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

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Running head: *Escherichia coli* Virulence Plasmid
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, to UPEC strain HE300, and to donor strain APEC O2 using various assays, including lethality for chick embryos, growth in human urine, and ability to cause urinary tract infection in mice. The transconjugant killed significantly more chick 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.
Key words: *Escherichia coli*, avian colibacillosis, urinary tract infection, virulence plasmid, UPEC, APEC, UTI
Introduction

Avian colibacillosis, which is caused by avian pathogen *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 (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 co-transfer 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* 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 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 strain (NC); and their transconjugant
(NC/pAPEC-O2). The models used included a chick embryo lethality assay,
chosen because its results have been shown to positively correlate with an
isolate’s ability to cause lethality in three-week old chickens (32) and morbidity
and mortality in subcutaneous and intravenous chicken challenge models (12).
Also, lethality to chick 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
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.
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 Dr. Soren Schubert, from 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 chick embryos (44); and the transconjugant, NC/pAPEC-O2, produced from the mating of the 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 hr. 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 co-transfers with pAPEC-O2-R, which encodes ampicillin and other resistances (26). Presumptive transconjugant colonies (ampicillin and streptomycin resistant) were picked from the selector plates, and their identities were confirmed with plasmid profiles, genotypes, antimicrobial resistance patterns, and abilities to produce ColV and aerobactin.

**Plasmid isolation.** Plasmid DNA was harvested according to 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 polymerase chain reaction (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 modification of the method of Fredericq (11, 56). 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/L 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 hrs. 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.

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

Growth in human urine. 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 um 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 PBS prior to inoculation (100 µl of inoculum into 4.9 ml of urine) to achieve an approximate starting concentration of $10^2$ to $10^3$ CFUs 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.

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 NC and NC/pAPEC-O2, each of which had been grown individually overnight in LB broth at 37°C. The challenge inocula contained ~2 x 10^9 total CFU, which is standard in use of this model (22). After 48h, mice were euthanatized, and their urine, bladders, and kidneys were collected steriley. Organ homogenates and urine 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 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 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 between embryo lethality among the strains were evaluated for statistical significance using a two-sample test of proportions (47). The growth rates of each *E. coli* strain were 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 that where 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 ANOVA to place the strains into classes based upon 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 out-competed the other (with McNemar’s test used for significance testing). The criterion for statistical significance was P < .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 chick embryos (44), and lacks the traits and genes known to be associated with pAPEC-O2-ColV (Table 1). Using standard procedures, NC was mated with TC, a K-12 derivative that contains the two large plasmids from 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 representing 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. NC/pAPEC-O2 also had acquired ampicillin resistance and a 101-kb plasmid, consistent with co-transfer 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, 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 NC/pAPEC-O2. Three different assay systems, i.e. chick 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 chick embryos (Table 2). That is, 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 < .0001). Further, the difference in embryo lethality between NC/pAPEC-O2 and APEC O2 was not statistically significant.

In human urine, NC/pAPEC-O2 grew significantly faster than did NC (P < .05), but did not attain the growth rate of APEC O2, which was statistically indistinguishable from that of human UPEC strain HE300 (Figure 1, Table 3). In contrast, no differences in growth rates in LB broth were evident for NC, APEC O2, NC/pAPEC-O2, and HE300 (Figure 2).

Finally, NC and NC/pAPEC-O2 were compared by their abilities to colonize the murine urinary tract (Table 4). NC outcompeted NC/pAPEC-O2 in six of eight urine samples (NS) and 12 of 15 bladders (P = .035). However, the
transconjugant outcompeted the recipient in 17 of 18 culture-positive kidneys (P < .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 role of two large, co-transferring APEC plasmids in virulence using several models. We found that acquisition of pAPEC-O2-ColV and pAPEC-O2-R by an avian commensal strain (NC) was accompanied by an enhanced ability to kill chick embryos, grow in human urine, and colonize the murine kidney.

When NC/pAPEC-O2 was tested in chick 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 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, co-transferring 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
described large plasmids in avian *E. coli* (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, co-transferring R plasmids, such
as pAPEC-O2-R. This association may provide an additional means by which
APEC virulence plasmids are favored in nature. The relationship between R 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
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, NC/pAPEC-O2, APEC O2, NC, and
UPEC HE300 (50) were compared for their growth in human urine, 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* (40). Also, it is known that UPEC, as
compared to commensal *E. coli*, 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 recipient and transconjugant were compared for
their abilities to cause UTI in a mouse model. Although 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 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 NC in chick 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 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 \textit{E. coli}, 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 (38). In addition, others have demonstrated that plasmid-containing \textit{E. coli} from poultry may colonize human beings and that use of antimicrobials may facilitate this exchange (28, 29, 34, 53). Also, Levy and co-
workers (28) demonstrated that resistance plasmids and the *E. coli* containing them could be transferred from chicken to chicken and from chicken 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* (ExPEC) (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 to virulence of certain APEC plasmids. Although further work will be required to draw definitive conclusions about the role these plasmids in virulence, it is likely that these plasmids enable commensal *E. coli* strain NC to kill chick 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 UTI 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


### Table 1. Phenotypic Characteristics of Strains/Plasmids Used

<table>
<thead>
<tr>
<th>Strain/Plasmid</th>
<th>Relevant Characteristics *</th>
<th>Reference and/or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE300</td>
<td>Positive Control for Urine Growth</td>
<td>Human UTI (50)</td>
</tr>
<tr>
<td>TC</td>
<td>Donor of pAPEC-O2-ColV and pAPEC-O2-R; is St&lt;sup&gt;+&lt;/sup&gt;, Lac&lt;sup&gt;-&lt;/sup&gt;</td>
<td>K12 strain (27)</td>
</tr>
<tr>
<td>NC</td>
<td>Low virulence to chick embryos; is St&lt;sup&gt;+&lt;/sup&gt;, Lac&lt;sup&gt;-&lt;/sup&gt;; lacks iss, tsh, and the aerobactin, ColV, and iro operons</td>
<td>Healthy Chicken (44)</td>
</tr>
<tr>
<td>NC/pAPEC-O2</td>
<td>Transconjugant produced by the mating of TC and NC; contains pAPEC-O2-ColV and pAPEC-O2-R; St&lt;sup&gt;+&lt;/sup&gt;, Lac&lt;sup&gt;-&lt;/sup&gt;</td>
<td>This Study</td>
</tr>
<tr>
<td>APEC O2</td>
<td>Virulent to chick embryos; original source of pAPEC-O2-ColV, pAPEC-O2-R</td>
<td>Diseased Chicken (27)</td>
</tr>
<tr>
<td>pAPEC-O2-ColV</td>
<td>ColV plasmid of APEC O2; contains iss, tsh, and traT genes, along with the aerobactin, ColV, sit and iro operons</td>
<td>(27)</td>
</tr>
<tr>
<td>pAPEC-O2-R</td>
<td>R plasmid encoding resistance to multiple antimicrobials</td>
<td>(26)</td>
</tr>
</tbody>
</table>

* Abbreviations: St, streptomycin; Tc, tetracycline; r, resistant; s, sensitive; ColV, colicin V; Lac, lactose fermentation
Table 2. Embryo Lethality Four Days Post Infection

<table>
<thead>
<tr>
<th>Strain</th>
<th>Embryos</th>
<th>Deaths</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt; vs. NC</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt; vs. APEC 02</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>66</td>
<td>10</td>
<td>----</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>APEC O2</td>
<td>64</td>
<td>48</td>
<td>&lt; .001</td>
<td>----</td>
</tr>
<tr>
<td>NC/pAPEC-O2</td>
<td>66</td>
<td>41</td>
<td>&lt; .001</td>
<td>&gt; .10</td>
</tr>
</tbody>
</table>

<sup>a</sup> As calculated using a two-sample test of proportions (47)
Table 3. Growth in Human Urine

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth Rate Mean(^a) (Standard Error)</th>
<th>Growth Rate Class(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.507 (0.012)</td>
<td>A</td>
</tr>
<tr>
<td>NC/pAPEC-O2</td>
<td>0.547 (0.007)</td>
<td>B</td>
</tr>
<tr>
<td>APEC O2</td>
<td>0.633 (0.012)</td>
<td>C</td>
</tr>
<tr>
<td>HE300</td>
<td>0.623 (0.012)</td>
<td>C</td>
</tr>
</tbody>
</table>

\(^a\) Growth rate mean was calculated in log\(_{10}\) CFUs per hour

\(^b\) Growth rates with the same letter are not significantly different, whereas growth rates with a different letter are significantly different (P < .05), calculated using a one-way ANOVA (Fisher's Least Significant Difference)
Table 4. Comparative Urovirulence of NC and NC/pAPEC-O2 in Mice

<table>
<thead>
<tr>
<th>Site Cultured</th>
<th>Total No.</th>
<th>No.(%) with NC/pAPEC-O2 &lt; NC</th>
<th>No.(%) with NC/pAPEC-O2 &gt; NC</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>8</td>
<td>2 (25)</td>
<td>6 (75)</td>
<td>&gt; .10</td>
</tr>
<tr>
<td>Bladder</td>
<td>15</td>
<td>3 (20)</td>
<td>12 (80)</td>
<td>.035</td>
</tr>
<tr>
<td>Kidney</td>
<td>18</td>
<td>17 (94)</td>
<td>1 (6)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

* Cultures were unavailable for seven urinary and twelve kidney samples.

* P value by McNemar’s test.
Growth in urine of APEC O2 (the source of the plasmids of interest); recipient *E. coli* strain, NC; the transconjugant, NC/pAPEC-O2; and UPEC HE300. Growth curves were determined by measuring viable counts (CFU ml$^{-1}$) and reflect an average of three trials for each strain.
Figure 2.

Growth in LB broth of APEC O2 (the source of the plasmids of interest); recipient strain, NC; the transconjugant, NC/pAPEC-O2; and (human pyelonephritis isolate) UPEC HE300. Growth curves were determined by measuring viable counts (CFU ml$^{-1}$) and are representative of three trials for each strain.