing temperature (°C) |
|--------|----------------|----------------|-----------------------------|
| EDL933 | TGACCGCTTACGCAGTTCGC | AATGGTTGCTGCCACGGCTC | 68 |
| FRIK-2001 | GGATGACGGTGCCTGTGCTG | GGCTGGCGGGAGCTACCTAA | 68 |
| TW14359 | ATCGGTGGCTGGAATGGGCT | AAGTGCAACTGGTGCGTGCT | 68 |
| ECI-1375 | CGCTCGCCCTAAGATGGGGA | TTCGACGGCTTTCGCTGACG | 68 |
| ECI-1911 | CACACTGTCCGGTGATGCCG | CGCGCTGTTCAGCATGAGGT | 68 |

**Table S2.** Primers and annealing temperatures used to quantify commensal *Escherichia coli* strains originally isolated from cattle. See the manuscript text for PCR-specific information.

| Strain | Forward primer | Reverse primer | Annealing temperature (°C) |
|---|---|---|---|
| 1303 | CATATAGTGGTGAACCCGATCC | ACGCCCTAAAGCAGTGAAA | 60 |
| 1304 | CGGACGGTCTATAACCTTGAAC | TTGTGTTGCCGCTGTACT | 58 |
| 1305 | CACCTGACCATCTCTCGTTTAT | GCGAATTGAGGCTTATGGTTTAG | 60 |
| 1306 | GCTCTTCTGCAACGGTTTATG | CGATGTGCCATATGGGTTTAATG | 59 |
| 1307 | CATTTCAGATGTGCGGGAAAG | GCCGTACCTCTAAGTTTCTGAG | 60 |
| 1308 | ATCTCATGTCAGACAAGGAGTTTAT | GAGACCAGACAGGTTTAGGTATTT | 58 |
| 1309 | ACCAGCCTTTCCAGTAAGTTC | CCACATCCCGTCACACAATA | 60 |
| 1310 | GCTGGGAGGATTCCCAATATC | AAGTGTTCGTTGTGGTGATAGA | 58 |
| 1312 | GACACGAACGGATGAAGGAA | TCGTTGTAACCATCGCCATAA | 58 |
| 1313 | CCGGTAATGGCGGTTGTAAT | GGAACAGACGATGCAGGTATTT | 60 |
| 1314 | GGCTGACGTACTGGATCATTTA | ACACTCGCTGAGGCTTTC | 60 |
| 1315 | GATGGTGACAGCCTTGTACTT | ACTTCTCGGCGACTGAAATG | 58 |
| 1316 | CCACTCACTTTGTCCTGGTT | GCTCTGAGGCGTTTGGTATTA | 60 |
| 1317 | CGTGCACAGATGGTTGATAGA | CCGACGAACTGATGATGTAACT | 58 |
| 1318 | TTCACCCGGGAATATCCTTTG | CCGCGATTACCTTGGTTACTAT | 58 |
| 1319 | GGTTTCGGGCTTACTTGTATCT | CGTCTAATGGAGGCATGGAAA | 58 |
| 1321 | GATGATGTATCTAGGCAGGGTTTAT | CAGTCTCTCTTCAAAGCATCCA | 60 |
| 1322 | CCATGAATTACGTTTCTCAGCTAAT | AAGGACGTAAGGCTCGTAGA | 58 |

**Table S3.** Health assessment scoring system.

| Category | Score | Definition |
| --- | --- | --- |
| General activity | 0 | Bright and alert |
|  | 1 | Slow to move, stays in nest area, restless/agitated or separated |
|  | 2 | Reluctant to move, not bright or alert |
|  | 3 | Reluctant to move even if prodded gently and depressed, hunched, eyes partly closed |
|  | 4 | Moribund |
| Hair coat appearance and grooming behavior | 0 | Fur coat shiny and smooth, regular grooming behavior |
|  | 1 | Reduced grooming behavior, fur coat dry, beginning to huddle |
|  | 2 | Huddled, fur coat rough, dry, and stands up |
|  | 3 | Huddled, ungroomed, severe piloerection observed |
| Vocalization | 0 | No vocalization |
|  | 1 | Occurrence of vocalization |

**Table S4.** Predicted commensal *Escherichia coli* strain phenomics (subtype designations).

| | Conventional MLST | | | | | | | | | Phylotype | | | Serotype prediction | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain | adk | fumC | gyrB | icd | mdh | purA | recA | ST | Clonal complex | Profile | Clermont phylotype | O-Type | H-Type | Serotype |
| 1303 | 6 | 445 | 326 | 12 | 282 | 178 | 247 | 3381 | | TspE4: –; arpA: +; chu: +; yjaA: + | E | O11 | H25 | O11:H25 |
| 1304 | 8 | 11 | 4 | 8 | 8 | 9 | 2 | 1495 | | TspE4: –; arpA: +; chu: -; yjaA: + | A | O133 | H4 | O133:H4 |
| 1305 | 6 | 4 | 32 | 16 | 12 | 8 | 7 | 164 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O45 | H42 | O45:H42 |
| 1306 | 34 | 36 | 39 | 87 | 67 | 16 | 4 | 349 | ST349 | TspE4: +; arpA: +; chu: +; yjaA: – | D | O166 | H15 | O166:H15 |
| 1307 | 119 | 150 | 12 | 16 | 24 | 8 | 6 | 661 | | TspE4: +; arpA: +; chu: –; yjaA: – | B1 | O174 | H2 | O174:H2 |
| 1308 | 34 | 36 | 28 | 25 | 28 | 16 | 4 | 70 | | TspE4: –; arpA: +; chu: +; yjaA: – | D | O23 | H15 | O23:H15 |
| 1309 | 43 | 41 | 15 | 18 | 11 | 7 | 6 | 101 | ST101 | TspE4: +; arpA: +; chu: –; yjaA: – | B1 | O21 | H21 | O21:H21 |
| 1310 | 6 | 4 | 4 | 18 | 24 | 5 | 14 | 56 | ST155 | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O25 | H16 | O25:H16 |
| 1312 | 6 | 19 | 5 | 1 | 335 | 8 | 7 | — | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | – | H2 | –:H2 |
| 1313 | 6 | 19 | 3 | 16 | 11 | 8 | 6 | 1727 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O83 | H7 | O83:H7 |
| 1314 | 6 | 95 | 4 | 18 | 11 | 7 | 14 | 1304 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O163 | H7 | O163:H7 |
| 1315 | 76 | 195 | 19 | 13 | 17 | 28 | 467 | 6527 | | TspE4: +; arpA: –; chu: +; yjaA: + | B2 | O2 | H10 | O2:H10 |
| 1316 | 6 | 23 | 4 | 18 | 7 | 7 | 6 | 1001 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O8 | H2 | O8:H2 |
| 1317 | 83 | 12 | 155 | 30 | 1 | 1 | 17 | 1629 | | TspE4: –; arpA: +; chu: +; yjaA: – | E | – | H31 | –:H31 |
| 1318 | 9 | 4 | 33 | 18 | 11 | 8 | 6 | 336 | ST278 | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O151/O118 | H27 | O151/O118:H27 |
| 1319 | 9 | 4 | 5 | 88 | 9 | 8 | 7 | 8620 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O8 | H16 | O8:H16 |
| 1321 | 6 | 19 | 14 | 16 | 11 | 12 | 2 | 1079 | | TspE4: +; arpA: +; chu: -; yjaA: – | B1 | O6 | H49 | O6:H49 |
| 1322 | 46 | 26 | 208 | 6 | 4 | 16 | 4 | 1567 | | TspE4: –; arpA: +; chu: +; yjaA: – | D | – | H48 | –:H48 |

**Table S5.** Predicted commensal *Escherichia coli* strain phenomics (antimicrobial resistance).

| | Antimicrobial resistance prediction | | |
|---|---|---|---|
| **Strain** | **Genotype** | **Resistance** | **Plasmid** |
| 1303 | None | Sensitive | None |
| 1304 | None | Sensitive | None |
| 1305 | None | Sensitive | IncFIA, IncFIB(AP001918) |
| 1306 | None | Sensitive | None |
| 1307 | None | Sensitive | None |
| 1308 | None | Sensitive | IncFIA |
| 1309 | aph(3″)-Ib, aph(6)-Id, sul2, tet(B) | StrR, KanR, SulR, TetR[a] | IncFIB(pB171) |
| 1310 | aph(3″)-Ib, aph(6)-Id, sul2, tet(B) | StrR, KanR, SulR, TetR[a] | None |
| 1312 | None | Sensitive | IncX1 |
| 1313 | None | Sensitive | None |
| 1314 | None | Sensitive | IncFIA |
| 1315 | None | Sensitive | IncFIB(AP001918), IncX1 |
| 1316 | None | Sensitive | IncFIB(pB171) |
| 1317 | None | Sensitive | IncFIB(AP001918), IncI2(Delta) |
| 1318 | None | Sensitive | IncFIB(AP001918) |
| 1319 | None | Sensitive | None |
| 1321 | None | Sensitive | IncFIA, IncFIB(AP001918) |
| 1322 | None | Sensitive | IncFIA |

[a]Str, streptomycin; Kan, kanamycin; Sul, sulfisoxazole; Tet, tetracycline

**Table S6.** Predicted commensal *Escherichia coli* strain phenomics (virulence factors).

| Strain | Predicted virulence factors[a] |
|---|---|
| 1303 | *chuA; eilA; espY2:000868321; fdeC; fimH; hlyE; nlpI; ompT; terC; terC; traJ; traT; yehA; yehB; yehC; yehD* |
| 1304 | *capU; csgA; fimH; fimH; hlyE; iss; nlpI; sitA; terC; terC; yehA; yehB; yehC; yehD* |
| 1305 | *csgA; fdeC; fimH; hlyE; iss; lpfA; nlpI; ompT; ompT; terC; terC; traJ; traT; yehA; yehB; yehC; yehD* |
| 1306 | *aslA; chuA; eilA; fimH; hlyE; iss; kpsE; kpsMII_K5; nlpI; shiB; terC; terC; yehB; yehC; yehD* |
| 1307 | *csgA; fdeC; fimH; hlyE; iss; lpfA; lpfA; nlpI; terC; terC; yehA; yehB; yehC; yehD* |
| 1308 | *aslA; astA; chuA; eilA; fimH; gad; gad; hlyE; kpsE; kpsMII; kpsMII; nlpI; papC; shiB; terC; terC; tia; traJ; traT; yehA; yehB; yehC; yehD* |
| 1309 | *F17A; F17C; F17D; F17G; afaA; afaB; afaB; csgA; fdeC; fimH; hlyE; iss; lpfA; nlpI; ompT; ompT; shiB; terC; terC; tia; traT; yehA; yehB; yehC; yehD* |
| 1310 | *F17A; F17C; F17D; F17G; csgA; fdeC; fimH; fyuA; gad; hlyE; irp2; iss; lpfA; nlpI; ompT; terC; terC; tia; traT; yehA; yehB; yehC; yehD* |
| 1312 | *csgA; fdeC; fimH; gad; hlyE; lpfA; nlpI; terC; terC; yehA; yehB; yehC; yehD* |
| 1313 | *fimH; gad; hlyE; iss; lpfA; nlpI; ompT; shiB; terC; terC; yehA; yehC; yehD* |
| 1314 | *csgA; fdeC; fimH; gad; hlyE; iss; lpfA; nlpI; ompT; terC; terC; traT; yehA; yehB; yehC; yehD* |
| 1315 | *aslA; cba; cdt-VB; chuA; cib; clbB; cma; cnf2; etsC; fimH; focC; focG; fyuA; gad; hlyA; hlyF; iroN; irp2; iss; kpsE; kpsMII_K1; mchB; mchC; mchF; mcmA neuC; nlpI; ompT; sfaD; sitA; terC; terC; traT; usp; vat; yehA; yehB; yehC; yehD; yfcV* |
| 1316 | *csgA; fdeC; fimH; hlyE; iss; lpfA; nlpI; ompT; terC; terC; traJ; traT; yehA; yehB; yehC; yehD* |
| 1317 | *air; chuA; eilA; eltIIAB-c2; espY2:000868321; fimH; hlyE; nlpI; ompT; terC; terC; traT; usp; yehB; yehC; yehD* |
| 1318 | *F17A; F17C; F17D; F17G; cdt-IIIB; cnf2; csgA; fdeC; fimH; hlyE; hra; iss; iucC; iutA; nlpI; ompT; ompT; terC; terC; traT; yehA; yehB; yehC; yehD* |
| 1319 | *csgA; fdeC; fimH; hlyE; iss; lpfA; nlpI; ompT; tcpC; terC; terC; yehA; yehB; yehC; yehD* |
| 1321 | *anr; astA; csgA; fdeC; fimH; hlyE; iss; lpfA; nlpI; ompT; ompT; shiB; terC; terC; traJ; traJ; traT; traT; yehA; yehB; yehC; yehD* |
| 1322 | *aslA; chuA; eilA; fimH; gad; hlyE; nlpI; ompT; terC; terC; traJ; traT; yehA; yehB; yehC; yehD* |

[a]See Joensen et al. [1] and Malberg Tetzschner et al. [2] for specific information on the virulence factors presented herein.

**Table S7.** Frequency of commensal *Escherichia coli* strains recovered from the propagation culture medium (i.e., for strains propagated communally).

| Strain | Frequency (%)[a] |
|--------|------------------|
| 1303 | 2.2 |
| 1304 | 7.7 |
| 1305 | 8.8 |
| 1306 | 1.1 |
| 1307 | 2.2 |
| 1308 | 1.1 |
| 1309 | 5.5 |
| 1310 | 2.2 |
| 1312 | 13.2 |
| 1313 | 1.1 |
| 1314 | 9.9 |
| 1315 | 3.3 |
| 1316 | 5.5 |
| 1317 | 4.4 |
| 1318 | 1.3 |
| 1319 | 9.9 |
| 1321 | 9.9 |
| 1322 | 4.4 |

[a]The total number of colonies examined was 300.

**References**

1.  Joensen, K.G.; Scheutz, F.; Lund, O.; Hasman, H.; Kaas, R.S.; Nielsen, E.M.; Aarestrup, F.M. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* **2014**, *52*, 1501-1510, doi:10.1128/JCM.03617-13.
2.  Malberg Tetzschner, A.M.; Johnson, J.R.; Johnston, B.D.; Lund, O.; Scheutz, F. In silico genotyping of *Escherichia coli* isolates for extraintestinal virulence genes by use of whole-genome sequencing data. *J Clin Microbiol* **2020**, *58*, doi:10.1128/jcm.01269-20.