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Complete DNA Sequence, Comparative Genomics, and Prevalence of an IncHI2 Plasmid Occurring among Extraintestinal Pathogenic *Escherichia coli* Isolates▽†

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We have sequenced a large plasmid that occurs among avian pathogenic *Escherichia coli* isolates. This plasmid, pAPEC-O1-R, is a 241,387-bp IncHI2 plasmid which is cotransmissible via bacterial conjugation with a ColBM virulence plasmid, encodes resistance to eight antimicrobial agents, and appears to occur at low rates among extraintestinal *E. coli* isolates.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) organisms cause significant disease in both humans and animals. Large plasmids are often a part of the ExPEC genome (8, 9, 10, 14). However, these plasmids are underrepresented in the sequence database; and such sequences are necessary to better understand their roles in virulence, resistance, and horizontal gene transfer (5). No complete sequences of IncH plasmids from *E. coli* are available, although such plasmids are well described in other organisms (6, 16). The only fully sequenced IncHI2 plasmid, R478, was isolated from *Serratia marcescens* in the United States in 1969 (11). Here, we present the first complete sequence of an IncHI2 plasmid from an *E. coli* isolate, and we describe the use of comparative genomics to further define the backbone components of IncHI2 plasmids and to explore their evolution. Additionally, the prevalence of the genes of IncHI2 plasmids and the resistance that they encode are determined for a broad collection of ExPEC and commensal *E. coli* isolates in order to evaluate their host range and impact.

Overview of pAPEC-O1-R. Approximately 3,000 sequencing reads from the genomic library of avian pathogenic *E. coli* (APEC) O1 were used to assemble the complete sequence of pAPEC-O1-R at approximately ninefold coverage. The closed, circular plasmid had a size of 241,387 bp (Fig. 1). The overall G+C content of pAPEC-O1-R was 46.3%. Analysis of the sequence revealed 225 open reading frames (ORFs) (see the supplemental material). Of the ORFs identified, 15 (6.7%) were classified as hypothetical proteins with no matches in the nonredundant database, 94 (41.8%) were classified as conserved hypothetical proteins with matches to proteins of unknown function, 34 (15.1%) were identified as plasmid transfer proteins, 33 (14.7%) were matched with antimicrobial resistance proteins, and 17 (7.6%) were matched with mobile genetic elements.

The transmissibility of pAPEC-O1-R was confirmed by mating APEC O1 with *E. coli* DH5α, a plasmidless recipient strain (15), and transconjugants were produced at a rate of approximately 5×10^{-5} transconjugants/donor at 25°C.

Comparative genomics. In an effort to better define the IncH plasmid backbone, pAPEC-O1-R was compared to the only other fully sequenced IncHI2 plasmid, pR478, and its close relatives, *Salmonella enterica* subsp. *enterica* serovar Typhi strain CT18 plasmid pHCM1 and *Salmonella* serovar Typhi plasmid R27 (13, 16). Comparison of the nucleotide sequences of pR478 and pAPEC-O1-R revealed that they were extremely similar to one another (Fig. 2). Regions common to pR478 and pAPEC-O1-R, which might define the IncHI2 backbone, included the Tra1 and Tra2 transfer regions; the copper, silver, and tellurite resistance regions; and the IncHI2 replicon (7). The DNA not common to both plasmids included insertion sequence (IS) elements, different class 1 integrons in both plasmids, and the arsenic and mercury resistance regions in pR478. If, as suggested previously (6), IncH plasmid evolution is mediated by a series of acquisition events, these two plasmids are likely derived from a common ancestor that had the core "backbone" components described above.

A four-way comparison of pAPEC-O1-R, pR478, and IncHI1 plasmids pHCM1 and pR27 revealed that pR27 and pHCM1 shared much less nucleotide sequence homology to pAPEC-O1-R than they did to pR478 (Fig. 2). At a lower level of identity, the regions common to all four plasmids included the Tra1, Tra2, and replication regions (Fig. 2), which might define the general backbone for IncH plasmids. Future sequencing studies involving other IncH plasmids will surely increase our understanding of their evolution and host range.

Antimicrobial resistance encoded by pAPEC-O1-R. pAPEC-O1-R was found to contain genes encoding resistance to eight different antimicrobial agents (Table 1); some of these genes were located within a class 1 integron (1). When pAPEC-O1-R was transferred into *E. coli* DH5α, the transconjugant acquired the ability to resist streptomycin, tetracycline, gentamicin, sulfisoxazole, potassium tellurite, silver nitrate, copper sulfate,

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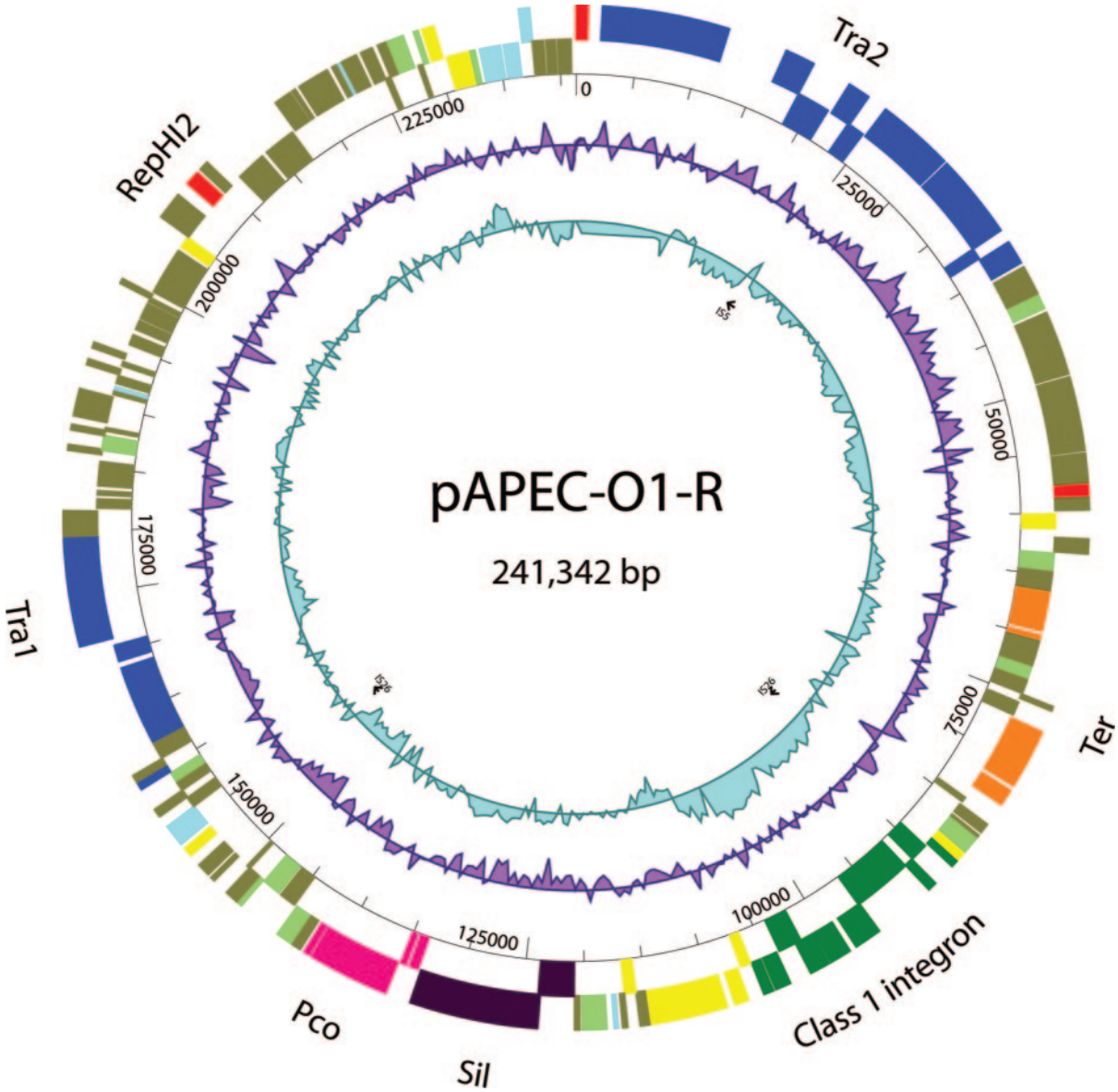

FIG. 1. Map of pAPEC-O1-R. The outer two circles show ORFs in the forward and the reverse orientations, respectively. Colors correspond to the following: blue, plasmid transfer; red, replication, olive, unknown function; yellow, mobile genetic elements; orange, tellurite resistance; pink, copper resistance; black, silver resistance; green, class 1 integron; light green, plasmid maintenance; light blue, cell metabolism. The third circle shows a scale (in base pairs). The fourth circle shows the G+C content plotted against the average G+C content for the plasmid of 49.6% (purple). The fifth circle plots the G+C skew in a 500-bp sliding window (blue). IS elements are depicted inside the fifth circle. The map was created by using GenVision from DNASTAR.

and benzylkonium chloride. Recent attention has been given to the possibility that the *E. coli* isolates of production animals serve as reservoirs of drug resistance for humans (17). Several derivatives of the antimicrobial compounds described above are currently approved for use as growth promoters and/or for the prevention of disease in the U.S. layer and broiler industries (12). Thus, opportunities exist for the selection of multidrug-resistant APEC, such as APEC O1 and APEC O2, and producers should take caution before they use such drugs (9, 10, 11).

Prevalence of pAPEC-O1-R-related genes among ExPEC and commensal *E. coli*. Multiplex PCR was performed to determine the prevalence of pAPEC-O1-R-associated resistance genes and the RepHI2 replicon among APEC, human uropathogenic *E. coli* (UPEC), and human and avian commensal *E. coli* populations (Tables 1 and 2). The results are presented in Table 3. The genes of the pAPEC-O1-R resistance region and the IncHI2 replicon occurred infrequently or rarely among the extraintestinal and commensal *E. coli* isolates tested. These findings are supported by the findings in the literature. That is, while IncH plasmids appear to occur among *Salmonella*, *Klebsiella*, and *Serratia* species, they have been found infrequently among *E. coli* isolates (2, 3, 7). The low rate of occurrence of these plasmids among *E. coli* isolates, the differential codon usage from that of the host, and other genetic differences

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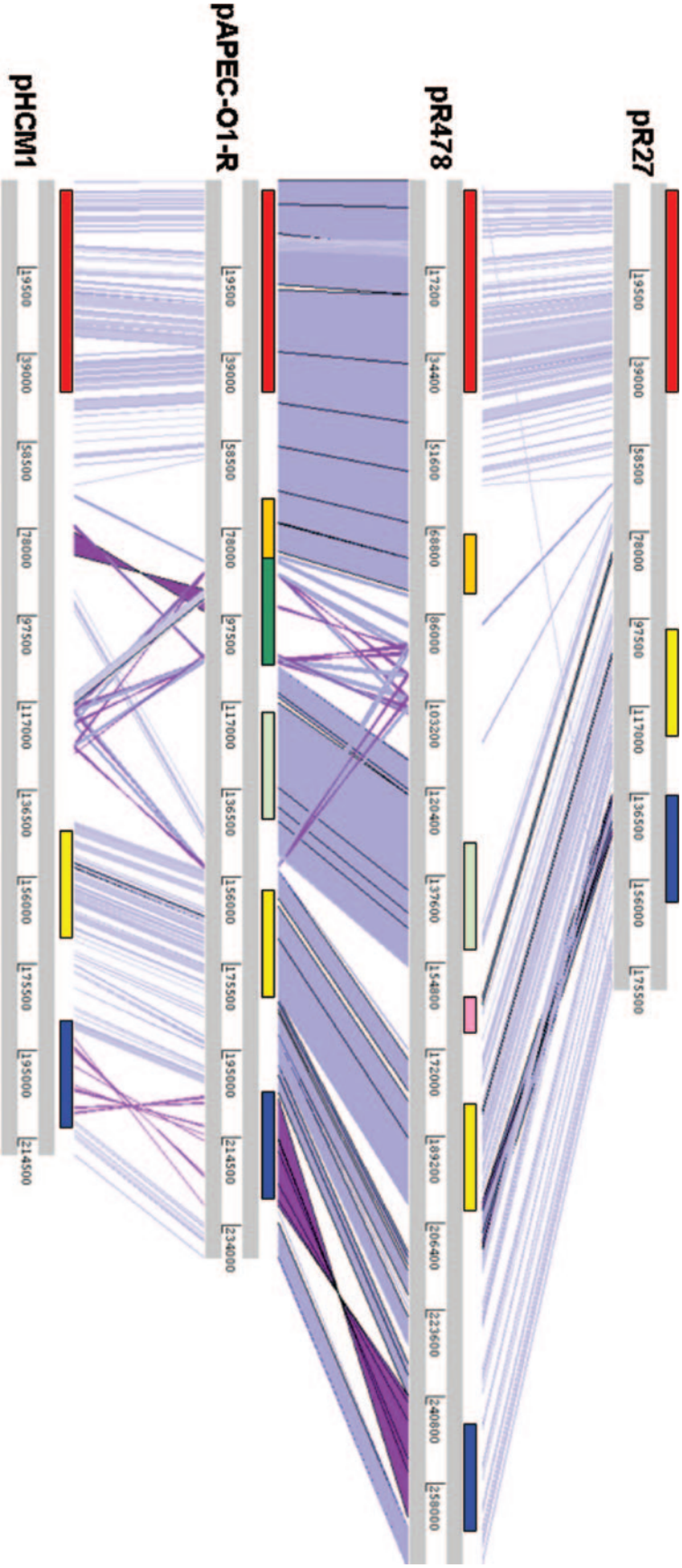

FIG. 2. Four-way nucleotide comparison of pAPEC-O1-R with pR27 ($n = 33$), pR478 ($n = 11$), and pHCM1 ($n = 26$). Light purple rays, direct nucleotide homology at a cutoff value of 50%; dark purple rays, inverted nucleotide homology. Colored blocks correspond to the following regions: red, Tra2 region; orange, tellurite resistance region; dark green, class 1 integron unique to pAPEC-O1-R; light green, copper and silver resistance regions; yellow, Tra1 region; pink; arsenic resistance region of pR478; and blue, IncH12 replicon and adjacent regions.

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TABLE 1. Summary of antimicrobial resistances encoded by pAPEC-O1-R

Gene(s)	Functional resistance
terY3Y2XY1W, *terZABCDEF*	Potassium tellurite
silESRCBAP	Silver nitrate
pcoEABCDRSE	Copper sulfate
aadA	Streptomycin
aac3-VI	Gentamicin
tetAR	Tetracycline
qacEΔ1	Benzylkonium chloride
SulI	Sulfisoxazole

between pAPEC-O1-R and its host *E. coli* chromosome suggest that this plasmid was likely acquired from another source. One potential source for such plasmids is the chicken gut, as it is thought that APEC strains that cause disease in poultry originate from the fecal flora (4). Evidence exists for the conjugal transfer of R plasmids in the chicken gut, and the implications of the gut as a "mixing vessel" which facilitates horizontal gene transfer merits further attention.

In sum, this study presents the complete sequence of an IncHI2 plasmid that occurs among APEC isolates. pAPEC-O1-R encodes resistance to antibiotics and heavy metals, possesses a class 1 integron, and is transferable to plasmidless strains of *E. coli*. This plasmid shares remarkable similarities with an IncHI2 plasmid from *S. marcescens*, and comparative

TABLE 3. Occurrence of pAPEC-O1-R-associated genes among APEC, UPEC, avian commensal *E. coli*, and human commensal *E. coli* isolates

Gene(s)	% Occurrence[a]			
	APEC (n = 451)	AFEC (n = 104)	UPEC (n = 200)	HFEC (n = 101)
pcoE	19.0	6.8	5.0	25.5
pcoA	19.2	6.8	18.0	10.8
pcoD	20.6	5.7	6.0	9.7
terX	4.4	4.9	0.5	0.0
terF	7.0	11.7	0.5	0.0
terY3	5.1	4.9	0.5	0.0
terD	8.2	11.7	0.5	0.0
silE	20.8	6.6	5.5	10.8
silP	22.4	5.7	5.5	11.0
repA (IncHI2)	3.3	3.9	0.0	0.0
groEL	23.7	20.8	1.0	0.0
aadA	29.4	12.3	1.5	1.0
aac3-VI	7.7	7.6	0.5	1.0

[a] AFEC, avian commensal *E. coli*; HFEC, human commensal *E. coli.*

TABLE 2. Primers used in this study[a]

Primer	Gene	Sequence (5′ to 3′)	$T_{annealing}$	Amplicon size (bp)
PCOE F	*pcoE*	GTGGGGCAGCTTTTGCTCAGTCCAGTGA	63	385
PCOE R		CGAAGCTTTCTTGCCTGCGTCTGATGTG		
PCOA F	*pcoA*	ATCCGGAAGGTCAGCACCGTCCATAGAC	63	507
PCOA R		GACCTCGCGGATGTCAGTGGCTACACCT		
PCOD F	*pcoD*	GGCGCCCAGAATGATAATCGCAACA	63	502
PCOD R		GGGCGTGGCGCTGGCTACACTT		
TERX F	*terX*	ATGCGCCGCCTGCCTGTTTACCTTGTTA	63	576
TERX R		CGCGCTTGTGCTGCCGGAAGACA		
TERF F	*terF*	CCGACAAACTTCCAGAAGATGGGGTAGT	63	428
TERF R		GAGGCAGCGGTTGCATTTGTACTTGACG		
TERY3 F	*terY3*	CCTGGGGCCGTCAGCGGACCTG	63	302
TERY3 R		TCCTTGCTGGTGGCCGTTCATACTTCAT		
TERD F	*terD*	CCACTGCGCGGAATTTCCACTCACCAT	63	231
TERD R		ACGCCGTCCCGTCTGATGTTGACAAG		
SILE F	*silE*	TCGGCCTGGGCCACTGAAACCGTGAATA	63	364
SILE R		GGCGGTGCGCTTCGGCCATAGCCTGATG		
SILP F		ACACCCCGGCCTGGGCTCCTT	63	603
SILP R	*silP*	TGCGGGCACGGGAACAAACCTC		
AAC3-VI F	*aac3-VI*	GGGCAAGCGCCGCGTCACTTATT	63	302
AAC3-VI R		CGCGGCGTTGTTTCGGCTTCA		
AADA F	*aadA*	TAACGGCGCAGTGGCGGTTTTCA	63	365
AADA R		AAGCTCGCCGCGTTGTTTCATCAAG		
GROEL F	*groEL*	CGCCGGCATGAACCCGATGGACCTCA	63	318
GROEL R		TCGGCCTGCATCGACTGCGGGTTGTTG		
HI2 F	*repA*	TTTCTCCTGAGTCACCTGTTAACAC	63	644
HI2 R		GGCTCACTACCGTTGTCATCCT		

[a] The source of all primers is this study.

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genomics between these and other IncH plasmids make it possible to define IncH plasmid backbone components and provide insight into the likely evolutionary development of these plasmids. The sequence of pAPEC-O1-R, along with that of another APEC R plasmid, pAPEC-O2-R, illustrates the potential impact that these transferable, multidrug resistance-encoding plasmids have within the poultry environment.

Nucleotide sequence accession number. The complete, annotated sequence of pAPEC-O1-R is deposited in GenBank under accession number DQ517526.

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