11-2006

Acquisition of Avian Pathogenic Escherichia coli Plasmids by a Commensal E. coli Isolate Enhances Its Abilities To Kill Chicken Embryos, Grow in Human Urine, and Colonize the Murine Kidney

Jerod A. Skyberg
Iowa State University

Timothy J. Johnson
Iowa State University

James R. Johnson
University of Minnesota

Connie Clabots
University of Minnesota

Catherine M. Logue
North Dakota State University, cmlogue@iastate.edu

See next page for additional authors

Acquisition of Avian Pathogenic Escherichia coli Plasmids by a Commensal E. coli Isolate Enhances Its Abilities To Kill Chicken Embryos, Grow in Human Urine, and Colonize the Murine Kidney

Abstract

We have found an avian pathogenic *Escherichia coli* (APEC) plasmid, pAPEC-O2-ColV, which contains many of the genes associated with APEC virulence and also shows similarity in content to a plasmid and pathogenicity island of human uropathogenic *E. coli* (UPEC). To test the possible role of this plasmid in virulence, it was transferred by conjugation along with a large R plasmid, pAPEC-O2-R, into a commensal avian *E. coli* strain. The transconjugant was compared to recipient strain NC, UPEC strain HE300, and donor strain APEC O2 using various assays, including lethality for chicken embryos, growth in human urine, and ability to cause urinary tract infection in mice. The transconjugant killed significantly more chicken embryos than did the recipient. In human urine, APEC O2 grew at a rate equivalent to that of UPEC strain HE300, and the transconjugant showed significantly increased growth compared to the recipient. The transconjugant also significantly outcompeted the recipient in colonization of the murine kidney. These findings suggest that APEC plasmids, such as pAPEC-O2-ColV, contribute to the pathogenesis of avian colibacillosis. Moreover, since avian *E. coli* and their plasmids may be transmitted to humans, evaluation of APEC plasmids as possible reservoirs of urovirulence genes for human UPEC may be warranted.

Disciplines

Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments

This article is from *Infection and Immunity* 74, no. 11 (November 2006): 6287–6292, doi:10.1128/IAI.00363-06.

Authors

Jerod A. Skyberg, Timothy J. Johnson, James R. Johnson, Connie Clabots, Catherine M. Logue, and Lisa K. Nolan

Infection and
Immunity

Acquisition of Avian Pathogenic *Escherichia coli* Plasmids by a Commensal *E. coli* Isolate Enhances Its Abilities To Kill Chicken Embryos, Grow in Human Urine, and Colonize the Murine Kidney

Jerod?A.? Skyberg, Timothy J. Johnson, James R. Johnson, Connie Clabots, Catherine M. Logue and Lisa K. Nolan

Infect. Immun. **2006, 74(11):6287. DOI: 10.1128/IAI.00363-06.**
Published Ahead of Print 5 September 2006.

INFECTION AND IMMUNITY, Nov. 2006, p. 6287–6292 Vol. 74, No. 11
0019-9567/06/$08.00+0 doi:10.1128/IAI.00363-06

Acquisition of Avian Pathogenic *Escherichia coli* Plasmids by a Commensal *E. coli* Isolate Enhances Its Abilities To Kill Chicken Embryos, Grow in Human Urine, and Colonize the Murine Kidney▽

Jerod A. Skyberg,[1]† Timothy J. Johnson,[1] James R. Johnson,[2] Connie Clabots,[2]
Catherine M. Logue,[3] and Lisa K. Nolan[1]*

Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011[1]; Mucosal and Vaccine Research Center, VA Medical Center, and Department of Medicine, University of Minnesota, Minneapolis, Minnesota[2]; and Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, North Dakota 58105[3]

Received 6 March 2006/Returned for modification 22 May 2006/Accepted 17 August 2006

We have found an avian pathogenic *Escherichia coli* (APEC) plasmid, pAPEC-O2-ColV, which contains many of the genes associated with APEC virulence and also shows similarity in content to a plasmid and pathogenicity island of human uropathogenic *E. coli* (UPEC). To test the possible role of this plasmid in virulence, it was transferred by conjugation along with a large R plasmid, pAPEC-O2-R, into a commensal avian *E. coli* strain. The transconjugant was compared to recipient strain NC, UPEC strain HE300, and donor strain APEC O2 using various assays, including lethality for chicken embryos, growth in human urine, and ability to cause urinary tract infection in mice. The transconjugant killed significantly more chicken embryos than did the recipient. In human urine, APEC O2 grew at a rate equivalent to that of UPEC strain HE300, and the transconjugant showed significantly increased growth compared to the recipient. The transconjugant also significantly outcompeted the recipient in colonization of the murine kidney. These findings suggest that APEC plasmids, such as pAPEC-O2-ColV, contribute to the pathogenesis of avian colibacillosis. Moreover, since avian *E. coli* and their plasmids may be transmitted to humans, evaluation of APEC plasmids as possible reservoirs of urovirulence genes for human UPEC may be warranted.

Avian colibacillosis, which is caused by avian pathogenic *Escherichia coli* (APEC), is one of the most significant and widespread infectious diseases occurring in production birds. It is responsible for large financial losses for the poultry industry each year due to mortality, lost production, and condemnations (1, 16, 17). A better understanding of the virulence mechanisms of the causative APEC strains is needed to guide the development of preventive measures.

Large plasmids occur commonly among APEC strains (7, 37) and may be a defining feature of the APEC pathotype (37). Certain APEC plasmids harbor a number of virulence genes (8, 27, 52) and are transmissible to other bacterial strains by conjugation (8, 14, 27). Such putative virulence plasmids may cotransfer with large multidrug resistance-encoding R plasmids, as seen with pAPEC-O2-R and pAPEC-O2-ColV (27), which may provide a mechanism for their selection and maintenance among the *E. coli* strains causing disease in production birds.

Despite the fact that these large plasmids contain many of the genes or operons thought to contribute to *E. coli* virulence, we and others have reported that their acquisition by *E. coli* K-12 strains does not necessarily result in increased virulence of the recipient (14, 25, 30). Ginns and colleagues (14) thought that this phenomenon might be due to a deficiency in the chromosomal background of the K-12 recipients, making them ill suited to cause disease. Therefore, in this study, we used an avian commensal *E. coli* strain (NC) as a recipient with the idea that it would be better adapted for survival in the avian host than a K-12 strain and would therefore provide a better background in which to assess the contributions of these plasmids to virulence and also because curing APEC O2 of its plasmids by standard methods (14, 18, 36) had proved problematic (data not shown).

To determine the contributions of pAPEC-O2-ColV and pAPEC-O2-R to virulence, we used three different models to compare the virulence of an APEC plasmid donor strain (APEC O2), which contains pAPEC-O2-ColV and pAPEC-O2-R; the recipient, an avian commensal *E. coli* strain (NC); and their transconjugant (NC/pAPEC-O2). The models used included a chicken embryo lethality assay, chosen because its results have been shown to positively correlate with an isolate's ability to cause lethality in 3-week old chickens (32) and morbidity and mortality in subcutaneous and intravenous chicken challenge models (12). Also, lethality to chicken embryos is a common characteristic of *E. coli* causing extraintestinal infections in humans (30). Additionally, since pAPEC-O2-ColV shows considerable sequence homology to a plasmid and pathogenicity island (PAI) of human uropathogenic *E. coli* (UPEC) and since there is rising concern that UPEC may colonize the human colon following ingestion of contaminated

---

* Corresponding author. Mailing address: Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Phone: (515) 294-3534. Fax: (515) 294-8500. E-mail: lknolan@iastate.edu.

† Present address: Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59718.

▽ Published ahead of print on 5 September 2006.

TABLE 1. Phenotypic characteristics of *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)[a]	Source and/or reference
Strains		
HE300	Positive control for growth in urine	Human UTI (50)
TC	Donor of pAPEC-O2-ColV and pAPEC-O2-R; is St^s Lac$^-$	K-12 strain (27)
NC	Low virulence to chicken embryos; is St^r Lac$^+$; lacks *iss*, *tsh*, and the aerobactin, ColV, and *iro* operons	Healthy chicken (44)
NC/pAPEC-O2	Transconjugant produced by the mating of TC and NC; contains pAPEC-O2-ColV and pAPEC-O2-R; St^r Lac$^+$	This study
APEC O2	Virulent to chicken embryos; original source of pAPEC-O2-ColV and pAPEC-O2-R	Diseased chicken (27)
Plasmids		
pAPEC-O2-ColV	ColV plasmid of APEC O2; contains *iss*, *tsh*, and *traT* genes, along with the aerobactin, ColV, *sit* and *iro* operons	27
pAPEC-O2-R	R plasmid encoding resistance to multiple antimicrobials	26

[a] Abbreviations: St^r, streptomycin resistant; St^s, streptomycin sensitive; ColV, colicin V; Lac, lactose fermentation.

food, such as poultry (21, 24, 39), two assays of urovirulence, namely, growth in human urine (40) and a murine model of ascending urinary tract infection (UTI) (22), were also used to assess these organisms.

(This research was done by Jerod A. Skyberg in partial fulfillment of the requirements for the Ph.D. degree from North Dakota State University, Fargo, 2006.)

MATERIALS AND METHODS

Media and bacterial strains. All bacterial strains were stored in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 20% glycerol at −80°C prior to use (42). In preparation for amplification, bacterial strains were grown on either MacConkey or nutrient agar (Difco) overnight at 37°C. *E. coli* strains and plasmids used in this study (Table 1) included APEC O2, isolated from the joint of a chicken with colibacillosis and the original source of pAPEC-O2-ColV and pAPEC-O2-R (27); UPEC HE300 (kindly provided by Soren Schubert at the Max von Pettenkofer Institut), isolated from a human case of acute pyelonephritis (50); TC, a transconjugant produced from the mating of APEC O2 and *E. coli* DH5α (27); NC, isolated from the feces of an apparently healthy chicken, which is of low virulence to chicken embryos (44); and the transconjugant, NC/pAPEC-O2, produced from mating TC with NC.

Conjugation protocol. Transconjugants were produced using techniques described previously (31). The plasmid donor strain used was TC, which is a transconjugant itself. Use of TC as the intermediate donor of pAPEC-O2 plasmids facilitated identification of transconjugants, as TC does not ferment lactose, whereas APEC O2 and the recipient NC do. TC contains pAPEC-O2-ColV and pAPEC-O2-R (26, 27), both of which were previously transferred into TC from the original donor strain, APEC O2 (27). To obtain the transconjugant, 0.2 ml of an exponentially grown culture of TC was mixed with 1.8 ml of an overnight culture of NC in antibiotic medium 3 broth (Difco). Mixtures were incubated without shaking at 25°C, 37°C, and 42°C for 18 h. Transconjugants were selected by their ability to resist streptomycin (65 μg/ml; Amresco, Solon, OH) (TC inhibiting) and ampicillin (100 μg/ml; Amresco) (NC inhibiting). Ampicillin resistance was chosen for transconjugant selection, as pAPEC-O2-ColV cotransfers with pAPEC-O2-R, which encodes resistance to ampicillin and other antimicrobials (26). Presumptive transconjugant colonies (ampicillin and streptomycin resistant) were picked from the selector plates, and their identities were confirmed by plasmid profiles, genotypes, antimicrobial resistance patterns, and the abilities to produce ColV and aerobactin.

Plasmid isolation. Plasmid DNA was harvested by the method of Wang and Rossman (55). The DNA obtained was separated in 0.8% agarose gels using horizontal gel electrophoresis.

Virulence genotyping. To determine whether test and control organisms contained the pAPEC-O2-ColV-like virulence cluster, they were examined for relevant constituent genes by using a previously described multiplex PCR assay (44). Targets included *iss*, the increased serum survival gene (2); *tsh*, encoding a temperature-sensitive hemagglutinin (35); *iucC*, a gene involved in aerobactin synthesis (4); and *cvi*, the immunity gene of the ColV operon (13).

ColV production. Isolates were screened for ColV production using a modified version (56) of the method of Fredericq (11). The controls included *E. coli* 23559 (American Type Culture Collection [ATCC], Rockville, MD), which does not produce colicin and is sensitive to the action of colicins, and *E. coli* 23558 (ATCC), which elaborates ColV. Organisms to be tested were stab inoculated into nutrient agar (Difco) and incubated overnight at 37°C. Organisms were killed by inverting the plates over chloroform-soaked filter paper for 30 min. Then, 10 ml of half-strength nutrient agar, containing the indicator organisms, *E. coli* 23559 and *E. coli* ATCC 23561 (56), was poured over the chloroform-killed colonies. Plates were incubated overnight at 37°C and examined for zones of growth inhibition of the indicator organism around the test stabs.

Aerobactin production. Isolates were also assessed for aerobactin production as described by Vidotto et al. (54). Low-iron agar assay plates, composed of M-9 minimum salts, containing 200 μM 2,2′-dipyridyl (Sigma, St. Louis, MO) and 0.2% glucose (Sigma), were seeded with 1 ml/liter of an overnight culture of the indicator organism, *E. coli* LG1522, which is incapable of producing aerobactin but can use exogenously produced aerobactin. APEC O2, NC, NC/pAPEC-O2, a known aerobactin-producing organism (*E. coli* LG1315), and a negative-control organism (*E. coli* HB101 [ATCC 33694]), were stab inoculated into the agar, and the plates were incubated at 37°C for 24 h. Following incubation, plates were observed for growth of the indicator organism around the stabs as evidence of aerobactin elaboration by the test isolates.

Virulence assays. (i) Embryo lethality assay. Strains APEC O2, NC, and NC/pAPEC-O2 were assessed for lethality in chicken embryos by inoculation of overnight-washed bacterial cultures (~500 CFU [CFU]) into the allantoic cavities of 12-day-old, embryonated, specific-pathogen-free eggs (32). Phosphate-buffered saline-inoculated and -uninoculated embryos were used as controls. Embryo deaths were recorded for 4 days.

(ii) Growth in human urine. Strains APEC O2, NC, and NC/pAPEC-O2 were compared by their ability to grow in human urine, as described elsewhere (40). Human UPEC strain HE300 was also included in this assessment (50). Urine samples from five volunteers (who were healthy, not taking antibiotics, and reported never experiencing a UTI) were collected, individually filter sterilized with 0.2-μm filters, pooled, and stored at −20°C. The strains to be tested were grown overnight in 2 ml of Luria-Bertani (LB) broth. The next day, the cell density was estimated by spectrophotometry, and cultures were diluted in phosphate-buffered saline prior to inoculation (100 μl of inoculum into 4.9 ml of urine) to achieve an approximate starting concentration of 10^2 to 10^3 CFU per ml, which was confirmed by viable counts (this concentration of bacteria represents the lower end of what is considered a significant indicator of UTI in symptomatic young women [51]). Mixtures were incubated at 37°C with shaking, and aliquots were removed at set intervals for determination of viable counts. Results were analyzed as the average of three trials.

(iii) Mouse UTI assay. A competition model of ascending murine UTI was used, as previously described (22). Female CBA/J mice were anesthetized and inoculated via the urethra under non-reflux-inducing conditions with a mixture containing approximately equal concentrations of *E. coli* strains NC and NC/pAPEC-O2, each of which had been grown individually overnight in LB broth at 37°C. The challenge inocula contained $\sim 2 \times 10^9$ total CFU, which is standard in use of this model (22). After 48 h, mice were euthanatized, and urine samples and their bladders and kidneys were collected sterilely. Organ homogenates and urine samples were cultured quantitatively on agar with or without ampicillin to determine the relative proportions of the strains. Additionally, from each positive culture, a representative colony (as available) was subjected to PCR analysis

TABLE 2. Abilities of *E. coli* strains to cause chicken embryo lethality 4 days postinfection

Strain	No. of embryos	No. of deaths	P value[a]	
			NC	APEC O2
NC	66	10		<0.001
APEC O2	64	48	<0.001	
NC/pAPEC-O2	66	41	<0.001	>0.10

[a] P value of the number of deaths caused by *E. coli* strains compared to the values for strains NC and APEC O2 calculated by using a two-sample test of proportions (47).

for one or more of the constituent virulence genes of pAPEC-O2-ColV to provide molecular confirmation of plasmid content and strain identity. Dual-strain challenges were used to compare the colonizing abilities of strains NC and NC/pAPEC-O2, as intra-animal competition assessments minimize the impact of mouse-to-mouse variation and maximize the ability to identify differences among test strains (22). Colonies from the cultures were similarly analyzed to define the relative abundance of the two test strains, and this proportion (the input ratio) was used to adjust the postmortem quantitative culture results (the output ratio) from the mouse infection experiments to obtain the competitive index.

Biostatistics. Differences in embryo lethality among the strains were evaluated for statistical significance using a two-sample test of proportions (47). The growth rate of each *E. coli* strain was determined as described previously (3, 10). All data were included from the point at which the cell concentration had increased to 150% of the inoculated concentration to the point at which the population density ceased to increase. Urine growth rate data were analyzed using linear regression analysis (Systat, Evanston, IL) to determine the specific growth rate for each strain. Differences among the mean growth rates were determined by using analysis of variance to place the strains into classes on the basis of growth rates (SAS Institute). Differences in colonization abilities as determined by the mouse UTI model were assessed as the proportion of positive cultures (by site) in which, according to the competitive index, either test strain outcompeted the other (with McNemar's test used for significance testing). The criterion for statistical significance was a P of <0.05.

RESULTS

Creation of transformant NC/pAPEC-O2. A fecal commensal *E. coli* isolate (strain NC) from an apparently healthy chicken was chosen as the recipient strain for these studies, since it was known to be of low virulence to chicken embryos (44) and it lacks the traits and genes known to be associated with pAPEC-O2-ColV (Table 1). By using standard procedures, strain NC was mated with TC, a K-12 derivative that contains the two large plasmids from an avian colibacillosis isolate, APEC O2. To verify that NC had acquired pAPEC-O2-ColV through conjugation, the presumptive NC transconjugant was compared to APEC O2 and NC with respect to several traits associated with pAPEC-O2-ColV and pAPEC-O2-R. Consistent with its being a transconjugant, NC/pAPEC-O2 was found to contain a 180-kb plasmid of the size of pAPEC-O2-ColV and to have acquired all the pAPEC-O2-ColV-associated traits assessed, namely, *iss*, *tsh*, *iucC*, *cvi*, and the abilities to produce ColV and aerobactin. Strain NC/pAPEC-O2 had also acquired ampicillin resistance and a 101-kb plasmid, consistent with cotransfer of the large R plasmid of donor strain APEC O2, i.e., pAPEC-O2-R (Table 1). In no case did we find a transconjugant containing only pAPEC-O2-R or pAPEC-O2-ColV, possibly because transconjugants containing both of the plasmids were selected for by the conditions used in our mating procedure. Transconjugants containing the R plasmid were selected for by plating the mating mixture on plates containing ampicillin. In addition, strain NC is sensitive to colicin V (data not shown); therefore, it is possible that all the recipient colonies that survive are those that have acquired the colicin V plasmid (and colicin V immunity), as the colicin V-sensitive recipient cells are eliminated by the action of colicin V that is produced by the plasmid donor in the mating mixture.

Virulence assessment of strain NC/pAPEC-O2. Three different assay systems, i.e., chicken embryo lethality, growth in human urine, and a murine model of ascending UTI, were used to compare the virulence of the transconjugant with that of its parents and human UPEC strain HE300. Acquisition of pAPEC-O2-ColV and pAPEC-O2-R by NC was associated with a significantly increased ability to kill chicken embryos (Table 2). That is, strain NC (lacking pAPEC-O2-ColV and pAPEC-O2-R) killed only 10 (15%) of 66 embryos, whereas the transformant NC/pAPEC-O2 killed 41 (62%) of 66 embryos ($P < 0.0001$). Further, the difference in embryo lethality between NC/pAPEC-O2 and APEC O2 was not statistically significant.

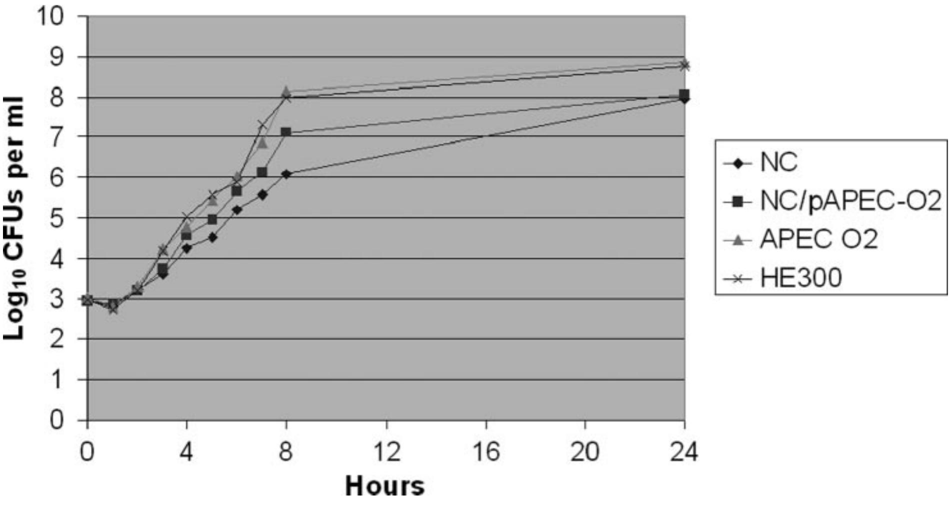

FIG. 1. Growth of *E. coli* strains in urine. Strains APEC O2 (the source of the plasmids of interest), recipient *E. coli* strain NC, the transconjugant NC/pAPEC-O2, and UPEC HE300 were studied. Growth curves were determined by measuring viable counts (CFU ml^{-1}) and represent an average of three trials for each strain.

TABLE 3. Growth of *E. coli* strains in human urine

Strain	Mean growth rate (SE) and statistical significance[a]
NC	0.507 (0.012) A
NC/pAPEC-O2	0.547 (0.007) B
APEC O2	0.633 (0.012) C
HE300	0.623 (0.012) C

[a] The growth rate was calculated in $\log_{10}$ CFU per hour. Growth rates with the same letter are not significantly different, whereas growth rates with different letters are significantly different ($P < 0.05$), calculated by one-way analysis of variance (Fisher's least significant difference).

TABLE 4. Comparative urovirulence of *E. coli* strains NC and NC/pAPEC-O2 in mice

Site cultured	Total no. of samples[a]	No. (%) of samples with the following urovirulence pattern[b]:		*P* value[c]
		NC/pAPEC-O2 > NC	NC/pAPEC-O2 < NC	
Urine	8	2 (25)	6 (75)	>0.10
Bladder	15	3 (20)	12 (80)	0.035
Kidney	18	17 (94)	1 (6)	<0.001

[a] Cultures were unavailable for 7 urine and 12 kidney samples.

[b] Number of samples that were colonized more strongly by strain NC than by strain NC/pAPEC-O2 (NC/pAPEC-O2 < NC) or vice versa (NC/pAPEC-O2 > NC).

[c] *P* values of the numbers of samples with the urovirulence pattern shown by McNemar's test.

In human urine, strain NC/pAPEC-O2 grew significantly faster than did strain NC ($P < 0.05$) but did not attain the growth rate of APEC O2, which was statistically indistinguishable from that of human UPEC strain HE300 (Fig. 1 and Table 3). In contrast, no differences in growth rates in LB broth were evident for strains NC, APEC O2, NC/pAPEC-O2, and HE300 (Fig. 2).

Finally, the abilities of strains NC and NC/pAPEC-O2 to colonize the murine urinary tract were compared (Table 4). NC outcompeted NC/pAPEC-O2 in six of eight urine samples (not statistically significant) and 12 of 15 bladders ($P = 0.035$). However, the transconjugant outcompeted the recipient in 17 of 18 culture-positive kidneys ($P < 0.001$).

DISCUSSION

Since large plasmids appear to be one of the most common features of APEC and may be a defining characteristic of the APEC pathotype (37), this study sought to determine the roles of two large, cotransferring APEC plasmids in virulence using several models. We found that acquisition of pAPEC-O2-ColV and pAPEC-O2-R by an avian commensal *E. coli* strain (NC) was accompanied by enhanced abilities to kill chicken embryos, grow in human urine, and colonize the murine kidney.

When *E. coli* NC/pAPEC-O2 was tested in chicken embryos, it killed significantly more embryos than NC, whereas the difference in the proportion of embryos killed by NC/pAPEC-O2 versus wild-type strain APEC O2 was not statistically significant. Although we feel it is likely that this increase in virulence was due to acquisition of pAPEC-O2-ColV, interpretation of these results must be tempered by the fact that NC/pAPEC-O2 also contains a second APEC-derived plasmid, pAPEC-O2-R, that is absent from strain NC (26). Sequence analysis of this second cotransferring plasmid has shown that it lacks the cluster of putative virulence genes associated with pAPEC-O2-ColV (26, 27), suggesting that the enhanced virulence of NC/pAPEC-O2 may be due to acquisition of pAPEC-O2-ColV and not to pAPEC-O2-R. These results suggest that, in a suitable background, pAPEC-O2-ColV is a virulence plasmid that may contribute to the pathogenesis of *E. coli* infections in poultry. Such results are similar to what Smith found when transferring ColV plasmids into avirulent recipients (46). Others have also reported large plasmids in avian *E. coli* strains (7, 14, 25, 52), and it appears that genes localized to pAPEC-O2-ColV are widespread among APEC (37). Therefore, ColV plasmids similar to pAPEC-O2-ColV may be important contributors to APEC virulence generally. Also, of interest is the association of these pAPEC-O2-ColV-like plasmids with large, cotransferring R plasmids, such as pAPEC-O2-R. This association may

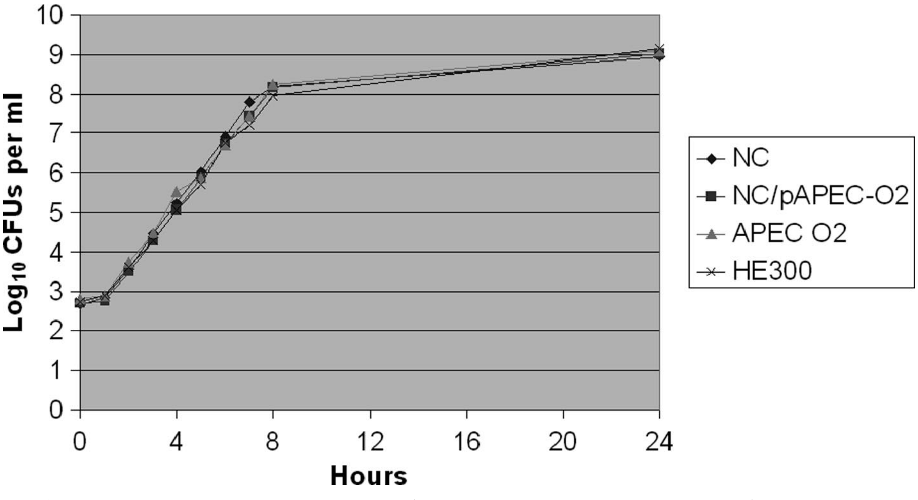

FIG. 2. Growth of *E. coli* strains in LB broth. Strains APEC O2 (the source of the plasmids of interest), recipient strain NC, the transconjugant NC/pAPEC-O2, and (human pyelonephritis isolate) UPEC HE300 were studied. Growth curves were determined by measuring viable counts (CFU ml^{-1}) and represent an average of three trials for each strain.

provide an additional means by which APEC virulence plasmids are favored in nature. The relationship between R plasmid and virulence plasmids should be further examined, as should the possible role of R plasmids in virulence.

Also of interest is the potential link that plasmids, such as pAPEC-O2-ColV, provide between APEC and UPEC virulence. For example, the *iro* operon located on pAPEC-O2-ColV lies in close approximation to the *iss* gene (27), which is similar to the arrangement described by Sorsa et al. for a plasmid from a human UPEC strain (49). In fact, the reported sequence around *iss* in the UPEC strain showed over 99% identity with pAPEC-O2-ColV (GenBank accession number AY567838). Additionally, pAPEC-O2-ColV shares certain similarities in both sequence and gene arrangement with PAI III of UPEC strain 536, which contains the *iro* operon, *tsh*, and remnants of the ColV operon (6).

These similarities of pAPEC-O2-ColV to UPEC plasmids and PAIs, as well as the documented transfer of APEC and their plasmids from birds to human beings (28, 45), prompted us to evaluate the potential contribution of pAPEC-O2-ColV to urovirulence in a mammalian host. First, the growth of strains NC/pAPEC-O2, APEC O2, NC, and UPEC HE300 (50) in human urine was compared, since Russo et al. (39) reported that microbial growth in urine was a good predictor of urovirulence and have used growth and gene expression in urine to screen for potential urovirulence factors among *E. coli* strains (40). Also, it is known that UPEC, compared to commensal *E. coli* strains, show shorter lag periods and doubling times when cultured in urine (15). The acquisition of pAPEC-O2-ColV and pAPEC-O2-R by the recipient strain resulted in a statistically significant increase in its growth rate in urine, suggesting that acquisition of these plasmids was responsible for the transconjugant's enhanced ability to grow in urine. In contrast, all of the strains grew at similar rates in LB broth, indicating that the plasmids confer a growth advantage specific to urine and are not a general growth promoter.

To further assess the potential of pAPEC-O2-ColV and pAPEC-O2-R to contribute to urovirulence, the abilities of the recipient and transconjugant to cause UTI in a mouse model were compared. Although strain NC outcompeted the transconjugant in colonization of the lower urinary tract (bladder), the transconjugant significantly outcompeted the recipient to an even greater extent in the kidney. These results suggest that the plasmids of strain APEC O2, most likely pAPEC-O2-ColV, have the potential to contribute to the pathogenesis of upper UTI in mammalian hosts.

Although future experiments will be required to definitively determine whether pAPEC-O2-ColV or pAPEC-O2-R is responsible for enhancing the disease-causing abilities of strain NC in chicken embryos and mice, pAPEC-O2-ColV is the more likely candidate. Several traits encoded by pAPEC-O2-ColV may contribute to virulence, including the ability to resist complement, a common characteristic among APEC and the UPEC causing upper UTIs (5, 20, 33), and the ability to acquire iron under limiting conditions, a characteristic associated with both APEC and UPEC virulence (5, 20). For instance, the aerobactin (4), *iro* (9), and *sit* operons, along with the *iss* and *tsh* genes (GenBank accession number AY545598), have all been found on pAPEC-O2-ColV (27). Interestingly, genes of the aerobactin, *sit*, and *iro* operons along with the *tsh* gene were upregulated in UPEC CFT073, an archetypical UPEC strain, during growth in human urine or in the mouse urinary tract (19, 48). Further study of pAPEC-O2-ColV or related plasmids may detect other candidate virulence genes contributing to the pathogenesis of both avian colibacillosis and human UTI.

Although these results suggest that pAPEC-O2-ColV has the potential to contribute to urovirulence, we did not determine whether *E. coli* strains containing pAPEC-O2-ColV-like plasmids colonize human beings. However, genes associated with pAPEC-O2-ColV are widely distributed among both APEC and human UPEC strains (38). In addition, others have demonstrated that plasmid-containing *E. coli* strains from poultry may colonize human beings and that use of antimicrobials may facilitate this exchange (28, 29, 34, 53). Also, Levy and coworkers (28) demonstrated that resistance plasmids and the *E. coli* strains containing these plasmids could be transferred from chicken to chicken and from chickens to humans.

So too, evidence exists that this microbial transfer from birds to human beings may involve potential human pathogens (24, 43). In addition, numerous recent studies have shown that extraintestinal pathogenic *E. coli* strains (41), a group encompassing UPEC and APEC, are fairly common in retail poultry (21, 23, 24). Therefore, it would seem that further study of APEC plasmids is warranted, both for their contributions to APEC virulence in poultry and for their potential to serve as reservoirs of urovirulence genes of significance to human health.

In summary, we used an avian fecal *E. coli* recipient strain to study the contributions of certain APEC plasmids to virulence. Although further work will be required to draw definitive conclusions about the roles of these plasmids in virulence, it is likely that these plasmids enable commensal *E. coli* strain NC to kill chicken embryos, suggesting that it plays a role in the pathogenesis of avian colibacillosis. Also, the transconjugant's enhanced ability to grow in urine and cause upper UTIs in mice suggest that APEC plasmids are capable of contributing to urovirulence in mammalian hosts. Together with their documented ability to transfer to human beings, the results of this study suggest that APEC plasmids could serve as reservoirs of urovirulence genes for *E. coli* that cause UTIs in humans.

REFERENCES

1. **Barnes, H. J., S. S. Vaillancourt, and W. G. Gross.** 2003. Colibacillosis, p. 631–652. *In* Y. M. Saif (ed.), Diseases in poultry. Iowa State University Press, Ames.
2. **Binns, M. M., D. L. Davies, and K. G. Hardy.** 1979. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. Nature **279:**778–781.
3. **Broughall, J. M., P. A. Anslow, and D. C. Kilsby.** 1983. Hazard analysis applied to microbial growth in foods: development of mathematical models describing the effect of water activity. J. Appl. Bacteriol. **55:**101–110.
4. **de Lorenzo, V., and J. B. Neilands.** 1986. Characterization of *iucA* and *iucC* genes of the aerobactin system of plasmid ColV-K30 in *Escherichia coli*. J. Bacteriol. **167:**350–355.
5. **Dho-Moulin, M., and J. M. Fairbrother.** 1999. Avian pathogenic *Escherichia coli* (APEC). Vet. Res. **30:**299–316.
6. **Dobrindt, U., G. Blum-Oehler, T. Hartsch, G. Gottschalk, E. Z. Ron, R. Funfstuck, and J. Hacker.** 2001. S-fimbria-encoding determinant *sfa*$_I$ is located on pathogenicity island III$_{536}$ of uropathogenic *Escherichia coli* strain 536. Infect. Immun. **69:**4248–4256.
7. **Doetkott, D. M., L. K. Nolan, C. W. Giddings, and D. L. Berryhill.** 1996. Large plasmids of avian *Escherichia coli* isolates. Avian Dis. **40:**927–930.
8. **Dozois, C. M., M. Dho-Moulin, A. Bree, J. M. Fairbrother, C. Desautels, and R. Curtiss III.** 2000. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. Infect. Immun. **68:**4145–4154.
9. **Dozois, C. M., F. Daigle, and R. Curtiss III.** 2003. Identification of pathogen-

specific and conserved genes expressed *in vivo* by an avian pathogenic *Escherichia coli* strain. Proc. Natl. Acad. Sci. USA **100:**247–252.

10. **Duffy, G., J. J. Sheridan, R. L. Buchanan, D. A. Mcdowell, and I. S. Blair.** 1994. The effect of aeration, initial inoculum and meat microflora on the growth-kinetics of *Listeria monocytogenes* in selective enrichment broths. Food Microbiol. **11:**429–438.
11. **Fredericq, P.** 1957. Colicins. Annu. Rev. Microbiol. **11:**7–22.
12. **Gibbs, P. S., S. R. Petermann, and R. E. Wooley.** 2004. Comparison of several challenge models for studies in avian colibacillosis. Avian Dis. **48:** 751–758.
13. **Gilson, L., H. K. Mahanty, and R. Kolter.** 1987. Four plasmid genes are required for colicin V synthesis, export, and immunity. J. Bacteriol. **169:** 2466–2470.
14. **Ginns, C. A., M. L. Benham, L. M. Adams, K. G. Whithear, K. A. Bettelheim, B. S. Crabb, and G. F. Browning.** 2000. Colonization of the respiratory tract by a virulent strain of avian *Escherichia coli* requires carriage of a conjugative plasmid. Infect. Immun. **68:**1535–1541.
15. **Gordon, D. M., and M. A. Riley.** 1992. A theoretical and experimental analysis of bacterial growth in the bladder. Mol. Microbiol. **6:**555–562.
16. **Gross, W. G.** 1994. Diseases due to *Escherichia coli* in poultry, p. 237–259. *In* C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom.
17. **Gyles, C. L.** 1993. *Escherichia coli*, p. 164–187. *In* C. L. Gyles and C. O. Thoen (ed.), Pathogenesis of bacterial infections in animals. Iowa State University Press, Ames.
18. **Heery, D. M., R. Powell, F. Gannon, and L. K. Dunican.** 1989. Curing of a plasmid from *E. coli* using high-voltage electroporation. Nucleic Acids Res. **17:**10131.
19. **Heimer, S. R., D. A. Rasko, C. V. Lockatell, D. E. Johnson, and H. L. Mobley.** 2004. Autotransporter genes *pic* and *tsh* are associated with *Escherichia coli* strains that cause acute pyelonephritis and are expressed during urinary tract infection. Infect. Immun. **72:**593–597.
20. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
21. **Johnson, J. R., A. C. Murray, A. Gajewski, M. Sullivan, P. Snippes, M. A. Kuskowski, and K. E. Smith.** 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. **47:**2161–2168.
22. **Johnson, J. R., S. Jelacic, L. M. Schoening, C. Clabots, N. Shaikh, H. L. Mobley, and P. I. Tarr.** 2005. The IrgA homologue adhesin Iha is an *Escherichia coli* virulence factor in murine urinary tract infection. Infect. Immun. **73:**965–971.
23. **Johnson, J. R., P. Delavari, T. T. O'Bryan, K. E. Smith, and S. Tatini.** 2005. Contamination of retail foods, particularly turkey, from community markets (Minnesota, 1999–2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog. Dis. **2:**38–49.
24. **Johnson, J. R., M. A. Kuskowski, K. Smith, T. T. O'Bryan, and S. Tatini.** 2005. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. J. Infect. Dis. **191:**1040–1049.
25. **Johnson, T. J., C. W. Giddings, S. M. Horne, P. S. Gibbs, R. E. Wooley, J. Skyberg, P. Olah, R. Kercher, J. S. Sherwood, S. L. Foley, and L. K. Nolan.** 2002. Location of increased serum survival gene and selected virulence traits on a conjugative R plasmid in an avian *Escherichia coli* isolate. Avian Dis. **46:**342–352.
26. **Johnson, T. J., K. E. Siek, S. J. Johnson, and L. K. Nolan.** 2005. DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic *Escherichia coli* transmissible R plasmid. Antimicrob. Agents Chemother. **49:**4681–4688.
27. **Johnson, T. J., K. E. Siek, S. J. Johnson, and L. K. Nolan.** 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J. Bacteriol. **188:**745–758.
28. **Levy, S. B., G. B. FitzGerald, and A. B. Macone.** 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature **260:**40–42.
29. **Linton, A. H., K. Howe, P. M. Bennett, M. H. Richmond, and E. J. Whiteside.** 1977. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. J. Appl. Bacteriol. **43:**465–469.
30. **Minshew, B. H., J. Jorgensen, M. Swanstrum, G. A. Grootes-Reuvecamp, and S. Falkow.** 1978. Some characteristics of *Escherichia coli* strains isolated from extraintestinal infections of humans. J. Infect. Dis. **137:**648–654.
31. **Nolan, L. K., R. E. Wooley, J. Brown, and J. B. Payeur.** 1991. Comparison of phenotypic characteristics of *Salmonella* spp. isolated from healthy and ill (infected) chickens. Am. J. Vet. Res. **52:**1512–1517.
32. **Nolan, L. K., R. E. Wooley, J. Brown, K. R. Spears, H. W. Dickerson, and M. Dekich.** 1992. Comparison of a complement resistance test, a chicken embryo lethality test, and the chicken lethality test for determining virulence of avian *Escherichia coli*. Avian Dis. **36:**395–397.
33. **Nolan, L. K., S. M. Horne, C. W. Giddings, S. L. Foley, T. J. Johnson, A. M. Lynne, and J. Skyberg.** 2003. Resistance to serum complement, *iss*, and virulence of avian *Escherichia coli*. Vet. Res. Commun. **27:**101–110.
34. **Ojeniyi, A. A.** 1989. Direct transmission of *Escherichia coli* from poultry to humans. Epidemiol. Infect. **103:**513–522.
35. **Provence, D. L., and R. Curtiss III.** 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. Infect. Immun. **62:**1369–1380.
36. **Riva, S., A. Fietta, M. Berti, L. G. Silvestri, and E. Romero.** 1973. Relationships between curing of the F episome by rifampin and by acridine orange in *Escherichia coli*. Antimicrob. Agents Chemother. **3:**456–462.
37. **Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan.** 2005. Characterizing the APEC pathotype. Vet. Res. **36:**241–256.
38. **Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, M. K. Fakhr, and L. K. Nolan.** 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology **151:**2097–2110.
39. **Russo, T. A., S. T. Jodush, J. J. Brown, and J. R. Johnson.** 1996. Identification of two previously unrecognized genes (*guaA* and *argC*) important for uropathogenesis. Mol. Microbiol. **22:**217–229.
40. **Russo, T. A., U. B. Carlino, A. Mong, and S. T. Jodush.** 1999. Identification of genes in an extraintestinal isolate of *Escherichia coli* with increased expression after exposure to human urine. Infect. Immun. **67:**5306–5314.
41. **Russo, T. A., and J. R. Johnson.** 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. Infect. Dis. **181:**1753–1754.
42. **Sanderson, K. E., and D. R. Zeigler.** 1991. Storing, shipping, and maintaining records on bacterial strains. Methods Enzymol. **204:**248–264.
43. **Shooter, R. A., E. M. Cooke, S. A. Rousseau, and A. L. Breaden.** 1970. Animal sources of common serotypes of *Escherichia coli* in the food of hospital patients. Possible significance in urinary-tract infections. Lancet **2:**226–228.
44. **Skyberg, J. A., S. M. Horne, C. W. Giddings, R. E. Wooley, P. S. Gibbs, and L. K. Nolan.** 2003. Characterizing avian *Escherichia coli* isolates with multiplex polymerase chain reaction. Avian Dis. **47:**1441–1447.
45. **Smith, H. W.** 1969. Transfer of antibiotic resistance from animal and human strains of *Escherichia coli* to resident *E. coli* in the alimentary tract of man. Lancet **1:**1174–1176.
46. **Smith, H. W.** 1974. A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. J. Gen. Microbiol. **83:**95–111.
47. **Snedecor, G. W., and W. G. Cochran..** 1980. Statistical methods, 7th ed. Iowa State University Press, Ames.
48. **Snyder, J. A., B. J. Haugen, E. L. Buckles, C. V. Lockatell, D. E. Johnson, M. S. Donnenberg, R. A. Welch, and H. L. Mobley.** 2004. Transcriptome of uropathogenic *Escherichia coli* during urinary tract infection. Infect. Immun. **72:**6373–6381.
49. **Sorsa, L. J., S. Dufke, J. Heesemann, and S. Schubert.** 2003. Characterization of an *iroBCDEN* gene cluster on a transmissible plasmid of uropathogenic *Escherichia coli*: evidence for horizontal transfer of a chromosomal virulence factor. Infect. Immun. **71:**3285–3293.
50. **Sorsa, L. J., S. Dufke, and S. Schubert.** 2004. Identification of novel virulence-associated loci in uropathogenic *Escherichia coli* by suppression subtractive hybridization. FEMS Microbiol. Lett. **230:**203–208.
51. **Stamm, W. E., G. W. Counts, K. R. Running, S. Fihn, M. Turck, and K. K. Holmes.** 1982. Diagnosis of coliform infection in acutely dysuric women. N. Engl. J. Med. **307:**463–468.
52. **Tivendale, K. A., J. L. Allen, C. A. Ginns, B. S. Crabb, and G. F. Browning.** 2004. Association of *iss* and *iucA*, but not *tsh*, with plasmid-mediated virulence of avian pathogenic *Escherichia coli*. Infect. Immun. **72:**6554–6560.
53. **van den Bogaard, A. E., N. London, C. Driessen, and E. E. Stobberingh.** 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J. Antimicrob. Chemother. **47:**763–771.
54. **Vidotto, M. C., E. E. Muller, J. C. de Freitas, A. A. Alfieri, I. G. Guimaraes, and D. S. Santos.** 1990. Virulence factors of avian *Escherichia coli*. Avian Dis. **34:**531–538.
55. **Wang, Z., and T. G. Rossman.** 1994. Large-scale supercoiled plasmid preparation by acidic phenol extraction. BioTechniques **16:**460–463.
56. **Wooley, R. E., P. S. Gibbs, H. W. Dickerson, J. Brown, and L. K. Nolan.** 1996. Analysis of plasmids cloned from a virulent avian *Escherichia coli* and transformed into *Escherichia coli* DH5 alpha. Avian Dis. **40:**533–539.

Editor: V. J. DiRita