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The Genome Sequence of Avian Pathogenic *Escherichia coli* Strain O1:K1:H7 Shares Strong Similarities with Human Extraintestinal Pathogenic *E. coli* Genomes▽

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***Escherichia coli* strains that cause disease outside the intestine are known as extraintestinal pathogenic *E. coli* (ExPEC) and include human uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC). Regardless of host of origin, ExPEC strains share many traits. It has been suggested that these commonalities may enable APEC to cause disease in humans. Here, we begin to test the hypothesis that certain APEC strains possess potential to cause human urinary tract infection through virulence genotyping of 1,000 APEC and UPEC strains, generation of the first complete genomic sequence of an APEC (APEC O1:K1:H7) strain, and comparison of this genome to all available human ExPEC genomic sequences. The genomes of APEC O1 and three human UPEC strains were found to be remarkably similar, with only 4.5% of APEC O1's genome not found in other sequenced ExPEC genomes. Also, use of multilocus sequence typing showed that some of the sequenced human ExPEC strains were more like APEC O1 than other human ExPEC strains. This work provides evidence that at least some human and avian ExPEC strains are highly similar to one another, and it supports the possibility that a food-borne link between some APEC and UPEC strains exists. Future studies are necessary to assess the ability of APEC to overcome the hurdles necessary for such a food-borne transmission, and epidemiological studies are required to confirm that such a phenomenon actually occurs.**

Escherichia coli is among the world's most well-studied organisms and is often found at the forefront of advancing technology. Not surprisingly, *E. coli* is on the leading edge of an ongoing shift in the field of genomics (3, 6, 65). Now that at least one representative organism per species has been sequenced for most pathogens of interest, the focus in genomics has reoriented towards obtaining multiple sequences within a species. With more genomic sequences available for *E. coli* than for any other species, it leads this trend (3). Thus far, all of the pathogenic *E. coli* strains sequenced have originated from human hosts (6, 8, 10, 24, 47, 68). This bias has left a gap in our knowledge, as various *E. coli* strains cause significant and widespread disease in animals, including in those raised for human consumption (2, 13, 41). Consequently, while the genomic analysis of *E. coli* strains from animals can be justified solely on the basis of *E. coli*'s detrimental impact on animal agriculture, a broader justification would also include the potential link between animal-source *E. coli* and human disease.

Links between human and animal disease caused by *E. coli* are well established in some instances but remain speculative in others. For instance, recent reports of outbreaks of human urinary tract infections (UTIs) have stimulated interest in the potential that *E. coli* from animals has to cause human UTIs via the food supply (28, 41, 49). Since UTIs are among the world's most common bacterial infections (20), cause significant morbidity, and cost the health care system of the United States over a billion dollars annually (20, 55), this putative link deserves attention. Although it is generally agreed that the immediate source of uropathogenic *E. coli* (UPEC) causing human UTIs is an individual's own colonic flora (27), it is not completely understood how these virulent clones come to inhabit the colon. One hypothesis is that retail poultry harboring avian pathogenic *E. coli* (APEC) represents a food-borne source of *E. coli* clones capable of causing human UTIs (50, 52).

APEC cause colibacillosis in production birds, a widespread disease which is responsible for multimillion dollar annual losses for the world's poultry industries (2). APEC strains are a subset of extraintestinal pathogenic *E. coli* (ExPEC), a pathotypic category which also includes UPEC (55) and *E. coli* causing neonatal meningitis and septicemia (27, 34). As ExPEC, APEC and UPEC both cause extraintestinal disease, share virulence-associated traits, and have overlapping O serogroups and phylogenetic types (50). Additionally, there is a well-documented history of transfer of *E. coli* strains and their plasmids from poultry to humans (37, 38, 44, 67), and a recent report has shown that APEC plasmids can contribute to the urovirulence of *E. coli* for mammalian hosts (62). These observations have led to further speculation that APEC might be a source of

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UPEC, which gains access to the human colon following ingestion of contaminated poultry (50). Here, we begin to test the hypothesis that some APEC strains are a source of human UPEC by generating the first APEC genome sequence and comparing it to all of the human UPEC genomes currently available. This newly sequenced ExPEC strain, APEC O1 (an O1:K1:H7 strain), was chosen for study because its serotype, phylogenetic group, and virulence genotype were representative of 1,000 well-characterized APEC and human UPEC isolates.

MATERIALS AND METHODS

Choice of strain for sequencing. (i) Bacterial strains. The choice of APEC O1 for sequencing was based on its possession of traits characteristic of strains causing extraintestinal disease in poultry and humans. APEC isolates were obtained from lesions of chickens and turkeys clinically diagnosed with colibacillosis from various locations within the United States. The UPEC strains were isolated from cases of human UTIs; they were kindly provided by Paul Carson (Meritcare Hospital, Fargo, ND) or were from the VA Medical Center, Minneapolis, MN. A subset of both groups of isolates has been described previously (50, 51). For embryo lethality studies, three additional APEC isolates were used: APEC V1 (O nontypeable) and APEC V2 and V7 (both O2). For 1-day-old chick studies, three strains previously characterized as being of high, intermediate, and low lethality towards birds were used (53). All isolates were serogrouped through the *E. coli* Reference Center (Pennsylvania State University, University Park, PA) and were screened for traits associated with APEC and UPEC virulence, using the techniques described below. Organisms were stored at −80°C in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 10% (vol/vol) glycerol until use (57).

(ii) Virulence gene and phylogenetic typing. Test and control organisms were examined for the presence of a variety of genes known for their association with APEC or UPEC virulence by using multiplex PCR procedures which have been previously described (31, 32, 50, 51).

Genomic analysis. (i) Genomic sequencing of APEC O1. Two random shotgun libraries of APEC O1 were created: a 2- to 3-kb small-insert library and a 35- to 40-kb fosmid library. Shotgun sequencing was performed on the small-insert library by MWG Biotech (Hedersberg, Germany), using ABI 3700 and ABI 3730xl capillary sequencers. The fosmid library was also end sequenced. Finishing was accomplished using the pooled primer technique described by Tettelin et al. and used elsewhere (31, 32, 66). The final gaps were closed using standard PCR. Sequencing reads were assembled using SeqMan software from DNASTAR (Madison, WI). Open reading frames (ORFs) within the plasmid sequence were identified using GeneQuest from DNASTAR (Madison, WI), followed by manual inspection. The initiation codon giving the longest coding region was used, including GUG and UUG. ORFs corresponding to proteins larger than 66 amino acids in size were identified. Translated ORFs were then compared to known protein sequences using by BLAST (NCBI, September 2006). Those with greater than 90% amino acid sequence identity over greater than 90% of the sequence were considered matches. The DNA sequence was also analyzed to identify noncoding features. The G+C content of individual ORFs was analyzed using EMBOSS (58). Insertion sequences and repetitive elements were identified using IS FINDER (http://www-is.biotoul.fr/). tRNAs were identified using tRNA-Scan (39).

(ii) Comparative genomics. Each ORF of APEC O1 was compared to the predicted proteins of *E. coli* strains K-12 MG1655, CFT073, 536, and UTI89 by using the BLAST tool (1). The APEC O1 chromosome was aligned to the CFT073, 536, and UTI89 chromosomes by using MAVID (7), and these alignments were visualized by using GenomeViz (22). The GenomeViz output was incorporated into a circular genome map created by using GenVision from DNASTAR.

(iii) MLST. Multilocus sequence typing (MLST) was used to determine the genetic relatedness of all the fully sequenced *E. coli* genomes that are available to the public (http://www.mlst.net). Additionally, Fred Blattner and Guy Plunkett III of the University of Wisconsin, Madison, kindly provided access to the pertinent sequences of neonatal meningitis-associated *E. coli* strain RS218 for use in this study (http://www.genome.wisc.edu). Seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were extracted from the sequence database for all these genomic sequences, concatenated to produce a contiguous sequence of 9,015 bp, and aligned by the neighbor-joining method with 1,000 bootstrap iterations using ClustalW. A dendrogram of the groupings based on this alignment was created using MegAlign from DNASTAR. Sequences were also assigned sequence types based upon the scheme described at the *E. coli* MLST database (http://web.mpiib-berlin.mpg.de/mlst/dbs/Ecoli), which assigns allele designations to unique variants based on ~500-bp internal regions of each gene.

Virulence assays. (i) Embryo lethality assay. APEC O1 and several other well-characterized APEC strains were assessed for lethality in chicken embryos by inoculation of 500 CFU of overnight washed bacterial cultures into the allantoic cavities of 12-day-old, embryonated, specific-pathogen-free eggs (42). Phosphate-buffered saline-inoculated and uninoculated embryos were used as controls. Embryo deaths were recorded for 4 days.

(ii) Day-old chick lethality. The ability of APEC O1 to cause avian colibacillosis was confirmed in chicks, as described previously (35, 46, 53). Day-old chicks, vaccinated only against Marek's disease, were obtained from a commercial source and were divided into groups of 10 chickens each in Horsfall units at the Livestock and Infectious Disease Facility, Iowa State University. Chicks were provided food and water ad libitum. Chicks were inoculated intratracheally with 0.1 ml of a bacterial suspension containing ~10^7 CFU/ml of APEC O1 or with 0.5 ml of phosphate-buffered saline subcutaneously in the back of the neck. Chicks were monitored for 7 days. Deaths were recorded, and the survivors were euthanatized and examined for macroscopic lesions. Organisms were classified as being of low, intermediate, or high virulence based upon mortalities and gross lesions related to colibacillosis. Three APEC strains representing each of the pathogenicity groups, as kindly supplied by Sandra Cloud (University of Delaware), were used as controls (53).

Biostatistics. A cluster analysis of the genotyping results for the isolates tested was performed using the average linkage method based upon Jaccard's dissimilarity coefficient calculated from the presence of virulence genes (SAS Institute, Inc., 2004). In order to better discern patterns among the isolates, results of the cluster analysis along with the isolates' virulence genotypes were used to construct a single figure as described previously (50). Differences in embryo lethality between the strains were evaluated for statistical significance using a z-test for the equality of two binomial proportions (63). Differences in day-old-chick lethality were examined using Fisher's exact test (63).

Nucleotide sequence accession number. The complete sequence and annotation of APEC O1 has been deposited in GenBank under accession number NC_008563.

RESULTS AND DISCUSSION

Choice of strain. Extensive virulence genotyping was performed on large populations of APEC and UPEC strains in order to identify an APEC strain for sequencing. This was done in an effort to identify an APEC strain that was classified in a serogroup, phylogenetic group, and virulence genotype characteristic of both APEC and UPEC. Serogrouping, phylogenetic typing, and virulence genotyping for nearly 40 APEC- and ExPEC-associated genes were performed on 500 APEC and 500 UPEC isolates (Fig. 1). The genes examined included those encoding plasmid-associated virulence factors, such as the increased serum survival gene (*iss*) (19, 25, 43, 48), the aerobactin and salmochelin siderophore system genes (16, 31, 32), the autotransporter gene *tsh* (17, 31, 32), and certain colicin-associated genes (31, 32). Chromosomal virulence-associated genes were also sought, such as the vacuolating autotransporter gene (*vat*) (45), the *pap* (pyelonephritis-associated pilus) operon (36), the yersiniabactin siderophore system genes (55), and the *ireA* (iron-responsive element) gene (26, 36, 54). A cluster analysis of these genotyping results separated the APEC and UPEC isolates into two primary clusters and several subclusters (Fig. 1). One of the two major clusters contained primarily APEC strains, characterized by their possession of plasmid-associated virulence genes. The second major cluster contained primarily UPEC strains, characterized by virulence genes which have been localized to chromosome-encoded pathogenicity islands (PAIs). The remaining isolates fell into mixed multiple clusters, containing both APEC and

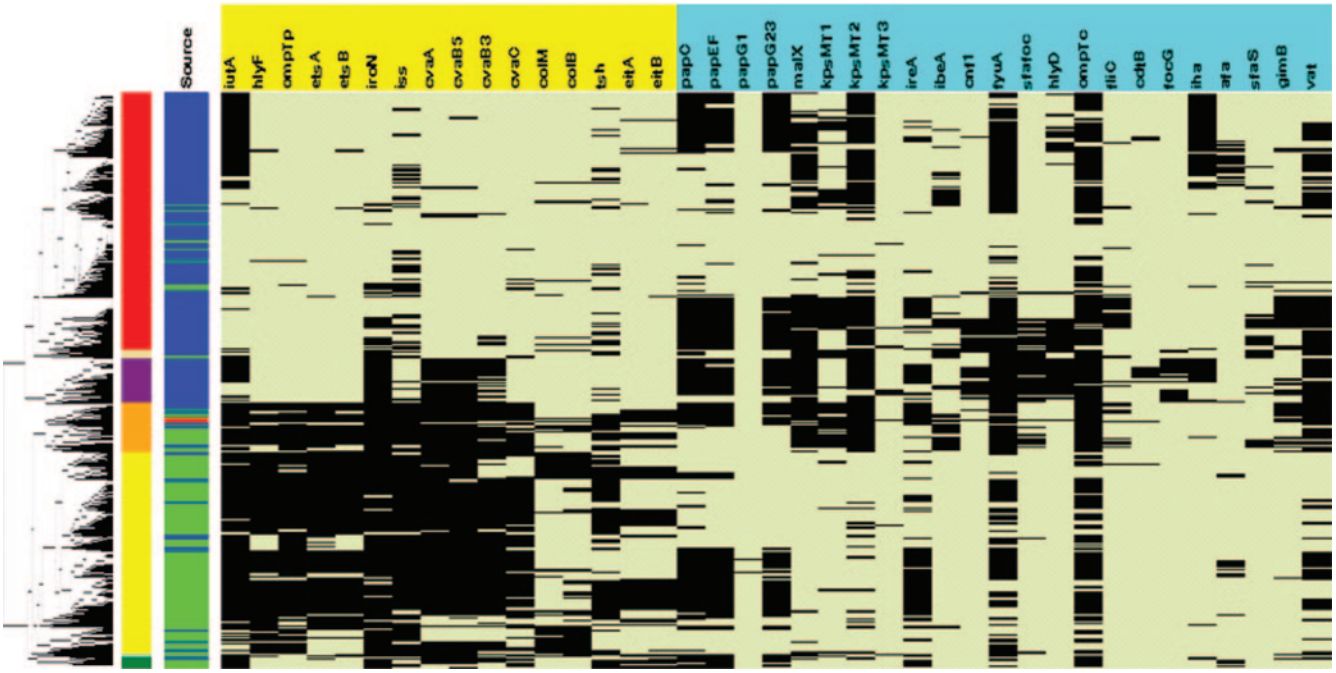

FIG. 1. Cluster analysis of 500 APEC and 500 UPEC strains for 39 ExPEC-associated traits. The leftmost portion of the figure is the dendrogram created from the average linkage cluster analysis on the presence of virulence factors. Just to the right of the dendrogram is column 1, which shows cluster membership based upon the dendrogram: red, cluster 1; cream, cluster 2; purple, cluster 3; orange, cluster 4; yellow, cluster 5; light blue, cluster 6; and green, cluster 7. Column 2 identifies an isolate as a UPEC strain (blue) or an APEC strain (green). Also, APEC O1 is identified in this column with a red bar. Columns 3 to 41 show the virulence genotype of each isolate tested. Each column in this group shows the results for a single gene. Black, gene is present; light green, gene is absent. Labels across the top of columns 3 to 41 show the APEC plasmid-linked genes (yellow) and the ExPEC chromosome-associated virulence genes (sky blue). ompTp, plasmid-encoded *ompT*; cvaB5, 5′ end of the *cvaB* gene; cvaB3, 3′ end of the *cvaB* gene; colM, *cma*; colB, *cbi*; papG1, *papG* allele 1; papG23, *papG* alleles 2 and 3. This method of analysis was first described by Rodriguez-Siek et al. (50) and has since been used in similar form elsewhere (8).

UPEC strains and possessing both plasmid- and chromosome-encoded virulence traits. APEC O1 was selected from one of these mixed clusters (Fig. 1). Within this mixed cluster were 85 isolates, of which 67% were found to be of the B2 phylotype. The most commonly occurring serogroups within this cluster were O1, O2, and O18.

APEC O1 was selected because it contained traits typical of both APEC and UPEC (50). APEC O1 is an O1:K1:H7 strain that was originally isolated from the lung of a chicken clinically diagnosed with colisepticemia. The O1 serogroup is one of the more commonly occurring serogroups among both APEC and UPEC strains (2, 29, 50, 51). O1 strains are also known to cause other important conditions in animals (5, 18, 21) and humans (4, 60), and a recent report of interhousehold spread of a UPEC O1:K1:H7 strain involving family members and their dog suggests that such strains may have zoonotic potential (30). Despite the widespread contributions of O1 strains to human and animal disease, no genomic sequence of an *E. coli* O1 strain had been described prior to this report.

APEC O1 is also classified in the B2 phylogenetic group, which is closely associated with extraintestinal virulence in *E. coli* (12, 50). Prior to sequencing, the ability of APEC O1 to cause disease in birds was confirmed by intratracheal inoculation of 1-day-old broiler chicks, according to the scheme used by Rosenberger et al. (53). Using this model, APEC O1 was categorized as being highly virulent for chickens (Table 1).

Overview of the APEC O1 genome. The genome of APEC O1 was assembled and finished at approximately eightfold coverage. It contained a 5,082,025-bp chromosome (Fig. 2) and, unlike other sequenced ExPEC strains, contained four plasmids totaling 565,600 bp in size (Table 2), giving it a total genome size of 5,647,625 bp. The presence of these plasmids was not surprising, considering that plasmids are a defining trait of the APEC pathotype (32, 33, 51). Such plasmids have also been identified among UPEC strains, although to a lesser

TABLE 1. Lethality for 1-day-old chicks

Organism[a]	Mean lesion score[b]	*P* value[c]
APEC O1	2.69	
Controls		
High	2.75	0.54
Medium	0.89	<0.0001
Low	0.17	<0.0001

[a] APEC O1 and the control organisms (one each for high, intermediate, and low pathogenicity) were classified by their virulence towards chicks (53).

[b] Average of lesion scores (ranked from 0 to 3 based upon occurrence of airsacculitis, pericarditis, and perihepatitis) for 12 birds for each organism tested.

[c] *P* value generated using Fisher's exact test (63). There was no statistically significant difference between the lesion scores following inoculation with APEC O1 and the high-pathogenicity group control. However, the lesion scores obtained with APEC O1 did differ significantly from those in birds inoculated with the medium- and low-pathogenicity control organisms. Collectively, these results demonstrate that APEC O1 is classified as a "high-pathogenicity" organism.

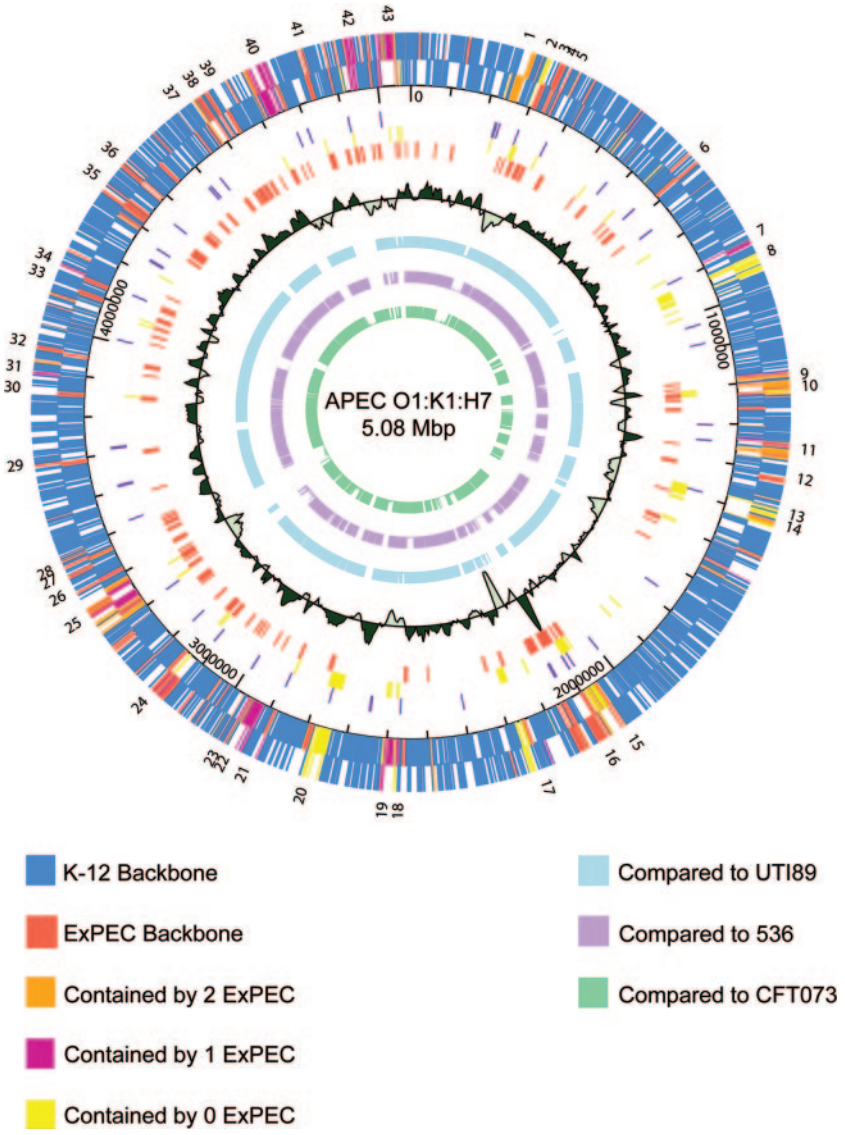

FIG. 2. Map of the APEC O1 chromosome and comparison of APEC O1's genome to those of other ExPEC strains. The outer ring shows genomic islands, as numbers (1 to 43) corresponding to those in Table 5; the 2nd and 3rd rings are coding regions in forward and reverse orientation (blue, backbone ORFs also present in K-12; dark orange, ORFs absent from K-12 but present in all other sequenced ExPEC strains; light orange, ORFs absent from K-12 but present in APEC O1 and two other ExPEC strains; lavender, ORFs absent from K-12 but present in APEC O1 and one other ExPEC strain; yellow, ORFs present only in APEC O1); the 4th ring depicts the scale in base pairs; the 5th ring depicts tRNA genes in dark purple; the 6th ring depicts ORFs unique to APEC O1 in yellow; the 7th ring depicts ORFs common to all sequenced ExPEC strains in orange; the 8th ring shows sliding G+C content compared to the overall average of 50.6%; and the 9th, 10th, and 11th rings show genome alignments of APEC O1 with UTI89 (light blue), 536 (light purple), and CFT073 (light green), respectively.

extent (64). Two of APEC O1's plasmids have been previously described: a 174,231-bp IncFIB, colicin-encoding virulence plasmid (32) and a 241,348-bp IncHI2 resistance plasmid encoding resistance to eight antimicrobial agents (33). The remaining two plasmids, pAPEC-O1-Cryp1 and pAPEC-O1-Cryp2, are 101 and 49 kb in size, respectively. These plasmids share limited identity with *Yersinia*-type plasmids, encode mostly hypothetical proteins, and do not confer any apparent phenotype.

The G+C content of the APEC O1 chromosome was found to be 50.5%, which is comparable to that of other *E. coli* strains (3). However, the plasmids of APEC O1 had a variable G+C content, ranging from 44.6% to 49.6% (Table 2). Within the chromosome, regional differences in G+C content could be discerned, with the differences occurring primarily within the genomic islands, which are defined here as regions of APEC O1 greater than 4 kb that are not present in *E. coli* K-12 MG1655. Codon adaptation indices reflected such differences in G+C content, with differential codon usage also occurring within the genomic islands.

APEC O1 is highly similar to human ExPEC. At the time of this publication, the genomic sequences of three human ExPEC strains were available for comparative genomics: UPEC strain CFT073 (O6:K2:H1), isolated from a patient with py-

TABLE 2. Overview of APEC O1 genome

Structure	Size (bp)	G+C content (%)	No. (%) of ORFs[a]			
			Total	Backbone	Common ExPEC	APEC O1 specific
Chromosome	5,082,025	50.55	4,467	3,497 (78.3)	3,890 (87.1)	202 (4.5)
Plasmids						
pAPEC-O1-ColBM	174,241	49.65				
pAPEC-O1-R	241,387	46.43				
pAPEC-O1-Cryp1	105,834	46.55				
pAPEC-O1-Cryp2	46,870	44.62				
Total genome[b]	5,650,357		5,042	3,497 (69.4)	3,901 (77.4)	721 (14.3)

[a] ORFs are defined as backbone, common ExPEC, or APEC O1 specific based on their comparison with *E. coli* strains K-12 MG1655, CFT073, UTI89, and 536.
[b] The total genome includes 93 tRNA genes and 22 rRNA genes.

elonephritis (68); UPEC strain 536 (O6:K15:H31), isolated from a patient with a complicated UTI (8); and UPEC UTI89 (O18:K1:H7), isolated from a patient with uncomplicated cystitis (10). Comparison of APEC O1 to these *E. coli* sequences was quite revealing (Table 3). Overall, APEC O1 shared the greatest nucleotide and protein similarities with UTI89, followed by 536 and CFT073. APEC O1 has a chromosome which is 149 kb smaller than that of CFT073, 143 kb larger than that of 536, and 16 kb larger than that of UTI89 (Table 3). Within the APEC O1 chromosome, 4,467 ORFs were identified, 78.3% of which were a part of a backbone common among all sequenced *E. coli* strains. This comparison also revealed the presence of a common ExPEC backbone (K-12-like sequences plus common ExPEC sequences), accounting for 87.1% of the ORFs of APEC O1. Of these ORFs, 9% were common to all sequenced ExPEC strains but absent from *E. coli* K-12 MG1655. Such a backbone of genes might be essential in the ability of ExPEC to cause extraintestinal disease or to survive in the extraintestinal environment, as genes within this ExPEC backbone include loci thought to play a role in ExPEC virulence and/or fitness. Some of the regions identified within this ExPEC backbone include the yersiniabactin and *pap* operons, *ireA*, *vat*, the lipopolysaccharide core synthesis region, the *sit* iron/manganese transport system, and the *auf* fimbrial operon (9, 36, 54, 56, 59) (Table 4). In addition to the characterized islands within the common ExPEC backbone, 16 other islands were identified and were simply termed "ExPEC islands," encoding mostly hypothetical proteins (Table 5). Future studies will be needed to ascertain their functions and roles in virulence and persistence, if any.

TABLE 3. Comparison of the fully sequenced ExPEC strains

Strain	Chromosome size (bp)	Plasmid size(s) (kb)	Content of APEC O1 ORFs (%)
APEC O1	5,082,025	240, 174,106, 47	100
UPEC UTI89	5,065,741	114	93
UPEC CFT073	5,231,428		90
UPEC 536	4,938,875		87
ExPEC[a]	3,942,842	NA[b]	86
E. coli K-12 MG1655	4,639,221		78

[a] All completely sequenced ExPEC strains.
[b] NA, not applicable.

The APEC O1 chromosome contained a total of 43 genomic islands greater than 4 kb in size that were not present in the K-12 genome. Of these, a total of 21 islands were greater than 10 kb in size and 17 were greater than 20 kb (Table 5). Of the 43 total islands, only 4 (9%) were APEC specific compared to other sequenced ExPEC strains, while 25 islands (58%) were possessed by all ExPEC strains. All of the APEC-specific islands either were composed of prophage-like elements or were found adjacent to prophage-like elements. These phage-related, APEC O1-specific regions will require further study in order to determine if they might be used as markers to distinguish APEC from human ExPEC. Failure to identify a marker specific to avian ExPEC which is not present in human strains would lend credence to the hypothesis that some APEC strains possess zoonotic potential. Additionally, these APEC O1-specific regions might harbor unknown virulence factors that contribute to this strain's ability to cause extraintestinal disease. Consequently, future studies to ascertain the prevalence and distribution of these APEC O1-specific regions among ExPEC strains and determine their functions may prove helpful in testing the hypothesis that APEC strains are linked to human disease.

Among the 43 chromosomal islands and four plasmids of APEC O1, five putative PAIs were identified by their possession of multiple virulence genes, differential G+C content, and

TABLE 4. Virulence-associated genes of APEC O1

Gene(s)	Description	Presence in:			
		APEC O1	CFT073	UTI89	536
papI to *-G*	*pap* fimbrial operon	+	+	+	+
ireA	Iron-regulated element	+	+	−	−
fyuA	Yersiniabactin siderophore gene	+	+	+	+
iutA	Aerobactin siderophore gene	+	+	−	−
iroN	Salmochelin siderophore gene	+	+	+	+
sitA to *-D*	Iron/manganese transport genes	+	+	+	+
iss	Serum survival gene	+	−	−	−
vat	Vacuolating autotransporter gene	+	+	+	+
tsh	Autotransporter/adhesin gene	+	−	−	−
cdtB	Cytolethal distending toxin gene	+	−	−	−
ibeA	Invasion gene	+	−	+	−
tia	Invasion gene	+	−	−	−

TABLE 5. Genomic islands of APEC O1

Island no.	Start (bp)	Stop (bp)	Description	Size (bp)	Presence in strain:			
					K-12 MG1655	CFT073	UTI89	536
1	271626	243408	PAI $II_{APEC\ O1}$ near tRNA-Asp	28,218	−	−	+	+
2	314347	295179	PAI $III_{APEC\ O1}$ near tRNA-Thr; contains *vat*	19,168	−	+	+	+
3	328228	339123	ExPEC island	10,895	−	+	+	+
4	347107	353093	ExPEC island	5,986	−	+	+	+
5	370099	375196	ExPEC island	5,097	−	+	+	−
6	682227	691198	ExPEC island	8,971	−	+	+	+
7	888045	897875	ExPEC island	9,830	−	−	+	−
8	919803	958265	Prophage	38,462	−	−	−	−
9	1182688	1221584	Prophage	38,896	−	+	+	−
10	1224376	1229282	ExPEC island containing *sitABCD* operon	4,906	−	+	+	+
11	1336568	1377916	Prophage	41,348	Partial	Partial	+	−
12	1414877	1421687	ExPEC island	6,810	−	+	+	+
13	1470904	1515743	Prophage	44,839	Partial	−	−	−
14	1515743	1522998	ExPEC island containing *cdt* locus	7,255	−	−	−	−
15	2054786	2080866	Prophage	26,080	−	Partial	Partial	Partial
16	2080867	2168373	PAI $IV_{APEC\ O1}$ near tRNA-Asn; contains yersiniabactin operon	87,506	−	+	+	+
17	2281456	2255598	Prophage	25,858	−	−	−	−
18	2554458	2560406	ExPEC island	5,948	−	+	+	+
19	2608989	2570656	Prophage	38,333	−	−	Partial	−
20	2777929	2738458	Prophage	39,471	−	−	−	−
21	2879008	2888332	ExPEC island near tRNA-Arg	9,324	−	−	+	−
22	2947835	2914107	Prophage	33,728	−	−	+	−
23	2950455	2957275	ExPEC island	6,820	−	−	+	−
24	3154123	3116205	ExPEC island near tRNA-Met	37,918	−	Partial	+	Partial
25	3387657	3304326	PAI $I_{APEC\ O1}$ near tRNA-Phe; contains *ireA*, *tia*, *pap* operon	83,331	−	Partial	Partial	Partial
26	3404606	3413814	ExPEC island	9,208	−	+	+	+
27	3443933	3450091	ExPEC island	6,158	−	+	+	+
28	3461178	3471122	ExPEC island	9,944	−	+	+	+
29	3688691	3696219	ExPEC island	7,528	−	+	+	+
30	3857622	3848199	ExPEC island containing *auf* fimbrial operon	9,423	−	+	+	+
31	3893727	3897975	ExPEC GimB island	4,248	−	−	−	+
32	3944861	3953813	ExPEC island containing *chu* locus	8,952	−	+	+	+
33	4089354	4098487	Lipopolysaccharide core synthesis region	9,133	−	+	+	+
34	4122439	4135802	ExPEC island	13,363	−	+	+	+
35	4311724	4334676	ExPEC island	22,952	−	+	+	+
36	4377338	4385895	ExPEC island	8,557	−	+	+	+
37	4548317	4553013	ExPEC island	4,696	−	+	+	+
38	4591752	4607443	ExPEC island	15,691	−	+	+	+
39	4636683	4642553	ExPEC island	5,870	−	+	+	+
40	4769233	4711722	Ethanolamine utilization island near tRNA-Phe	57,511	−	+	−	−
41	4833450	4842949	ExPEC island	9,499	−	+	+	+
42	4955192	4931724	ExPEC island containing *ibeA*	23,468	−	−	+	−
43	5047045	5002685	Prophage	44,360	−	−	+	−

size (Table 5) (23). Four of these were chromosomal and contained genes of the yersiniabactin operon, *vat*, *ireA*, the *pap* operon, and an invasion determinant, *tia*. A fifth PAI occurred within the virulence plasmid of APEC O1, pAPEC-O1-ColBM, and contained *iss*, the *sitABCD* operon, the aerobactin operon, the salmochelin operon, and *hlyF* (32). The occurrence of these traits on a plasmid-encoded PAI is one of few differences between APEC O1 and the human ExPEC examined, although many of the traits of this plasmid-located APEC PAI, such as the aerobactin, *sit*, and salmochelin operons, have been found in chromosomal PAIs of human ExPEC (8, 10, 14, 15, 68).

There have been previous reports of virulence-associated genes and/or operons occurring at specific locations in the APEC genome. Comparison of these regions with the genomic islands of APEC O1 and other sequenced ExPEC strains helps to further define these groups. For instance, Lymberopoulos et al. (40) recently described the Stg fimbrial operon occurring in an APEC O78 strain. This operon contributed to the ability of that strain to colonize the respiratory tract and was present in 45% of the APEC strains examined. Also, this operon was inserted between the *glmS* and *pstS* genes in APEC χ7122. However, this operon was absent in APEC O1 and the other sequenced ExPEC strains, and no other insertions were found within this region. Similarly, Chouikha et al. (11) described a virulence-associated genomic island at the *selC* locus in an APEC O2 strain. In APEC O1 and the three other sequenced ExPEC strains, though, a different island is inserted at this location, whereas the island described by Chouikha et al. is absent (genomic island 34 in Table 5).

TABLE 6. Embryo lethality at 4 days post infection

Strain	VF count[b]	No. of:		*P* value[a] vs DH5α	Kill class[c]
		Embryos	Deaths		
DH5α	0	20	1		A
APEC O1	28	20	11	0.001	B
APEC V1	14	20	12	0.0004	B
APEC V7	19	20	12	0.0004	B
APEC V2	20	20	16	<0.0001	C

[a] As calculated using Fisher's exact test (63).
[b] Total number of ExPEC virulence-associated traits detected, based on Fig. 1.
[c] As determined using a one-way analysis of variance (63).

Parreira and Gyles described a novel PAI near the *thrW* locus of an APEC O2 strain, which contained the *vat* gene (45). Interestingly, *vat* is present in APEC O1 and all of the other sequenced ExPEC strains at the same locus. However, in all of these sequenced strains, *vat* occurs within different genomic islands (genomic island 2 in Table 5). Further investigation of this and the other genomic islands will likely reveal much about their roles in the evolution of ExPEC virulence.

Based upon virulence genotyping, APEC O1 had one of the highest counts of virulence genes of all the ExPEC strains we examined (Fig. 1; Table 6). Despite its complement of virulence genes, it was significantly less virulent in an embryo assay of virulence than other APEC isolates (61), which contained fewer ExPEC-associated virulence genes (Table 6). Therefore, it would seem that the number of PAIs and/or virulence genes in an ExPEC strain is, in itself, insufficient to predict virulence, at least in this model. Instead, these results may be related to some minimal ExPEC backbone that is required for virulence. Additionally, virulence gene regulation is likely to play an important role in ExPEC's ability to cause disease, and studies to determine if correlations between gene expression and virulence can be discerned are under way. Certainly, such results make it clear that the genomic basis of ExPEC virulence remains incompletely understood.

As knowledge of the *E. coli* genome has expanded, the *E. coli* pan-genome (total nonredundant proteins occurring among all *E. coli* genomes) has increased, although the number of new ORFs contributed by each newly sequenced strain is rapidly declining. Also, the number of ORFs thought to be strain specific has decreased. For instance, CFT073 was estimated to have nearly 20% strain-specific ORFs in its chromosome at the time of publication of its sequence (68), but with the completion of subsequent ExPEC genomes, it has become apparent that many of these ORFs are shared (8, 10). Only 4.5% of APEC O1's chromosome is absent from other sequenced *E. coli* genomes. Thus, comparative analysis of the APEC O1 and other ExPEC genomes suggests that as more ExPEC sequences become available, fewer differences between individual ExPEC strains will be found. As a result, it seems likely that a common ExPEC backbone, containing sequences not found in other pathotypes, will become increasingly better defined.

APEC O1 and other sequenced ExPEC are phylogenetically related. Protein BLAST searches of the ORFs of APEC O1 indicated that it is very closely related to other ExPEC strains. MLST was used to determine the potential phylogenetic relationships among all sequenced *E. coli* strains (Fig. 3). APEC O1 was found to be very closely related to *E. coli* UTI89, a strain isolated from a case of human cystitis (10); 536, a strain isolated from a case of human UTI (8); and F11, also originating from a human cystitis case (GenBank draft sequence; accession number AAJU00000000). *E. coli* RS218, an O18:K1:H7 strain implicated in human neonatal meningitis, was also very closely related to APEC O1 (69). Not surprisingly, *E. coli* strains K-12 MG1655, K-12 W3110 (a second K-12 strain recently sequenced), and O157 EDL933 were more distantly related to APEC O1 than the aforementioned genomes (6, 47). CFT073, a human pyelonephritis isolate (68), appeared to be divergent from the other sequenced ExPEC strains based on the MLST results. This divergence is also illustrated by genome alignment of APEC O1 with other sequenced ExPEC. That is, of the sequenced ExPEC strains, CFT073 shared the least homology with APEC O1 (Fig. 2). Overall, these results demonstrate that the sequenced APEC and UPEC genomes are more closely related to each other than to other *E. coli* subtypes in terms of sequence identity and phylogeny. Such results suggest that there is no convincing genetic support for host- or syndrome-specific pathotypes (e.g., APEC and UPEC) within the broader ExPEC group. These results also further emphasize the importance of pathogenomics in better understanding highly heterogeneous groups such as ExPEC.

Conclusions. Overall, this study provides compelling evidence that the genomes of APEC O1 and several sequenced human ExPEC strains are very similar, thereby providing suggestive support for the hypothesis that at least some APEC strains are a source of UPEC causing human UTI. However, these findings must be interpreted within their context. That is, APEC O1 was selected because it belonged to a serogroup and phylogenetic group implicated in both human and avian disease, and it belonged to a virulence genotyping cluster (Fig. 1) containing a mixture of APEC and UPEC strains that were typified by possession of several common ExPEC virulence factors. This work makes no claim that all, or even most, human extraintestinal infections are derived from poultry-source *E. coli*. Rather, this work provides evidence that at least some human and avian ExPEC strains are highly similar to one another, and it supports the possibility that a food-borne link exists between some APEC and UPEC strains. However, validating this hypothesis requires an assessment of the ability of APEC to survive and persist on retail poultry, to traverse the human intestinal tract, and to ascend and colonize the urinary

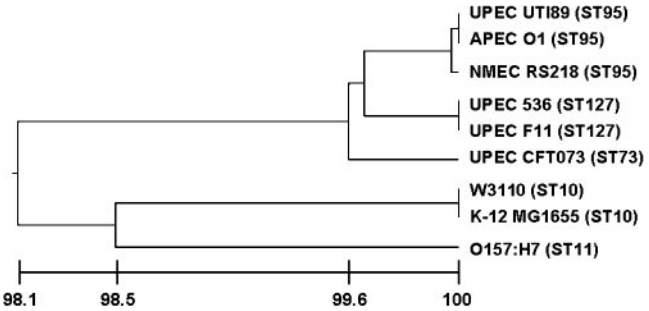

FIG. 3. Results of MLST of fully sequenced *E. coli* genomes. The bottom line indicates percent similarity between strains. Sequence types (STs) are indicated to the right of the strain's name.

tract. Furthermore, epidemiological studies will be required to confirm that such a phenomenon actually occurs.

While APEC O1 was highly similar to other sequenced human ExPEC strains, it was not identical to them. As observed with all *E. coli* strains sequenced thus far, APEC O1 contained a percentage of "unique" genomic regions not occurring in the other sequenced *E. coli* genomes. Before definitive conclusions can be drawn about the validity of the hypothesis that some APEC strains are involved in zoonotic disease, the distribution and function of these APEC O1-specific regions should be addressed. It is possible that these regions limit the disease-causing ability of APEC O1, and perhaps other APEC strains, to avian hosts. However, the sequencing data available at this point imply that all *E. coli* strains possess at least some unique regions, regardless of their source. A case in point is the four UPEC genomes which have been sequenced. While the chromosomes of these strains were quite heterogeneous, they were isolated from patients with similar types of disease. Thus, a better understanding of ExPEC genomics and a more lucid definition of the ExPEC "subpathotypes" are necessary in the future.

In conclusion, this study provides the first genomic sequence of an APEC isolate and of any animal source *E. coli* isolate. Based on comparative genomic analyses, it appears that APEC O1 shares extensive similarities with human ExPEC, and study of its sequence has resulted in a refined understanding of the *E. coli* pan-genome and the ExPEC backbone. These extensive comparisons of APEC and UPEC leave open the possibility that some APEC strains represent a potential food-borne source of human UPEC. Regardless, this study provides an APEC genomic sequence on which further testing of this hypothesis can be effectively based. Thus, the sequence of APEC O1 is another stepping stone towards controlling ExPEC-caused diseases in avian and human hosts.

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