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# Complete genomes of *Escherichia coli* isolates encoding the EatA autotransporter from pediatric sources

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**ABSTRACT** Virulence in *Escherichia coli* is multifactorial, with additional virulence factors continuously being examined and characterized. We describe the complete genomes of 12 *E. coli* isolates obtained from subjects enrolled in the Global Enteric Multicenter Study, each containing the virulence-associated enterotoxigenic *E. coli* autotransporter A gene (*eatA*).

**KEYWORDS** *Escherichia coli*, ETEC, virulence, EatA, plasmid, genome

While not considered a canonical virulence gene of enterotoxigenic *Escherichia coli* (ETEC), there is mounting evidence that the ETEC autotransporter A (*eatA*) plays a critical role in ETEC virulence (1). We describe the complete genomes of 12 *E. coli* isolates (2) containing the *eatA* gene (1, 3) from each of the seven geographic sites of the Global Enteric Multicenter Study (4). Phylogenomically diverse *eatA*-containing isolates from cases and controls, as well as male and female subjects, were selected for complete genome sequencing (Table 1).

Initial isolation is described by Panchalingam et al. (5). Briefly, feces or rectal swabs (transported in cold containers) were inoculated into Cary–Blair transport media then selective growth media within 6 and 18 hours of collection. Several lactose-fermenting colonies (red/pink) were selected from McConkey agar (incubated for 48 hours at 37°C) and sub-cultured in motility indole ornithine medium. Suspected *E. coli* isolates [motility(–), indole ornithine(–), methyl-red(+), Voges–Proskauer(–), citrate(–)] were lacking canonical pathotype genes as determined by multiplex PCR (6). Verified *E. coli* isolates were frozen in 20% glycerol and maintained at −80°C. Frozen cultures were provided to, and stored at, the Center for Vaccine Development, University of Maryland School of Medicine.

Isolates were resurrected from frozen culture on lysogeny agar overnight at 37°C. Genomic DNA was extracted using Qiagen's DNeasy Blood & Tissue Kit, and sequencing libraries for both sequencing technologies were generated with unsheared genomic DNA. Nanopore libraries were prepared using Oxford Nanopore Technologies' (ONT) Genomic DNA by Ligation Kit (7) and run on Nanopore R9 flow cells with the MinION Mk1B sequencer. Basecalling was performed using Guppy (v4.2.2) (8). Illumina libraries (150 bp paired end) were prepared using the Kapa Library Kit (Roche/Illumina) and sequenced on the Illumina HiSeq 4000 platform. Quality control and adapter trimming were performed with bcl2fastq (v2.20.0.445) (9) and porechop (v0.2.3_seqan2.1.1) (8) for Illumina and ONT sequencing, respectively. Hybrid assembly using both Illumina and ONT reads was conducted with Unicycler (v0.4.8) (10). Final assembly statistics were recorded with QUAST (5.0.2) (11). Genomes were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (v6.10) (12). All software was run with default values.

Isolate information, relevant sequencing statistics, and GenBank accession numbers are included in Table 1. Average genome coverage was 339.3× (range 257–489×), and the assembled genomes have an average genome size of 5,316,437 bp (range 5,129,814–

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**TABLE 1** Isolate and genome metrics

| Isolate information | | | | | | | | Sequencing statistics | | | | | | | | | | Accession numbers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Country | Case/control | Sex | Year of isolation | Phylogenetic group | Serotype | ST | Molecule name | Size (bp) | Illumina read pairs[a] | Illumina bases[a] | ONT trimmed reads[a] | ONT trimmed bases[a] | ONT N50 | Genome coverage[a] | No. of contigs | Total genome length (bp) | GC (%) | BioProject | Assembly | SRA (Illumina) | SRA (ONT) |
| 100117 | Gambia | Case | Female | 2008 | Cryptic | OND:H45 | 485 | E100117 | 5,053,006 | 3,198,198 | 983,510,344 | 157,308 | 513,659,526 | 3,299.5 | 273.3 | 6 | 5,478,516 | 50.25 | PRJNA1321367 | JBRERF010000001 | SRR35329244 | SRR35329229 |
| | | | | | | | | pE100117_127 | 127,487 | | | | | | | | | | | JBRERF010000002 | | |
| | | | | | | | | pE100117_123 | 123,295 | | | | | | | | | | | JBRERF010000003 | | |
| | | | | | | | | pE100117_121[b] | 121,255 | | | | | | | | | | | JBRERF010000004 | | |
| | | | | | | | | pE100117_50 | 50,722 | | | | | | | | | | | JBRERF010000005 | | |
| | | | | | | | | pE100117_3 | 2,751 | | | | | | | | | | | JBRERF010000006 | | |
| 100341 | Gambia | Case | Female | 2008 | A | O7:H4 | 484 (168) | E100341 | 4,860,512 | 3,530,593 | 1,086,152,515 | 179,773 | 762,931,855 | 4,265.0 | 357.5 | 5 | 5,172,020 | 50.53 | PRJNA1322554 | JBRERQ010000001 | SRR35332983 | SRR35332980 |
| | | | | | | | | pE100341_124[b] | 123,858 | | | | | | | | | | | JBRERQ010000002 | | |
| | | | | | | | | pE100341_119 | 119,093 | | | | | | | | | | | JBRERQ010000003 | | |
| | | | | | | | | pE100341_66 | 66,469 | | | | | | | | | | | JBRERQ010000004 | | |
| | | | | | | | | pE100341_2 | 2,088 | | | | | | | | | | | JBRERQ010000005 | | |
| 200086 | Mali | Control | Male | 2008 | Cryptic I | OND:H45 | 485 | E200086 | 5,179,901 | 3,063,393 | 939,867,671 | 612,423 | 1,861,035,025 | 3,057.0 | 489.1 | 9 | 5,727,105 | 50.28 | PRJNA1322554 | JBRERP010000001 | SRR35332982 | SRR35332979 |
| | | | | | | | | pE200086_121[b] | 121,281 | | | | | | | | | | | JBRERP010000002 | | |
| | | | | | | | | pE200086_120 | 120,396 | | | | | | | | | | | JBRERP010000003 | | |
| | | | | | | | | pE200086_110 | 110,583 | | | | | | | | | | | JBRERP010000004 | | |
| | | | | | | | | pE200086_79 | 77,979 | | | | | | | | | | | JBRERP010000005 | | |
| | | | | | | | | pE200086_59 | 59,766 | | | | | | | | | | | JBRERP010000006 | | |
| | | | | | | | | pE200086_49 | 49,559 | | | | | | | | | | | JBRERP010000007 | | |
| | | | | | | | | pE200086_59 | 4,885 | | | | | | | | | | | JBRERP010000008 | | |
| | | | | | | | | pE200086_3 | 2,755 | | | | | | | | | | | JBRERP010000009 | | |
| 200139 | Mali | Case | Female | 2008 | B1 | O104:H2 | 678 | E200139 | 4,932,310 | 3,362,912 | 1,034,895,605 | 1,126,528 | 1,325,135,994 | 1,204.3 | 442.0 | 7 | 5,339,051 | 50.66 | PRJNA1322554 | JBRERO010000001 | SRR35332971 | SRR35332978 |
| | | | | | | | | pE200139_148 | 148,227 | | | | | | | | | | | JBRERO010000002 | | |
| | | | | | | | | pE200139_112[b] | 112,455 | | | | | | | | | | | JBRERO010000003 | | |
| | | | | | | | | pE200139_78 | 77,987 | | | | | | | | | | | JBRERO010000004 | | |
| | | | | | | | | pE200139_60 | 60,142 | | | | | | | | | | | JBRERO010000005 | | |
| | | | | | | | | pE200139_5 | 4,686 | | | | | | | | | | | JBRERO010000006 | | |
| | | | | | | | | pE200139_3_2 | 3,244 | | | | | | | | | | | JBRERO010000007 | | |
| 203449 | Mali | Control | Female | 2009 | A | O8:H10 | 5386 | E203449 | 4,985,798 | 3,738,736 | 1,151,163,899 | 451,976 | 687,167,023 | 1,546.2 | 348.2 | 6 | 5,280,152 | 50.63 | PRJNA1322554 | JBRERN010000001 | SRR35332968 | SRR35332977 |
| | | | | | | | | pE203449_121 | 121,928 | | | | | | | | | | | JBRERN010000002 | | |
| | | | | | | | | pE203449_106[b] | 106,280 | | | | | | | | | | | JBRERN010000003 | | |
| | | | | | | | | pE203449_57 | 57,717 | | | | | | | | | | | JBRERN010000004 | | |
| | | | | | | | | pE203449_4_2 | 4,232 | | | | | | | | | | | JBRERN010000005 | | |
| | | | | | | | | pE203449_4_1 | 4,197 | | | | | | | | | | | JBRERN010000006 | | |

(*Continued on next page*)

**TABLE 1** Isolate and genome metrics (*Continued*)

| Isolate information | | | | | | | | Sequencing statistics | | | | | | | | | | | Accession numbers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Country | Case/control | Sex | Year of isolation | Phylogenetic group | Serotype | ST | Molecule name | Size (bp) | Illumina read pairs[a] | Illumina bases[a] | ONT trimmed reads[a] | ONT trimmed bases[a] | ONT N50 | Genome coverage[a] | No. of contigs | Total genome length (bp) | GC (%) | BioProject | Assembly | SRA (Illumina) | SRA (ONT) |
| 300010 | Mozambique | Control | Female | 2007 | D | O23:H15 | 70 | E300010 | 5,055,009 | 4,002,638 | 1,233,426,158 | 239,953 | 439,745,417 | 1,852.5 | 309.4 | 9 | 5,408,625 | 50.48 | PRJNA1322554 | JBRERM010000001 | SRR35332967 | SRR35332976 |
| | | | | | | | | pE300010_105 | 105,941 | | | | | | | | | | | JBRERM010000002 | | |
| | | | | | | | | pE300010_86 | 86,575 | | | | | | | | | | | JBRERM010000003 | | |
| | | | | | | | | pE300010_79 | 79,468 | | | | | | | | | | | JBRERM010000004 | | |
| | | | | | | | | pE300010_68[b] | 68,198 | | | | | | | | | | | JBRERM010000005 | | |
| | | | | | | | | pE300010_5 | 5,271 | | | | | | | | | | | JBRERM010000006 | | |
| | | | | | | | | pE300010_4 | 4,510 | | | | | | | | | | | JBRERM010000007 | | |
| | | | | | | | | pE300010_2 | 2,101 | | | | | | | | | | | JBRERM010000008 | | |
| | | | | | | | | pE300010_1 | 1,552 | | | | | | | | | | | JBRERM010000009 | | |
| 300082 | Mozambique | Case | Male | 2008 | B1 | O91:H10 | 12313 | E300082 | 4,572,448 | 3,688,114 | 1,134,203,909 | 145,832 | 489,282,805 | 3,401.0 | 311.9 | 7 | 5,205,800 | 50.56 | PRJNA1322554 | JBRERL010000001 | SRR35332966 | SRR35332975 |
| | | | | | | | | pE300082_261 | 261,732 | | | | | | | | | | | JBRERL010000002 | | |
| | | | | | | | | pE300082_153[b] | 153,406 | | | | | | | | | | | JBRERL010000003 | | |
| | | | | | | | | pE300082_104 | 104,543 | | | | | | | | | | | JBRERL010000004 | | |
| | | | | | | | | pE300082_56 | 56,826 | | | | | | | | | | | JBRERL010000005 | | |
| | | | | | | | | pE300082_50 | 50,961 | | | | | | | | | | | JBRERL010000006 | | |
| | | | | | | | | pE300082_6 | 5,884 | | | | | | | | | | | JBRERL010000007 | | |
| 300446 | Mozambique | Control | Female | 2008 | Cryptic I | OND:H45 | 485 | E300446 | 5,019,886 | 3,356,876 | 1,030,449,026 | 268,043 | 821,078,065 | 3,070.8 | 348.6 | 7 | 5,311,476 | 50.29 | PRJNA1322554 | JBRERK000000001 | SRR35332965 | SRR35332974 |
| | | | | | | | | pE300446_128 | 128,494 | | | | | | | | | | | JBRERK010000002 | | |
| | | | | | | | | pE300446_81[b] | 81,849 | | | | | | | | | | | JBRERK010000003 | | |
| | | | | | | | | pE300446_72 | 72,150 | | | | | | | | | | | JBRERK010000004 | | |
| | | | | | | | | pE300446_5 | 5,099 | | | | | | | | | | | JBRERK010000005 | | |
| | | | | | | | | pE300446_3 | 2,757 | | | | | | | | | | | JBRERK010000006 | | |
| | | | | | | | | pE300446_1 | 1,241 | | | | | | | | | | | JBRERK010000007 | | |
| 400634 | Kenya | Case | Female | 2008 | Cryptic I | OND:H45 | 485 | E400634 | 5,038,625 | 3,906,639 | 1,201,154,153 | 246,927 | 660,754,750 | 2,716.9 | 349.7 | 7 | 5,323,749 | 50.15 | PRJNA1322554 | JBRERJ010000001 | SRR35332964 | SRR35332973 |
| | | | | | | | | pE400634_103 | 103,452 | | | | | | | | | | | JBRERJ010000002 | | |
| | | | | | | | | pE400634_87[b] | 87,445 | | | | | | | | | | | JBRERJ010000003 | | |
| | | | | | | | | pE400634_48 | 48,180 | | | | | | | | | | | JBRERJ010000004 | | |
| | | | | | | | | pE400634_33 | 32,917 | | | | | | | | | | | JBRERJ010000005 | | |
| | | | | | | | | pE400634_8 | 7,952 | | | | | | | | | | | JBRERJ010000006 | | |
| | | | | | | | | pE400634_5 | 5,178 | | | | | | | | | | | JBRERJ010000007 | | |

(*Continued on next page*)

**TABLE 1** Isolate and genome metrics (*Continued*)

| Isolate information | | | | | | | | Sequencing statistics | | | | | | | | | | Accession numbers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Country | Case/control | Sex | Year of isolation | Phylogenetic group | Serotype | ST | Molecule name | Size (bp) | Illumina read pairs[a] | Illumina bases[a] | ONT trimmed reads[a] | ONT trimmed bases[a] | ONT N50 | Genome coverage[a] | No. of contigs | Total genome length (bp) | GC (%) | BioProject | Assembly | SRA (Illumina) | SRA (ONT) |
| 500002 | India | Case | Male | 2007 | Cryptic I | O ND:H45 | 485 | E500002 | 4,973,609 | 3,538,598 | 1,085,172,960 | 226,565 | 501,934,622 | 2,246.4 | 301.4 | 6 | 5,266,097 | 50.2 | PRJNA1322554 | JBRERI010000001 | SRR35332963 | SRR35332972 |
| | | | | | | | | pE500002_123[b] | 123,337 | | | | | | | | | | | JBRERI010000002 | | |
| | | | | | | | | pE500002_112 | 112,330 | | | | | | | | | | | JBRERI010000003 | | |
| | | | | | | | | pE500002_49 | 49,554 | | | | | | | | | | | JBRERI010000004 | | |
| | | | | | | | | pE500002_5 | 5,178 | | | | | | | | | | | JBRERI010000005 | | |
| | | | | | | | | pE500002_2 | 2,089 | | | | | | | | | | | JBRERI010000006 | | |
| 600003 | Bangladesh | Case | Male | 2007 | Cryptic I | O ND:H45 | 485 | E600003 | 4,933,499 | 2,785,935 | 840,338,429 | 203,157 | 486,787,717 | 2,431.0 | 257.5 | 6 | 5,154,834 | 50.17 | PRJNA1322554 | JBRERH010000001 | SRR35332962 | SRR35332970 |
| | | | | | | | | pE600003_100 | 100,740 | | | | | | | | | | | JBRERH010000002 | | |
| | | | | | | | | pE600003_68[b] | 67,796 | | | | | | | | | | | JBRERH010000003 | | |
| | | | | | | | | pE600003_47 | 46,687 | | | | | | | | | | | JBRERH010000004 | | |
| | | | | | | | | pE600003_4 | 4,011 | | | | | | | | | | | JBRERH010000005 | | |
| | | | | | | | | pE600003_2 | 2,101 | | | | | | | | | | | JBRERH010000006 | | |
| 700025 | Pakistan | Control | Male | 2008 | D | O166:H15 | 349 (349) | E700025 | 4,964,031 | 3,633,291 | 1,115,663,409 | 145,654 | 338,604,055 | 2,375.5 | 283.5 | 5 | 5,129,814 | 50.63 | PRJNA1322554 | JBRERG010000001 | SRR35332981 | SRR35332969 |
| | | | | | | | | pE700025_154[b] | 154,569 | | | | | | | | | | | JBRERG010000002 | | |
| | | | | | | | | pE700025_5_7 | 5,775 | | | | | | | | | | | JBRERG010000003 | | |
| | | | | | | | | pE700025_4 | 3,887 | | | | | | | | | | | JBRERG010000004 | | |
| | | | | | | | | pE700025_1_5 | 1,552 | | | | | | | | | | | JBRERG010000005 | | |

[a]Values refer to the complete genome project.
[b]Gray highlight indicates *eatA*-containing plasmid.

5,727,105 bp) and an average GC% of 50.4% (range 50.15%–50.7%). Analysis of these 12 *eatA*-positive isolates determined the sequence type (MLST) (13, 14) and molecular serotype (Serotype Finder v1.1 [15, 16]). Together, these isolates represent seven distinct sequence types and serotypes (Table 1).

The plasmid-encoded *eatA* gene was harbored on plasmids ranging from 68 to 153 kb, indicating *eatA* is carried on diverse plasmids and is phylogenomically distributed.

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| National Institutes of Health | U19AI110820 | David Rasko<br>Bernadette A. Hritzo<br>Jane M. Michalski Wilhelm |


## DATA AVAILABILITY

All data have been released in BioProjects PRJNA1321367 and PRJNA1322554. The associated SRA and genome assembly accession numbers for each isolate are listed in Table 1.

## ETHICS APPROVAL

This study received approval from the University of Maryland Baltimore Institutional Review Board (protocols HP-00040030 and HP-00059433-7).

The clinical protocol was approved by ethics committees at the University of Maryland Baltimore and at each GEMS field site (4). Written informed consent was obtained from the parent or primary caretaker of each participant before the initiation of study activities (4).

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