

Supplementary Material

Table S1. General Features of Sequenced Strains of pathogenic-hybrid *E. coli*.

Strain	Source	Phylogroup	Genome Size Mb	G+C Content %	N50	Number of Contigs	Serogroup	MLST
Ec-25.2	Healthy donor	A ¹	5,327,597	50.6	95056	224	O153:H2	ST 69
Ec-36.1	Healthy donor	B2 ¹	5,233,542	50.8	77241	276	H4	ST 4238
Ec-36.4	Healthy donor		5,171,155	49.9	77634	249	H4	

¹Mendez-Moreno et al., 2022

Table S2. Genomes used as control for phylogenetic analysis.

Genome	Control Strain			Accession Number
	Pathotype	Adherence Phenotype		
APEC102026	APEC	Diffuse and aggregative		ASM2875269v1
NMECO18	NMEC	Diffuse		SAMN02666437
CFT073	UPEC	“Train wagon”		AE014075
AIECLF82	AIEC	Adherente-Invasive		PRJNA487828
DAEC SK1144	DAEC	Diffuse		AP018784.2
EPEC E2348/69	EPEC	Localized adhesion		FM180568
EAEC	EAEC	Aggregative		SAMEA7457016
EIEC53638	EIEC	Adherente-Invasive		AAKB02000001.1
<i>E. coli</i> O145:H28	EHEC	Localized adhesion		AP019703.1
ETECH10407	ETEC	Aggregative		NC_017633.1
K12	Commensal	Commensal		MG1655
Ec-25.2	aEPEC / ETEC	Localized and aggregative		JAYSGN000000000
Ec-36.1	aEPEC / ETEC / DAEC	Localized, aggregative and diffuse		JAYMYX000000000
Ec-36.4	aEPEC / ETEC	Localized and aggregative		JAYSGM000000000

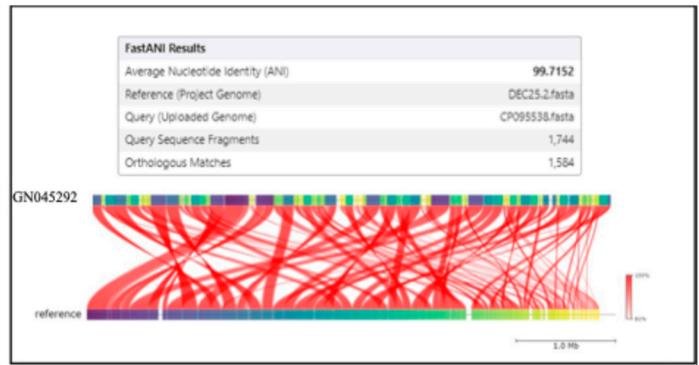
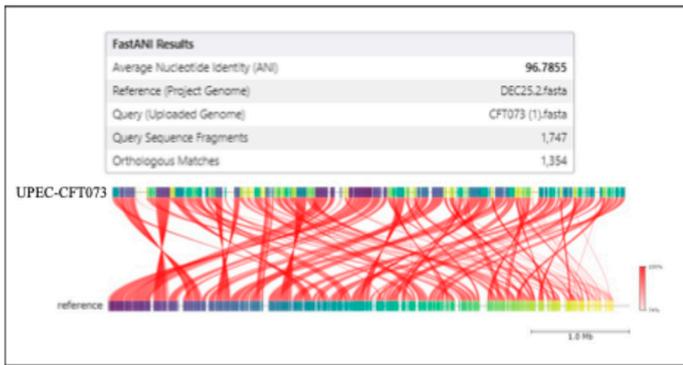
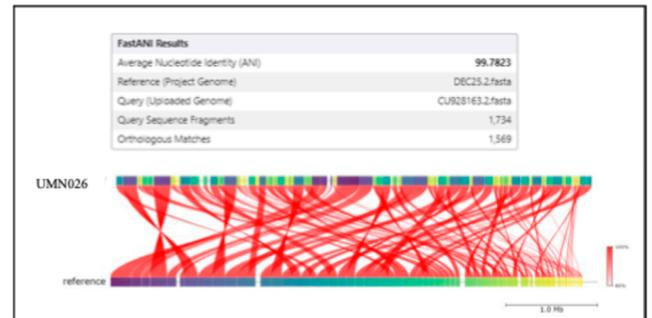
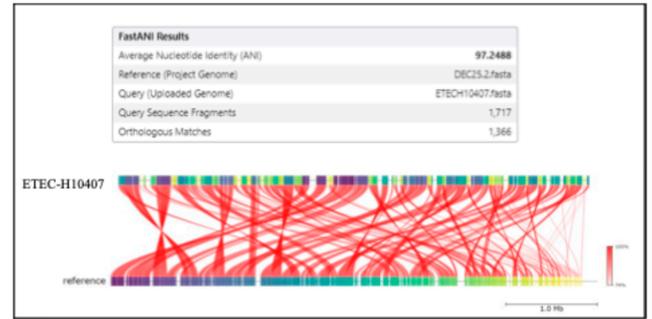
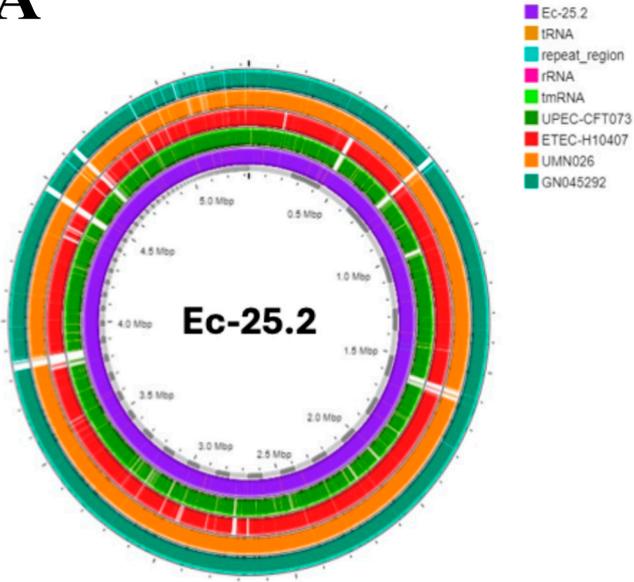
Table S3. Representative Genes of The Different Genomic Islands Found in the Sequenced Strains of pathogenic-hybrid *E. coli* (IslandViewer⁴).

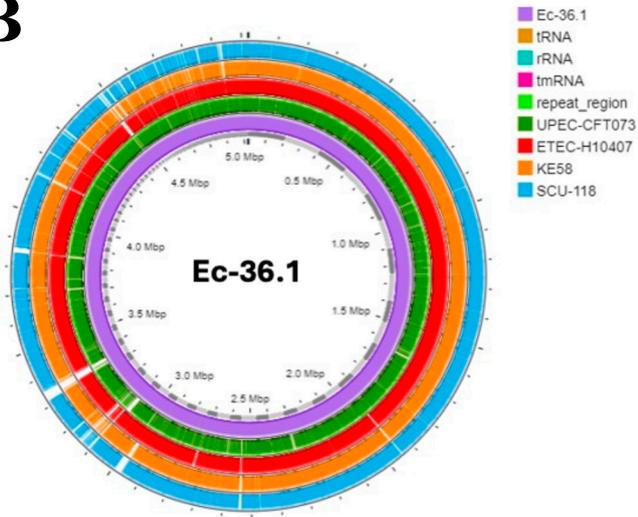
Strain Ec-25.2	
Phages Origin	
GI5	Phage integrase, phage tail tip specificity protein J, phage tail fiber, side tail fiber protein Stf
GI7	Phage host-killing protein Ki, Phage superinfection exclusion protein B, Phage transcriptional regulator Cro
GI9	Bacteriophage-encoded homolog of DNA replication protein DnaC, Phage head, head-DNA stabilization protein D, Phage activator protein cII, Phage repressor protein cI
GI11	Phage head, terminate DNA packaging protein A, Phage head, head-tail joining protein W
GI12	Phage-encoded host-cell envelope protein Lom, Phage tail fiber, side tail fiber protein Stf, Phage tail fiber, tail fiber assembly protein Tfa
GI16	Phage tail tip, assembly protein L
GI17	Phage lysozyme R, Phage endopeptidase
GI30	Phage major capsid protein, Phage head maturation protease, Phage head, portal protein B, Phage head, head-tail joining protein W
GI31	Phage terminate, small subunit, Phage-associated homing endonuclease, Putative prophage membrane protein
GI34	Phage integrase
GI51	Phage antitermination protein Q, Phage holin/antiholin component S, Phage tail, component G, Phage tail component T, Phage tail, component G Phage-encoded virulence determinant Bor, Phage tail, tail length tape-measure protein H
GI52	Phage protein YbcV and YbcW, Phage lysozyme R
GI56	Prophage Kil protein
GI57	Phage exclusion protein ren, Phage Rha protein
GI58	Phage protein NinE, Uncharacterized protein YbcO, Uncharacterized protein YdfB,
GI59	Phage head, head-tail joining protein FII and FI, Phage head, major capsid protein E, Phage tail, tail length tape-measure protein H, Phage tail, component T and G
Mobile Genetic Elements	
GI47	Transposase InsN for insertion sequence element IS911
GI63	IncF plasmid conjugative transfer regulator TraY, TraJ, TraM
Adhesion as Fimbriae	
GI21	Mannose-specific adhesin FimH, Type 1 fimbriae adaptor subunit FimG, Outer membrane usher protein FimD
GI41	Type-1 fimbrial protein, A chain precursor, Chaperone protein FimC precursor
GI48	Type 1 fimbriae regulatory protein FimB, Type 1 fimbriae regulatory protein FimE, Fimbrin-like protein FimI

Virulence	
GI24	Antigen 43, Aerobactin siderophore receptor IutA, IucD, IucC, IucA, IucB, Per-activated serine protease autotransporter enterotoxin EspC / autotransporter domain, T5aSS type secretion
GI54	PapE protein, PapG protein, YeeU protein (antitoxin to YeeV)
Antibiotic Resistance	
GI33	Multidrug efflux system EmrKY-TolC, inner-membrane proton/drug antiporter EmrY, EmrK
GI64	Aminoglycoside 3"-nucleotidyltransferase (AadA family), Small multidrug resistance (SMR) efflux transporter QacEΔ1, quaternary ammonium compounds, Dihydropteroate synthase type-2, Sulfonamide resistance protein
Strain Ec-36.1	
Phages Origin	
GI7	Phage ea22 protein, Phage recombination protein Bet, Phage host-nuclease inhibitor protein Gam, prophage Kil protein, Phage tail assembly chaperone, Phage tail assembly protein, Phage tail, tail length tape-measure protein H
GI13	Phage head, head-DNA stabilization protein D, Phage activator protein Cii, Phage holin/antiholin component S
GI15	Phage protein YbcV
GI16	Phage-encoded host-cell envelope protein Lom, Phage tail fiber, side tail fiber protein Stf, Phage tail fiber, tail fiber assembly protein Tfa,
GI35	Phage protein NinH and NinG
GI36	Phage antirepressor protein, prophage Kil protein, Phage recombination protein Bet
GI37	Phage ea22 protein, Phage DNA binding protein Roi, Phage antirepressor protein.
GI51	Phage capsid scaffolding protein GpO, Phage major capsid protein GpN, Phage tail protein GpX, Phage lysis regulatory protein LysA, LysB, LysC, Phage tail completion protein GpR, GpS.
GI60	Phage tail fiber protein, Phage terminase, large subunit (T4-like headful)
GI64	Phage head, portal protein B, Phage head, terminase DNA packaging protein A, Phage endopeptidase Rz
Mobile Genetic Elements	
GI26	Transposase / Integrase
GI46	Incl1 plasmid conjugative transfer ATPase PilQ, Incl1 plasmid conjugative transfer inner membrane protein PilR and PilS, Incl1 plasmid conjugative transfer pilus-tip adhesin protein PilV, Incl1 plasmid conjugative transfer protein TraI, TraS and TraU, transposase, Insertion element IS401 (<i>Burkholderia multivorans</i>) transposase
GI55	Integrase, Transposase InsN for insertion sequence element IS911, Transposase InsO for insertion sequence element IS911
Adhesion as Fimbriae	
GI4	CFA/I fimbrial auxiliary subunit, CFA/I fimbrial major subunit
Virulence	
GI3	T6SS component Hcp, Type VI secretion-related protein VasL, T6SS component TssM (IcmF/VasK)
GI10	Biofilm PGA synthesis N-glycosyltransferase PgaC, PgaB, PgaA
GI42	Iron (III) dicitrate transport system, periplasmic iron-binding protein FecB, FecC, FecD and FecE, Antigen 43,
GI66	Ferric enterobactin-binding periplasmic protein FepB, Enterobactin exporter EntS
Antibiotic Resistance	
GI23	Small multidrug resistance (SMR) efflux transporter
GI39	Multidrug efflux system EmrKY-TolC, inner-membrane proton/drug antiporter EmrY (MFS type)
Toxin-Antitoxin System	
GI9	YeeU protein (antitoxin to YeeV)
Strain Ec-36.4	
Phage Origin	
GI6	Phage ea22 protein, Phage recombination protein Bet, Phage host-nuclease inhibitor protein Gam, prophage Kil protein
GI8	Phage-encoded virulence determinant Bor, Phage protein YbcW and YbcV, Phage head, portal protein B, Phage tail assembly chaperone, Phage tail tip, assembly protein M and L
GI14	Prophage maintenance; modulation of host cell killing, Phage protein YdfU family, Phage protein YbcV
GI15	Phage protein YbcV
GI16	Phage-encoded host-cell envelope protein Lom, Phage tail fiber, side tail fiber protein Stf, Phage tail fiber, tail fiber assembly protein Tfa
GI19	Phage host-killing protein Kil, Phage host-nuclease inhibitor protein Gam, Phage recombination protein Bet
GI23	Phage-encoded host-cell envelope protein Lom, Phage tail fiber protein
GI34	Phage protein NinH, Phage recombination protein NinG
GI35	Prophage Kil protein, Phage recombination protein Bet
GI37	Phage ea22 protein, Phage DNA binding protein Roi
Mobile Genetic Elements	
GI26	Transposase, Integrase
GI41	Transposase InsO for insertion sequence element IS911
GI46	Incl1 plasmid conjugative transfer pilus-tip adhesin protein PilV, Incl1 plasmid conjugative transfer protein TraI, TraS and TraU, Insertion element IS401 (<i>Burkholderia multivorans</i>) transposase
GI52	Integrase, transposase, Transposase InsN for insertion sequence element IS911, Transposase InsO for insertion sequence element IS911

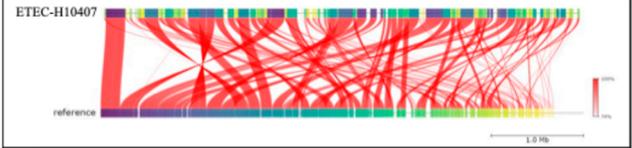
GI70	<i>IS</i> , phage, Tn; Transposon-related functions
Adhesion as Fimbriae	
GI43	Putative outer membrane usher protein YqiG
Virulence	
GI10	Biofilm PGA synthesis N-glycosyltransferase PgaC and PgaB, Biofilm PGA outer membrane secretin PgaA
GI22	DNA-damage-inducible protein I, Per-activated serine protease autotransporter enterotoxin EspC / autotransporter domain, T5aSS type secretion
GI40	Type III secretion bridge between inner and outer membrane lipoprotein (YscJ,HrcJ,EscJ, PscJ), Putative Type III secretion apparatus protein, Type III secretion cytoplasmic protein (YscF), Type III secretion inner membrane protein (YscU,SpaS,EscU,HrcU,SsaU, homologous to flagellar export components)
GI42	Antigen 43
GI56	Antigen 43
Antibiotic Resistance	
GI38	Multidrug efflux system EmrKY-TolC, inner-membrane proton/drug antiporter EmrY (MFS type), Multidrug efflux system EmrKY-TolC, membrane fusion component EmrK
Toxin-Antitoxin System	
GI9	YeeU protein (antitoxin to YeeV)
GI68	RelB/StbD replicon stabilization protein (antitoxin to RelE/StbE)

A

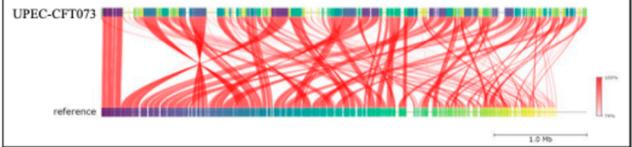


B**FastANI Results**

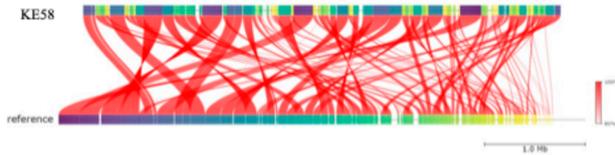
Average Nucleotide Identity (ANI)	99.0541
Reference (Project Genome)	DEC36.3 (3).fasta
Query (Uploaded Genome)	ETECH10407.fasta
Query Sequence Fragments	1,717
Orthologous Matches	1,413

**FastANI Results**

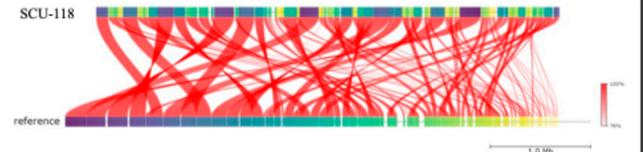
Average Nucleotide Identity (ANI)	96.6349
Reference (Project Genome)	DEC36.3 (3).fasta
Query (Uploaded Genome)	CFT073.fasta
Query Sequence Fragments	1,747
Orthologous Matches	1,327

**FastANI Results**

Average Nucleotide Identity (ANI)	99.5215
Reference (Project Genome)	DEC36.3 (3).fasta
Query (Uploaded Genome)	CP141075.1.fasta
Query Sequence Fragments	1,581
Orthologous Matches	1,412

**FastANI Results**

Average Nucleotide Identity (ANI)	99.5542
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Query (Uploaded Genome)	CP051716.fasta
Query Sequence Fragments	1,537
Orthologous Matches	1,404



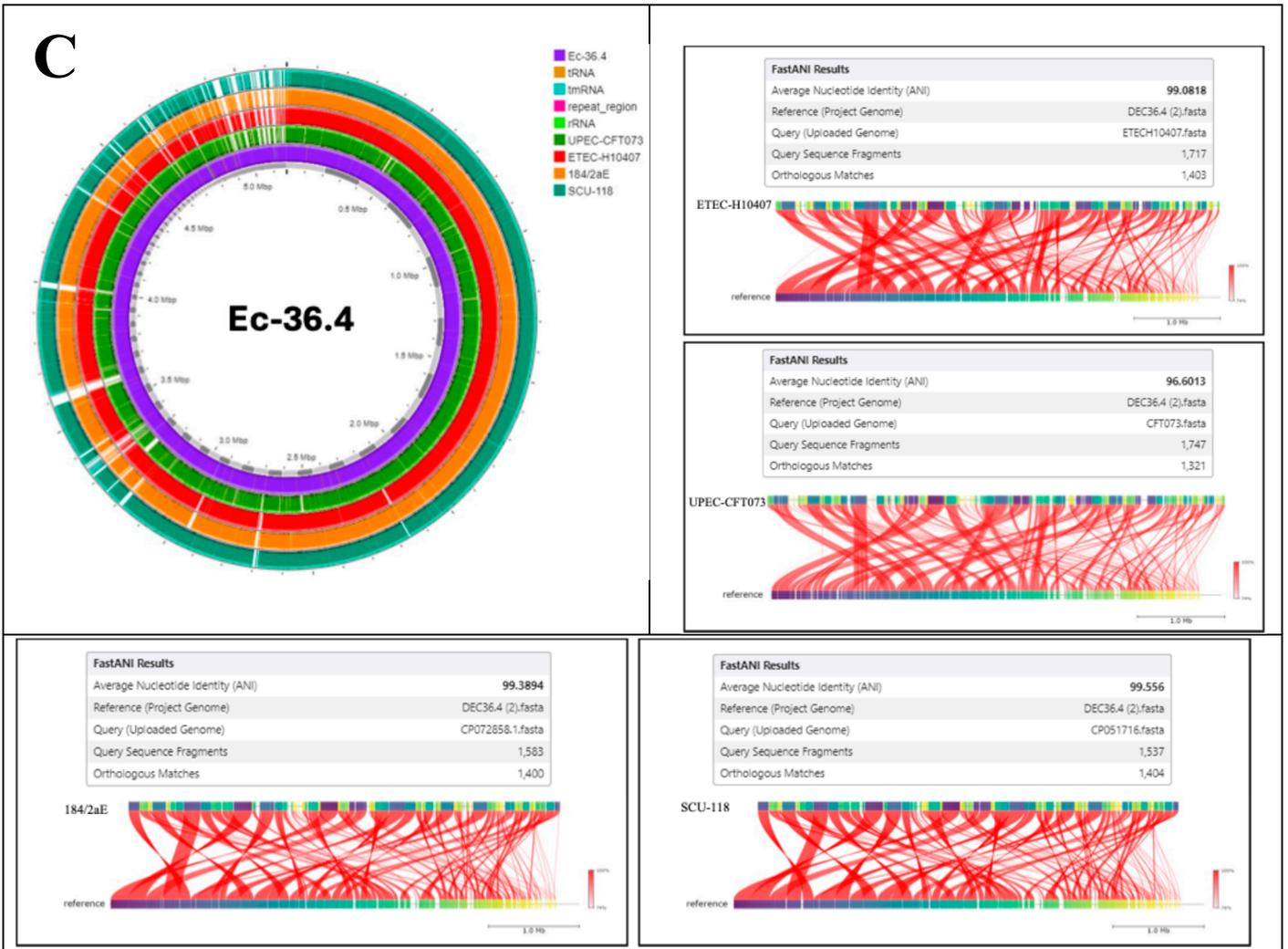


Figure S1. Maps constructed in Proksee, showing the comparison between the genomes of the present study with reference genomes previously reported in NCBI. A) Ec-25.2 genome; B) Ec-36.1 genome; C) Ec-36.4 genome. The reference genomes were selected based on characteristics that are considered to be pathogenic hybrids, therefore the reference genomes of the ETEC diarrhoeagenic pathotype and the ExPEC pathotype corresponding to UPEC are used. The other two genomes correspond to analyses performed in IslandViewer (orange) and BV-BCR (blue) where similar previously reported genomes were searched.

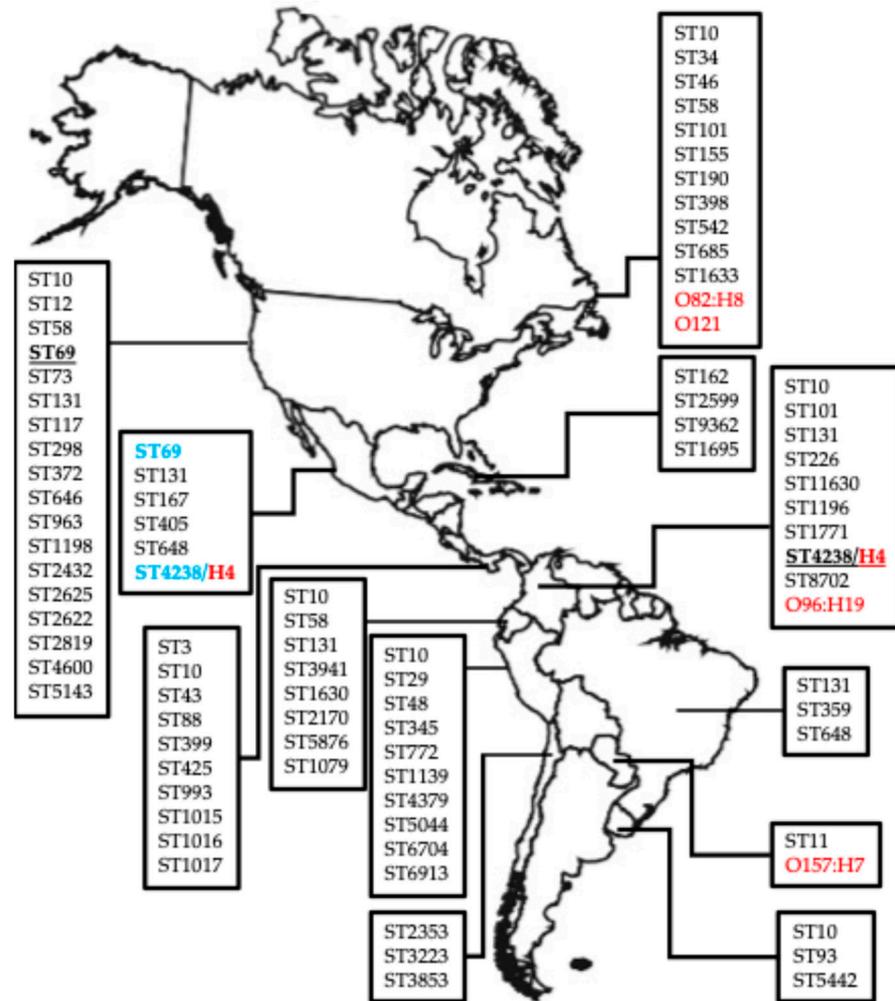


Figure S2. Regional distribution of *E. coli* Sequence Types (STs) and Serotypes in America. In bold shape and underlined are the ST previously reported corresponding with the ST identified in this study (highlighted in blue). In red, are serotypes reported in the last 5 years in the Americas region. The vector map is available at Vecteezy.com.

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