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Author(s): Subhashinie Kariyawasam, Timothy J. Johnson, Chitrita DebRoy, and Lisa K. Nolan
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Occurrence of Pathogenicity Island $I_{APEC-O1}$ Genes Among *Escherichia coli* Implicated in Avian Colibacillosis

Subhashinie Kariyawasam,[A] Timothy J. Johnson,[A] Chitrita DebRoy,[B] and Lisa K. Nolan[AC]

[A]Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, 1802 Elwood Drive, VMRI #2, Iowa State University, Ames, IA 50011
[B]Gastroenteric Disease Center, Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA 16802

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SUMMARY. Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is a leading cause of economic loss to the poultry industry worldwide. The ability of APEC to cause disease is determined by certain virulence markers, some of which are located on pathogenicity islands (PAIs). We recently described one such PAI in an APEC O1:K1 strain (APEC-O1). This PAI, termed PAI $I_{APEC-O1}$, carries the genes of the *pap* operon, a region similar to the *tia* invasion determinant of enterotoxigenic *E. coli*; *ireA*, a gene that encodes an iron-responsive element; and a novel 1.5-kb region, ORF 54. Here, the occurrence of six selected loci of PAI $I_{APEC-O1}$ (*papA, papC, papG, ireA, tia,* and ORF 54) among APEC and fecal *E. coli* strains from apparently healthy chickens (avian commensal *E. coli*) was determined using polymerase chain reaction (PCR) techniques. None of the commensal *E. coli* was positive for all six traits, whereas 7.2% of the APEC isolates were positive for all the traits. Although there was no significant difference in the occurrence of ORF 54 among APEC and commensal *E. coli*, *tia, ireA, papC*, and *papG* genes were predominantly present in APEC rather than in avian commensal *E. coli*. *papA* was detected in only 6.3% of APEC, perhaps because of the presence of allelic variants of the gene. Additionally, the presence of all six traits was tested with PCR in APEC isolates collected in the 1980s, and these results were compared with those obtained with the APEC isolated in the 1990s. There was no significant difference in the occurrence of *tia, ireA, papC, papG*, and ORF 54 between APEC isolates collected during the different decades. However, *papA* was more frequently present in APEC from the 1980s than it was in APEC from the 1990s. Phylogenetic group of an isolate did not correlate with pathogenicity or the presence of PAI traits, except that more APEC of the low-pathogenicity group belonged to the phylogenetic group B1. However, PAI traits occurred more frequently in isolates belonging to the intermediate- and high-pathogenicity groups than in isolates of low pathogenicity.

RESUMEN. Ocurrencia de los genes en la denominada Isla Patogénica $I_{APEC-O1}$ entre cepas de *Escherichia coli* implicadas en colibacilosis aviar.

La colibacilosis causada por cepas patógenas de *Escherichia coli* aviar es una de las mayores causas de pérdidas económicas en la avicultura a nivel mundial. La capacidad de las cepas patógenas aviares de *E. coli* para causar enfermedad está determinada por ciertos factores de virulencia, algunos de los cuales están localizados en las denominadas islas patogénicas. Recientemente descubrimos una de estas islas patogénicas en una cepa patógena aviar de *E. coli* denominada O1:K1 (APEC-O1). Esta isla patogénica, identificada como isla patogénica $I_{APEC-O1}$, contiene los genes del operón *pap*, una región similar al determinante de invasión *tia* para *E. coli* enterotoxigénica; *ireA*, un gen que codifica un elemento en la respuesta al hierro, y una nueva región de 1.5 Kb, con un marco abierto de lectura (ORF por sus siglas en Inglés) 54. Aquí, la ocurrencia de los seis genes de la isla patogénica $I_{APEC-O1}$ (*papA, papC, papG, ireA, tia*, y ORF 54) entre las cepas patógenas aviares de *E. coli* y las cepas de *E. coli* fecal de pollos aparentemente sanos (*E. coli* aviar comensal) fue determinada usando la técnica de reacción en cadena de la polimerasa (PCR por sus siglas en Inglés). Ninguna de las cápsulas de los *E. coli* comensales fue positiva para todas las seis características, mientras que el 7.2% de los aislamientos patógenos de *E. coli* aviar fueron positivos para estas seis características. Aunque no hubo diferencia significativa en la ocurrencia de ORF 54 entre las cepas patógenas aviares de *E. coli* y las cepas de *E. coli* comensales, los genes, *tia, ireA, papA*, y *papG*, se presentaron predominantemente en las cepas patógenas aviares de *E. coli* mas que en las cepas de *E. coli* comensales. El gen *papA* fue detectado sólo en 6.3% de las cepas patógenas aviares de *E. coli*, posiblemente debido a la presencia de variantes alélicas en el gen. Además, la presencia de todas las seis características fue estudiada por medio de PCR en aislamientos de cepas patógenas aviares de *E. coli* tomados en la década de 1980, comparando estos resultados con los obtenidos de aislamientos de cepas patógenas aviares de *E. coli* en la década de 1990. No se encontró diferencia significativa en la incidencia de *papA, papC, papG, ireA, tia*, y ORF 54 entre los aislamientos de cepas patógenas aviares de *E. coli* de las dos décadas. No obstante, *papA* fue detectado mas frecuentemente en los aislamientos de cepas patógenas aviares de *E. coli* de la década de 1980 que en las de la década de 1990. El grupo filogenético de un aislamiento no se relacionó con la patogenicidad o con la presencia de las características de las islas patogénicas, excepto que más cepas patógenas aviares de *E. coli* del grupo de baja patogenicidad pertenecieron al grupo filogenético B1. Sin embargo, las características de las islas patogénicas ocurrieron más frecuentemente en aislamientos pertenecientes a los grupos de patogenicidad intermedia y alta, que de los de baja patogenicidad.

Key words: avian pathogenic *Escherichia coli*, extraintestinal pathogenic *E. coli*, pathogenicity island, *pap* operon, *tia* gene, *ireA* gene, phylogenetic typing, polymerase chain reaction, emerging pathogen

Abbreviations: APEC = avian pathogenic *Escherichia coli*; ddH_2O = double-distilled water; ExPEC = extraintestinal pathogenic *Escherichia coli*; *ireA* = iron-responsive element; ORF = open reading frame; PAI = pathogenicity island; *pap* = pyelonephritis-associated pili; PCR = polymerase chain reaction; UPEC = human uropathogenic *Escherichia coli*

[C]Corresponding author.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a large subset of *E. coli* that differs from other *E. coli* by possession of distinct virulence determinants, such as colonizing factors, iron acquisition systems, toxins, serum resistance traits, and invasins (10). Avian pathogenic *E. coli* (APEC), an ExPEC (10), causes a variety of diseases in poultry, which are collectively known as colibacillosis (1,4). Of the diseases caused by APEC, respiratory tract infection/septicemia and cellulitis in broilers and peritonitis in layers are among the leading causes of losses to the poultry industry (1,4,22,23). In the United States alone, colibacillosis is responsible for annual multimillion dollar losses (1).

Since Hacker *et al.* (7) discovered the first pathogenicity island (PAI) in a human uropathogenic strain of *E. coli* (UPEC), a number of PAIs have been described in many pathogenic bacterial species (2,21). These horizontally acquired, large genomic regions carry virulence genes that are absent in nonpathogenic or less-pathogenic members of the species (2,21). The first PAI described in an APEC strain was reported to be about 22 kb in size and to carry a gene encoding a vacuolating cytotoxin, among others (16). Our laboratory has discovered another PAI of APEC (PAI $I_{APEC\text{-}O1}$) in a highly virulent strain of *E. coli* isolated from a turkey with colisepticemia (11). PAI $I_{APEC\text{-}O1}$ contains the pyelonephritis-associated pili (*pap*) operon that encodes the P pilus (4,12), a gene similar to the *tia* invasion determinant locus of enterotoxigenic *E. coli* (6), a gene that encodes an iron-responsive element (*ireA*) of ExPEC (20), and a region (open reading frame [ORF] 54) with no nucleotide sequence homology to published databases as of March 2006 (GenBank accession DQ105563). Because some of these genes have not previously been reported in APEC, we employed polymerase chain reaction (PCR) to ascertain the possible distribution of PAI $I_{APEC\text{-}O1}$ among APEC and *E. coli* isolated from the feces of apparently healthy chickens (avian commensal *E. coli*). Additionally, we screened 95 APEC isolates collected during the 1980s and 1990s to determine if PAI $I_{APEC\text{-}O1}$ is an emerging feature of APEC.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The 95 APEC and 95 avian commensal *E. coli* isolates that were used in this study to determine the distribution of PAI $I_{APEC\text{-}O1}$ regions and their association with APEC virulence were part of the culture collection of our laboratory and have previously been described using serogrouping (Table 1) and virulence genotyping (17,18). The APEC isolates were obtained from various locations within the United States and were isolated from different poultry species, anatomic sites within the sick host, and clinical syndromes. These APEC were originally isolated during the 1990s. The avian commensal *E. coli* isolates used in this study were from the feces of apparently healthy chickens during the 1990s from different farms and flocks in North Dakota and Georgia. Additionally, 95 APEC isolates collected from chickens with clinical colibacillosis in 1980s were used in the study and were kindly provided by Dr. Sandy Cloud (University of Delaware, Newark, DE). All the APEC from 1980s originated from the Delmarva Peninsula on the east coast of the United States, were classified into high-, intermediate-, or low-pathogenicity groups according to their effects on day-old broiler chickens, and were diverse in terms of their serogroups (Table 1) and other traits (19). All isolates were stored in Brain Heart Infusion Broth (Difco Laboratories, Detroit, MI) at −80 C under conditions designed to ensure their genetic stability since their original isolation.

Phylogenetic analysis of *E. coli* isolates. The APEC and avian commensal *E. coli* isolates were subjected to phylogenetic typing as described in Clermont *et al.* (3) and Rodriguez-Siek *et al.* (18). Briefly, isolates were assigned to one of four groups (A, B1, B2, and D) according to the presence or absence of two genes, *chuA* and *yiaA*, and the DNA fragment, TSPE4, as determined by a triplex PCR.

Table 1. Serogroups of the isolates used in the study.

Serogroup	Number of APEC from 1980s	Number of APEC 1990s	Number of Avian Commensal *E. coli*
O1	0	4	1
O2	22	6	3
O7	0	2	0
O8	0	1	0
O9	0	2	4
O11	1	0	0
O15	1	1	1
O18	1	1	0
O19	2	3	0
O20	0	3	4
O23	0	1	0
O25	0	3	1
O26	0	0	1
O27	0	0	1
O29	0	1	0
O32	0	1	3
O35	10	0	0
O37	0	0	1
O43	1	0	0
O45	1	0	1
O51	0	3	1
O60	0	0	1
O61	0	1	0
O69	0	0	1
O70/71	1	0	0
O73	0	0	5
O75	0	1	0
O77	0	0	1
O78	12	16	3
O82	0	1	0
O83	3	0	0
O85	0	1	0
O86	0	0	1
O88	0	0	1
O91	0	1	0
O100	0	1	0
O102	0	0	1
O103	1	0	0
O106	0	2	4
O108	0	0	3
O111	0	2	0
O115	1	0	0
O117	0	1	0
O119	0	0	1
O120	0	2	1
O135	1	0	0
O139	0	0	1
O144	0	0	1
O149	0	0	1
O150	2	0	0
O153	0	1	1
O158	0	2	2
O171	0	2	0
Untypable	35	29	44
Total	95	95	95

Comparison of PAI-associated traits among APEC and avian commensal *E. coli*. To determine the distribution of PAI $I_{APEC\text{-}O1}$-associated sequences among APEC and commensal *E. coli*, the occurrence of PAI $I_{APEC\text{-}O1}$ sequences in various APEC was determined by PCR. APEC and commensal *E. coli* isolates, from the 1990s, were examined for the presence of *papA, papC, papG, tia, ireA*, and ORF 54,

Table 2. Primers used for PCR amplifications.

Primer	Gene or sequence targeted	Primer sequence (5′-3′)	Product size (bp)	Reference
TiaF	*tia*	CCCTTCTGCATCCTTGTAAGACA	507	This study
TiaR		TATAAGGGCGGTGATAAAAACG		
PapAF	*papA*	GCTGCTCCAACTATTCCACAG	582	This study
PapAR		ACTGCAGAAAAAGCACCTTCA		
PapCF	*papC*	TGTTTACTGGCATGATGGTCA	1614	This study
PapCR		ACGCCGAAAGACGTATATCCT		
PapGIIF	*papG*	CCCAGCTTTGTTATTTTCCTTG	801	This study
PapGIIR		TTCTTACCATGGCTGTATGTCG		
IreAF	*ireA*	CCGTGATGTGTTCTGCAGTTAT	1162	This study
IreAR		AGAGAATCCGTTGCTGCATATT		
ORF 54F	Orf 54	AAAAGCAGAGCTGGTGAATGA	1420	This study
ORF 54R		TCCGCTGTTGTATACCCACTC		

all of which are present in PAI $I_{APEC-O1}$ (11). Primer pairs for each gene were designed on line with the targeted gene sequences (GenBank accession DQ105563) using the Primer3 software (Whitehead Institute, Cambridge, MA). These are shown in Table 2. Boiled preparations of the overnight cultures were used as template DNA for PCR amplifications (8). Twenty-five microliters of PCR reaction mixtures were used, containing 0.5 μM each of forward and reverse primers (Table 2), 0.2 mM deoxynucleotide triphosphate (USB, Cleveland, Ohio), 0.5 U of *Taq* DNA polymerase (Invitrogen Co., Carlsbad, CA), 2.5 μl of 10× PCR buffer (Invitrogen), 1.5 mM magnesium chloride ($MgCl_2$; Invitrogen), 2 μl of DNA template, and enough double-distilled water (ddH_2O) to bring the final volume to 25 μl. APEC O1 (O1:K1), which is known to contain PAI $I_{APEC-O1}$ (11), and ddH_2O were used as the positive and negative controls, respectively. *papG, papC,* and *tia* were amplified with an initial incubation at 94 C for 3 min, followed by 30 cycles of 94 C for 45 sec, 59 C for 30 sec, and 72 C for 45 sec, and a final extension at 72 C for 5 min. PCR cycling parameters for amplification of ORF 54, *papA*, and *ireA* genes were the same except that the 30-cycle extension time was 1 min instead of 45 sec. To verify the identity of PCR products, amplified products (two for each gene) were cloned into pGEM T-Easy vector (Promega Co., Madison, WI), transformed into *E. coli* JM109 strain (Promega Co.), and sequenced with the BigDye terminator chemistry (Applied Biosystems), using standard vector primers, at the Iowa State University DNA Sequencing and Synthesis Facility. Sequence analysis and homology comparisons were carried out using the Basic Local Alignment Search Tool (BLAST) program of the National Center for Biotechnology Information and software packages from DNAStar (Lasergene, Madison, WI).

Comparison of PAI $I_{APEC-O1}$-associated genes among APEC isolates from two decades. Ninety-five APEC isolates collected during 1980s were examined for the presence of *papA, papC, papG, tia, ireA*, and ORF 54 with PCR under the conditions described above. The results were compared with those of APEC isolates from 1990s.

Biostatistics. Statistical comparisons of the occurrence of each trait tested (*papA, papC, papG, ireA, tia*, and ORF 54) between APEC and commensal *E. coli* isolates, between APEC isolates from the two different decades, and among APEC of the different phylogenetic groups were done by Fisher's exact test using the SAS program (SAS Institute, Cary, NC). Also, Fisher's exact test was used to compare assignments of APEC from the 1980s to phylogenetic and pathogenicity groups. A $P \leq 0.05$ was considered significant.

RESULTS

Phylogenetic analysis of APEC and avian commensal *E. coli*. Phylogenetic typing results are shown in Tables 3, 4. Among the 95 avian commensal *E. coli* isolates analyzed, 65.27% belonged to phylogenetic groups A and B1, which according to the literature are thought to contain the less-pathogenic *E. coli* (3); 34.73% belonged to phylogenetic groups B2 and D, the groups thought to contain the more-pathogenic strains (3). Of the 95 APEC isolates collected during 1980s, 53.68% belonged to phylogenetic groups A and B1, and 46.32% belonged to groups B2 and D. Of the 95 APEC isolates from the 1990s, 49.47% belonged to A and B1 phylogenetic groups, whereas 50.53% belonged to the B2 and D groups. More APEC from 1990s (37.89%) and commensal *E. coli* (34.74%) were matched to group A than those of APEC isolates from 1980s (21.05%). Also, more APEC isolates from 1980s (32.63%) and commensal *E. coli* (30.53%) were matched to the group B1 than APEC from the 1990s (11.58%). There were no significant differences among the numbers of *E. coli* isolates of each category that belonged to phylogenetic group B2. However, more APEC from the 1980s (31.58%) and 1990s (27.37%) belonged to group D than those of commensal *E. coli* (13.68%).

Table 3. Phylogenetic groups of the *E. coli* isolates used in the study.

Category of *E. coli*[A]	Number of isolates in each phylogenetic group (%)[B]			
	A	B1	B2	D
Avian commensal *E. coli*	33 (34.74)[a]	29 (30.53)[a]	20 (21.05)[a]	13 (13.68)[b]
APEC from 1980s	20 (21.05)[b]	31 (32.63)[a]	14 (14.74)[a]	30 (31.58)[a]
APEC from 1990s	36 (37.89)[a]	11 (11.58)[b]	22 (23.16)[a]	26 (27.37)[a]

[A]Each category of *E. coli* contains 95 isolates.

[B]Values within a column followed by different lowercase, superscript letters differ significantly ($P \leq 0.05$). Percentages of isolates that fall within each phylogenetic group are shown in parentheses. *E. coli* isolates in phylogenetic groups B2 and D are more likely to be associated with disease than phylogenetic groups A and B1 (3).

Table 4. Comparison of APEC[A] assignments to phylogenetic and pathogenicity groups.

Phylogenetic group	Pathogenicity group[B]			
	High	Intermediate	Low	Total
A	12[a]	5[a]	2[a]	19
B1	14[a]	4[a]	12[b]	30
B2	12[a]	3[a]	1[a]	16
D	21[a]	7[a]	2[a]	30
Total	59	19	17	95

[A]Only APEC from the 1980s are included.

[B]Values within a column followed by different lowercase, superscript letters differ significantly ($P \leq 0.05$).

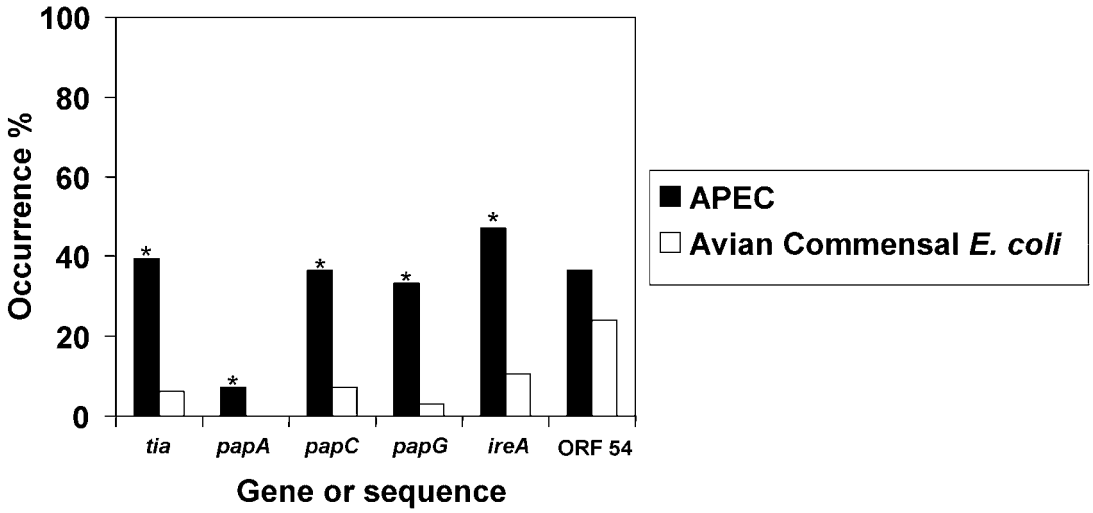

Fig 1. Occurrence of *tia, papA, papC, papG* and *ireA*, and ORF 54 among APEC and avian commensal *E. coli* isolates. Ninety-five APEC and 95 avian commensal *E. coli* were screened with PCR. Occurrence is indicated as a percentage of APEC or commensal *E. coli* positive for each trait tested. * = a statistically significant difference ($P < 0.05$).

Also, we compared the assignments of APEC from the 1980s to phylogenetic and pathogenicity groups (Table 4). There was no relationship between phylogenetic group and pathogenicity of the isolates except that most of the APEC with low pathogenicity belonged to the phylogenetic group B1.

Occurrence of PAI $I_{APEC\text{-}O1}$-associated genes and sequences in APEC and avian commensal *E. coli*. To ascertain whether the PAI-associated genes and sequences are predominantly present in APEC, as compared with commensal *E. coli*, a collection of 95 APEC and 95 commensal *E. coli* isolates were screened for the presence of six selected PAI-associated regions by PCR. *papC, papG, tia, ireA,* and ORF 54 were present in both APEC and commensal *E. coli* (Fig. 1). Only *papA*, which was amplified from just 6.3% of APEC, was present exclusively in APEC. The difference in occurrence of ORF 54 was not statistically significant between the two groups of *E. coli* ($P = 0.058$). However, *tia* ($P = 0.000001$), *papC* ($P = 0.000001$), *papG* ($P = 0.00000001$), and *ireA* ($P = 0.00000001$) were predominantly present in APEC, as compared with commensal *E. coli* (Fig. 1). No commensal *E. coli* isolate was positive for all the traits tested, whereas 7.2% of APEC possessed all six traits. On the other hand, 38.5% of APEC and 60% of commensal *E. coli* were negative for all six traits tested. About 31.6%, 5.3%, and 3.1% of commensal *E. coli* were positive only for one, two, or three of the traits tested, respectively. None of the commensal *E. coli* isolates screened carried more than three of the traits. However, nearly one-third (33.2%) of APEC contained four or more traits of the PAI $I_{APEC\text{-}O1}$ studied (Fig. 2).

Comparison of PAI $I_{APEC\text{-}O1}$-associated genes among APEC isolates from two decades. A collection of APEC isolates obtained during a period of two decades (1980s and 1990s) was analyzed by PCR for the presence of each of the selected traits (Table 5). The *papA* gene was found more frequently in APEC from the 1980s (16.84%) than from those of the 1990s (6.3%). There was no statistically significant difference in the occurrence of *tia, ireA, papC, papG*II, and ORF 54 between APEC isolates collected during the two decades.

Also, we studied the distribution of PAI-associated traits among APEC from three different pathogenicity groups (Fig. 3). Isolates

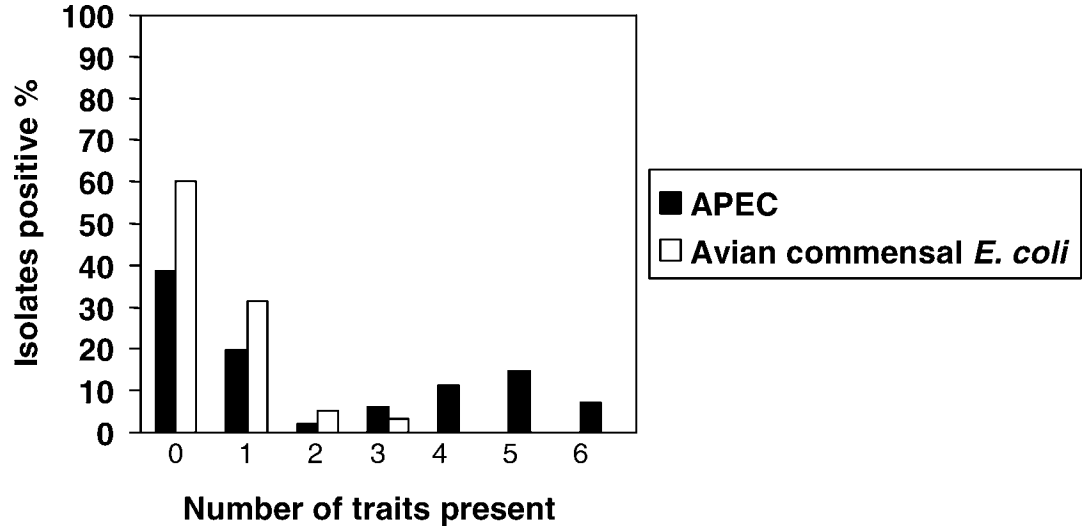

Fig. 2. Percentage of APEC and avian commensal *E. coli* isolates positive for a given number of traits as detected by PCR.

Table 5. Occurrence of PAI $I_{APEC-O1}$-associated traits among APEC isolated during the 1980s and 1990s.

Decade[A]	% of APEC positive[B]					
	tia	*ireA*	*papA*	*papC*	*papGII*	ORF 54
1980	36.84[a]	41.05[a]	16.84[a]	32.63[a]	33.68[a]	33.68[a]
1990	38.94[a]	45.26[a]	6.32[b]	35.78[a]	32.63[a]	35.78[a]

[A]Each decade was represented by 95 APEC isolates.
[B]Values within a column followed by different lowercase, superscript letters differ significantly ($P \leq 0.05$).

from high- and intermediate-pathogenicity groups contained more PAI traits than those of the low-pathogenicity group. None of the isolates from low-pathogenicity group was positive for all the six traits tested, whereas 11.86% and 10.52% isolates from high- and intermediate-pathogenicity groups, respectively, were positive for all six traits. Most of the APEC from the low-pathogenicity group (64. 7%) did not carry any of the six PAI-associated traits, whereas 29.41% carried only one of the traits. A small percentage of APEC (5.89%) from the low-pathogenicity group carried five of the traits that were tested in this study.

DISCUSSION

To determine whether PAI $I_{APEC-O1}$ is associated with virulent avian *E. coli*, as opposed to commensal *E. coli* of apparently healthy birds, the presence of six PAI $I_{APEC-O1}$-loci was detected by PCR in two collections of APEC and one of commensal *E. coli*. All collections were considered to be diverse, which was evidenced by previous serogrouping, virulence genotyping, pathogenicity testing, and/or cluster analysis (17,18,19). None of the PAI $I_{APEC-O1}$ tested markers, except for the *papA* gene, was exclusively present in APEC. This is not surprising, given the possibility that APEC may be an opportunistic or secondary pathogen that causes disease in immunocompromised hosts (1,4). However, only 3.1% of the commensal *E. coli* isolates contained at least three of the six markers tested, whereas 33.2% of the 1990s APEC were positive for more than four of the determinants. Similarly, the majority commensal *E. coli* did not carry any of the PAI-associated markers tested in the study, whereas only 38.5% of APEC lacked all six determinants. These observations suggest that PAI $I_{APEC-O1}$ is predominantly present in APEC rather than in commensal *E. coli*. On the other hand, the commensal *E. coli* that were positive for any one or a combination of genes may have the potential to cause disease in the ''right'' host, such as one that is immunocompromised. No effort was made in this study to assess that possibility. However, the content of PAI $I_{APEC-O1}$ genes among APEC of different pathogenicity groups was assessed. PAI traits were more common among APEC classified into intermediate- and high-pathogenicity groups than they were in those from the low-pathogenicity group. These results suggest that the PAI is associated with APEC's disease-causing abilities. Although most of the APEC of the low-pathogenicity group carried either none or one of the PAI-associated traits, 5.8% were positive for all five traits except for *papA*, suggesting that these isolates might have lost their capacity for virulence due to the loss over time of *papA* from the PAI. Also, the absence of certain PAI-associated genes in some APEC is not unexpected because of the extensive gene diversity demonstrated by APEC (14). Additionally, the possibility of excision of the entire PAI or individual genes within the PAI cannot be excluded, as PAIs may be unstable (13).

Among the genes tested by PCR, the *papA* gene, which encodes the major fimbrial protein of the P pilus (11), was present only in 6.3% of APEC. It is well known that the *papA* gene of the *pap* operon is antigenically diverse and exists in 11 serological variants (e.g., F7-1, F7-2, and F8–F16) (15). Perhaps, the rare occurrence of *papA* among APEC may be attributed to the genetic diversity of *papA*, given that the primer set used to amplify the gene in the present study specifically recognizes one or a limited number of allelic variants. Johnson *et al.* (9) also suggested that *papA* alleles can be horizontally transmitted between ExPEC, leading to continuous remodeling of PAIs. PapA subunit diversity has also been observed in canine and feline UPEC (5). Also, three allelic variants, namely alleles I, II, and III, exist for the *papG* gene, which encodes the tip-associated fimbrial adhesin (12). Numerous reports indicate that the majority of APEC carry *papG* allelic variant II (17,24). Sequence

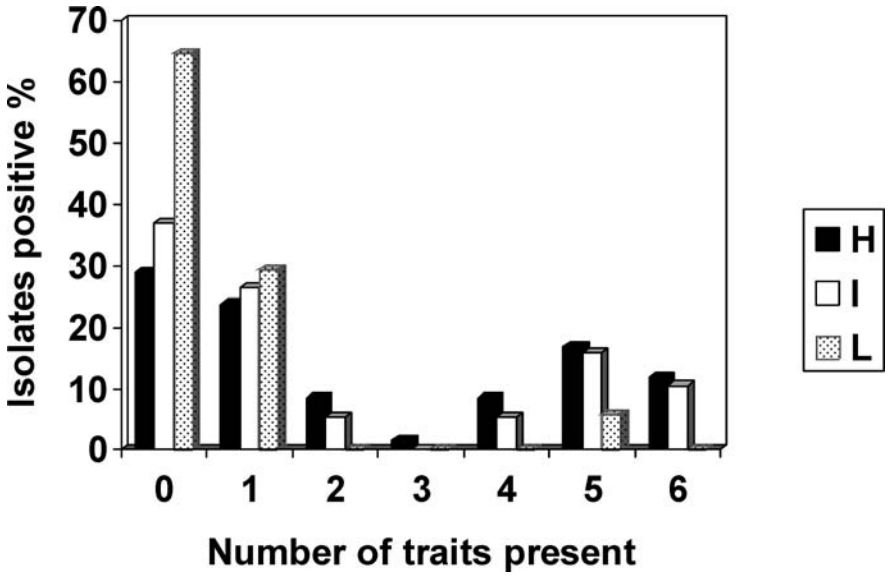

Fig. 3. Distribution of PAI-associated traits among APEC from three different pathogenicity groups. H = high-pathogenicity group; I = intermediate-pathogenicity group; L = low-pathogenicity group. Only APEC from the 1980s are included.

analysis of PAI $I_{APEC-O1}$ revealed that the *papG* gene, which resides within this PAI, resembles the *papG* allelic variant II, the predominant form found in APEC.

Analysis of PAI-associated genes in APEC isolates collected over two decades indicates that occurrence of *tia, ireA, papC, papG* genes, and ORF 54 has not changed from 1980s to 1990s. This result may suggest that PAI $I_{APEC-O1}$ may have been incorporated into the APEC genome before the 1980s. However, screening of more isolates representing different geographical locations and decades, including those collected before the 1980s, is necessary to prove this hypothesis. Interestingly, the results obtained with the isolates collected over the past two decades revealed that the *papA* gene is less frequently present in APEC from the 1990s than from those collected during 1980s. This result might indicate that over time *papA* has changed from one allelic variant to another or that the *papA* gene has been lost from the *pap* operon, resulting in loss of the ability to make functional P pili. Phylogenetic typing of avian *E. coli* isolates did not differentiate between pathogenic and commensal *E. coli* or correlate with pathogenicity group or possession of PAI traits. Such results suggest that phylogenetic typing may not prove helpful in typing avian *E. coli* isolates.

In summary, none of the commensal *E. coli* isolates contained all six PAI $I_{APEC-O1}$-associated markers tested, whereas 7.2% APEC were positive for all six traits. Except for the *papA* gene, which was detected only in APEC, the other five traits were present in both APEC and commensal *E. coli*, in variable frequencies. The occurrence of *tia, papC, papG,* and *ireA* was significantly higher in APEC than in commensal *E. coli*. Additionally, there was no difference in the occurrence of the traits tested, except for *papA*, between APEC from the 1980s and 1990s. It is interesting to note that the *tia, papC, papG*, and *ireA* genes are present in higher frequencies in APEC than they are in commensal *E. coli* and that they were associated with APEC classified as having intermediate-to-high pathogenicity. Hence, these genes may contribute to APEC virulence, and their presence may be exploitable in APEC detection and control. However, more extensive gene-prevalence studies, especially for the less-documented *tia* and *ireA* genes, should be carried out before concluding the validity of such an approach. This study also suggests that *tia, ireA, papA, papC, papG*, and ORF 54 are not required by some APEC to cause colibacillosis and that the pathogenicity of APEC is not determined exclusively by a few genes or a single PAI.

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