

Supplemental Data

Genomic diversity, antibiotic resistance, and maturation-dependent adhesion of F18 enterotoxigenic *Escherichia coli* strains in porcine intestinal cells

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Supplemental Figures

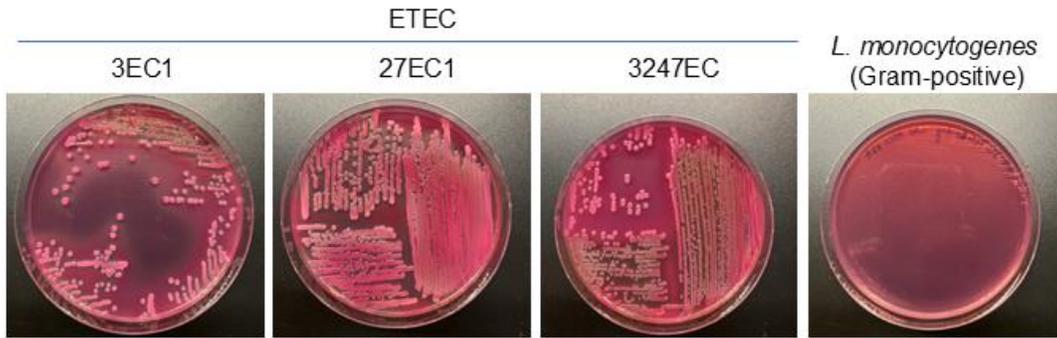


Figure S1. Growth characteristics of ETEC F18 clinical isolates on MacConkey agar plates.

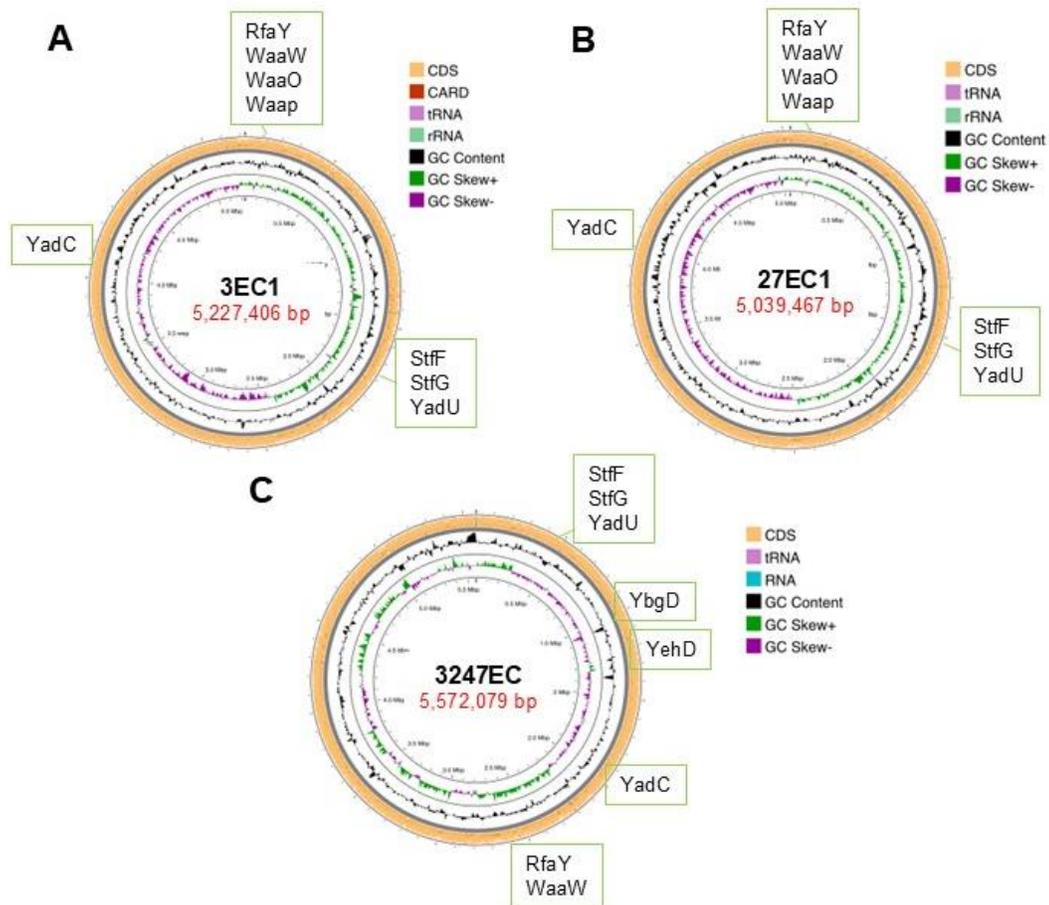


Figure S2. Genome sequence of enterotoxigenic *E. coli* (ETEC) F18 strains. Genomic map of ETEC (A) 3EC1, (B) 27EC1, and (C) 3247EC displaying the differential presence of genes encoding fimbriae or fimbriae-like proteins (box).

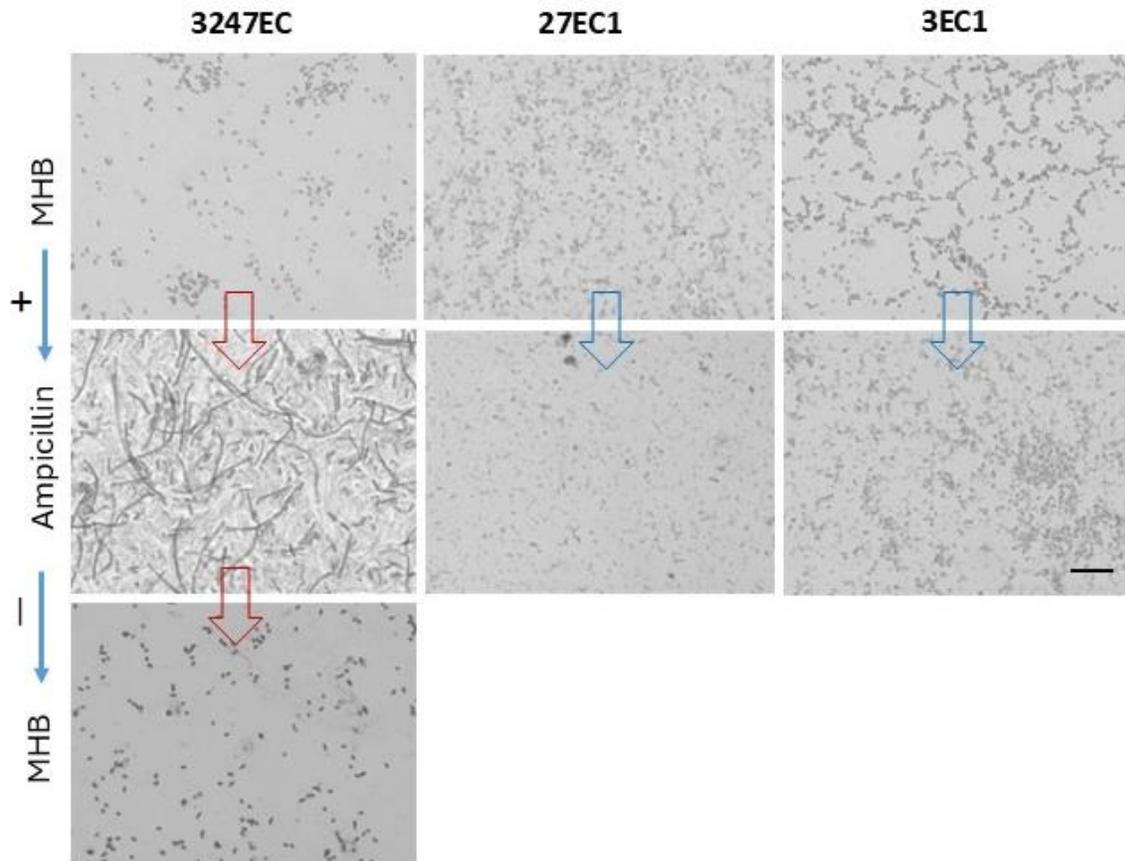


Figure S3. Morphology characterization of ETEC strains in the presence of antibiotics. Morphology of each ETEC strain cultured in Mueller-Hinton broth (MHB) with or without ampicillin (100 $\mu\text{g}/\text{mL}$). Morphology of strain 3247EC restored to the original when ampicillin was removed (Red Open Arrow). Blue Open Arrow: no apparent change in morphology. Magnification 1000x

Supplementary Tables

Table S1. List of plasmids identified in *Escherichia coli* strain 3247EC via PlasmidFinder.

Plasmid	Identity Query	Template length	Position in contig	Accession number
Col156	96.05	152 / 154	709583..709734	NC009781
IncB/O/K/Z	95	160 / 160	236371..236529	GU256641
IncFIA	99.74	388 / 388	297430..297816	AP001918
IncFIB(AP001918)	98.09	682 / 682	5437977..5438658	AP001918
IncFIB(pLF82)	98.39	560 / 560	5198181..5198740	CU638872
IncFII(pCoo)	96.18	262 / 262	5442916..5443177	CR942285
IncHI2	100	327 / 327	3006095..3006421	BX664015
IncHI2A	99.52	630 / 630	5265646..5266275	BX664015
IncQ1	99.81	529 / 796	5500779..5501306	M28829.1
IncY	98.95	765 / 765	5296268..5297032	K02380

Table S2. List of virulent genes identified in *Escherichia coli* strains 3EC1, 27EC1, and 3247EC via VirulenceFinder.

Isolate	Virulence factor	Identity	Query / Template length	Protein function	Accession number
3EC1	Stx2e	100	1236 / 1236	O139 S1191, variant e	M21534
	AslA	94.03	1657 / 1656		CP054236
	astA	99.15	117 / 117	Heat-stable enterotoxin EAST-1	AF161000
	csgA	100	456 / 456	curlin major subunit CsgA	CP069646
	fimH	100	489 / 489	Type 1 fimbriae	NA
	gad	99.93	1401 / 1401	Glutamate decarboxylase	CP002967
	gad	100	1401 / 1401	o139 S1191, variant e	U00096
	hlyE	97.71	918 / 918	Avian E. coli haemolysin	ECU57430
	iha	100	2091 / 2091	Adherence protein	AE005174
	kpsE	99.65	1149 / 1149	Capsule polysaccharide export inner-membrane protein	CP011134
	nlpl	99.77	885 / 885	lipoprotein Nlpl precursor	CP000243
	shiA	92.82	1044 / 1044	homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA	CP058222
	stx2e-O139-S1191	100	1236 / 1236	stx2e-O139-S1191	M21534
	terC	100	1041 / 1041	Tellurium ion resistance protein	CP006262
terC	98.74	714 / 714	Tellurium ion resistance protein	CP007491	

	terC	99.9	959 / 966	Tellurium ion resistance protein	MG591698
	traT	100	423 / 423	Outer membrane protein complement resistance	AKKX01000148
	traT	100	129 / 129	Outer membrane protein complement resistance	AMTE01000156
	yehA	100	1035 / 1035	Outer membrane lipoprotein, YHD fimbriael cluster	CP042934
	yehB	99.4	2481 / 2481	Usher, YHD fimbriael cluster	CP042934
	yehC	97.63	675 / 675	Chaperone, YHD fimbriael cluster	CP042934
	yehD	98.53	543 / 543	Major pilin subunit, YHD fimbriael cluster	CP042934
27EC1	csgA	98.9	456 / 456	curlin major subunit CsgA	CP069646
	fdeC	96.12	4254 / 4254	intimin-like adhesin FdeC	AP010953
	fimH	100	489 / 489	Type 1 fimbriae	NA
	gad	100	1401 / 1401	Glutamate decarboxylase	AP009240
	gad	99.79	1401 / 1401	Glutamate decarboxylase	AP009240
	hlyE	99.13	918 / 918	Avian E. coli haemolysin	ECU57430
	iha	100	2091 / 2091	Adherence protein	AE005174
	kpsE	95.56	1149 / 1149	Capsule polysaccharide export inner-membrane protein	CP000243
	kpsMII	99.23	777 / 777	Polysialic acid transport protein; Group 2 capsule	GG771940
	kpsMII	99.23	777 / 777	Polysialic acid transport protein; Group 2 capsule	QOOR01000049
	lpfA	100	573 / 573	Long polar fimbriae	AP010953
	nlpl	99.77	885 / 885	lipoprotein Nlpl precursor	CP000243
	papA_F19	100	372 / 372	Major pilin subunit F19	AP017620
	shiA	94.44	1044 / 1044	homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA	AF141323
	shiA	94.36	1028 / 1044	homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA	AF141323
	shiA	94.36	1028 / 1044	homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA	CP058222
	shiA	94.44	1044 / 1044	homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA	CP058222
	terC	100	1041 / 1041	Tellurium ion resistance protein	CP006262
	terC	100	714 / 714	Tellurium ion resistance protein	CP007491

	terC	99.28	966 / 966	Tellurium ion resistance protein	MG591698
	tia	100	738 / 738	Tia Invasion determinant	CP010172
	tia	99.86	738 / 738	Tia Invasion determinant	CP012112
	traT	100	423 / 423	Outer membrane protein complement resistance	AKKX01000148
	traT	100	129 / 129	Outer membrane protein complement resistance	AMTE01000156
	yehA	97.81	729 / 1035	Outer membrane lipoprotein, YHD fimbriael cluster	CP042934
	yehB	97.86	2481 / 2481	Usher, YHD fimbriael cluster	CP042934
	yehC	98.52	675 / 675	Chaperone, YHD fimbriael cluster	CP042934
	yehD	98.53	543 / 543	Major pilin subunit, YHD fimbriael cluster	CP042934
3247E C	afaA	100	384 / 384	Transcriptional regulator	GQ916596
	afaB	100	768 / 768	Periplasmic chaperone	GQ497943
	afaB	100	768 / 768	Periplasmic chaperone	GQ497943
	afaC	100	2589 / 2589	Outer membrane usher protein	AP017620
	afaD	100	420 / 420	Afimbrial adhesion	AF072900
	astA	100	117 / 117	Heat-stable enterotoxin EAST-1	AB042002
	cba	99.8	1536 / 1536	Colicin B	FJ664720
	cba	99.8	1536 / 1536	Colicin B	FJ664721
	cia	93.41	167 / 264	Colicin ia	UGDH01000002
	cma	99.88	816 / 816	Colicin M activity	FJ664718
	cma	99.88	816 / 816	Colicin M activity	FJ664720
	cma	99.88	816 / 816	Colicin M activity	FJ664744
	colE2	100	144 / 144	Colicin E2	D00021
	colE8	100	144 / 144	Colicin E8	X06119
	colE8	100	144 / 144	Colicin E8	X06119
	csgA	98.9	456 / 456	curlin major subunit CsgA	CP069646
	espP	99.6	2735 / 3903	Serine protease autotransporter EspP	AY258503
	faeH1	92.91	621 / 798	F4 (K88) Fimbria minor subunit, one of two copies in strain RHBSTW-00673	CP056356
	faeI	99.87	765 / 765	F4 (K88) Fimbria minor subunit	L05182
	faeJ	94.96	774 / 774	F4 (K88) Fimbria minor subunit	CP057646
fdeC	92.53	4258 / 4254	intimin-like adhesin FdeC	AP010953	

fimH	100	489 / 489	Type 1 fimbriae	NA
fyuA	99.95	2022 / 2022	Siderophore receptor	UINR0100001 7
gad	99.93	1401 / 1401	Glutamate decarboxylase	AP010953
gad	100	1401 / 1401	Glutamate decarboxylase	CU928160
hlyE	99.02	918 / 918	Avian E. coli haemolysin	ECU57430
irp2	100	6108 / 6108	High molecular weight protein 2 non-ribosomal peptide synthetase	QOEB0100002 3
iss	98.98	294 / 294	Increased serum survival	CP001509
iucC	100	1743 / 1743	Aerobactin synthetase	CBTO0100000 02
iutA	100	2202 / 2202	Ferric aerobactin receptor	CXZO0100005 6
lpfA	99.83	573 / 573	Long polar fimbriae	CP002185
nlpl	99.77	885 / 885	lipoprotein Nlpl precursor	CP000243
ompT	100	954 / 954	Outer membrane protease (protein protease 7)	UINH0100001 0
sitA	100	915 / 915	Iron transport protein	AEZP0200001 3
terC	100	714 / 714	Tellurium ion resistance protein	CP007491
terC	100	1041 / 1041	Tellurium ion resistance protein	KR653209
terC	99.28	966 / 966	Tellurium ion resistance protein	MG591698
traJ	98.99	690 / 690	Protein TraJ (Positive regulator of conjugal transfer operon)	AF550679
traT	95.53	627 / 627	Outer membrane protein complement resistance	AY686591
traT	95.53	627 / 627	Outer membrane protein complement resistance	MF156268
traT	100	831 / 831	Outer membrane protein complement resistance	NC_011964
yehA	96.42	1033 / 1035	Outer membrane lipoprotein, YHD fimbriae cluster	CP042934
yehB	98.15	2481 / 2481	Usher, YHD fimbriae cluster	CP042934
yehC	97.63	675 / 675	Chaperone, YHD fimbriae cluster	CP042934
yehD	98.71	543 / 543	Major pilin subunit, YHD fimbriae cluster	CP042934

Table S3. List of acquired antimicrobial resistance genes in *Escherichia coli* strains 3EC1, 27EC1, and 3247EC.

Isolate	Resistance gene	Accession no.	Identity	Phenotype
3EC1	aph(6)-IId	M28829	100	Streptomycin
	aph(3'')-Ib	AF024602, AF321551, AF313472, AF321550	100	Streptomycin
	aadA1	JQ414041	100	Spectinomycin, Streptomycin
	blaTEM-1B	AY458016	100	Amoxicillin, Ampicillin, Cephalothin, Piperacillin, Ticarcillin
	sul1	U12338	100	Sulfamethoxazole
	tet(A)	AJ517790	100	Doxycycline, Tetracycline
	dfrA1	AF203818, AJ238350, X00926	99.79	Trimethoprim
	bleO	AF051917	100	Bleomycin
27EC1	aph(6)-IId	M28829	100	Streptomycin
	aph(3'')-Ib	AF321551	100	Streptomycin
	floR	AF118107	98.19	Chloramphenicol, Florfenicol
	sul2	AY034138	100	Sulfamethoxazole
	tet(A)	AF534183	99.92	Doxycycline, Tetracycline
3247EC	aac(3)-IIId	EU022314	99.88	Apramycin, Gentamicin, Tobramycin, Dibekacin, Netilmicin, Sisomicin
	aph(6)-IId	M28829	100	Streptomycin
	aph(3')-Ia	V00359	100	Kanamycin, Neomycin, Lividomycin, Paromomycin, Ribostamycin,
	aph(3'')-Ib	AF321551	99.88	Streptomycin
	aadA5	AF137361	100	Spectinomycin, Streptomycin
	blaCTX-M-15	AY044436	100	Amoxicillin, Ampicillin, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Piperacillin, Ticarcillin
	sul2	AY034138	100	Sulfamethoxazole
	tet(A)	AJ517790	100	Doxycycline, Tetracycline
	dfrA17	FJ460238	100	Trimethoprim