Avian pathogenic *Escherichia coli* are similar to neonatal meningitis *E. coli* and are able to cause meningitis in the rat model of human disease.

Running head: APEC as a causative agent of neonatal meningitis

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Abstract

Escherichia coli causing avian colibacillosis and human neonatal meningitis, urinary tract infections and septicemia are collectively known as extraintestinal pathogenic E. coli (ExPEC). Characterization of ExPEC using various typing techniques has shown that they harbor many similarities despite their isolation from different host species leading to the hypothesis that ExPEC may have zoonotic potential. This study examined a subset of ExPEC: neonatal meningitis E. coli (NMEC) and avian pathogenic E. coli (APEC) belonging to the O18 serogroup, and found that they were not easily differentiated based on multilocus sequence typing, phylogenetic typing, or carriage of large virulence plasmids. Among the APEC examined, one strain was found to be an outlier, based on these typing methods, and demonstrated reduced virulence in murine and avian pathogenicity models. Some of the APEC strains tested in a rat model of human neonatal meningitis were able to cause meningitis, demonstrating APEC’s ability to cause disease in mammals, lending support to the hypothesis that APEC have zoonotic potential. In addition, some NMEC were able to cause avian colisepticemia, providing further support to this hypothesis. However, not all of the NMEC and APEC tested were able to cause disease in avian and murine hosts, despite their apparent similarities in known virulence attributes. Thus, it appears that a subset of NMEC and APEC harbor zoonotic potential, while
others do not, suggesting that unknown mechanisms underlie host specificity in some ExPEC.

Introduction

*Escherichia coli* causing extraintestinal disease are known as extraintestinal pathogenic *E. coli* (ExPEC) and include uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC) and avian pathogenic *E. coli* (APEC) subpathotypes. Recent studies have shown members of various ExPEC subpathotypes harbor similar virulence-associated genes despite their isolation from varied hosts and tissues (3, 8, 10, 20, 25, 27, 30, 32), and genomic sequencing of APEC O1 revealed that only 4.5% of the genome was not found in other sequenced ExPEC (17). More recently, a cluster of isolates from human and avian hosts, thought to represent potential zoonotic pathogens, has been identified (20).

Common among the isolates of this mixed cluster are genes associated with the conserved region of large virulence plasmids, which are a defining trait of the APEC subpathotype (15, 19, 24, 36, 37), and are essential for APEC virulence (5, 23). Interestingly, a closely related plasmid that was associated with high-level bacteremia in a neonatal rat meningitis model has also been described in an NMEC isolate (30).

Other virulence traits are also shared among ExPEC subpathotypes. Indeed, few traits, if any, appear to be exclusive to a particular ExPEC subpathotype, and in
fact, some traits that were thought to be exclusive have been shown to contribute to the pathogenesis of more than one condition (8).

Such similarities in the virulence traits found amongst APEC and other ExPEC subpathotypes have led to speculation that APEC has zoonotic potential (20, 25, 27) and may be a food-borne source of ExPEC causing disease in humans (10, 14, 18, 22). Indeed, ExPEC have been identified in retail foods and poultry products (7, 11, 12, 18), and at least one study has found avian isolates to be indistinguishable from human isolates (10). However, other studies showed that human ExPEC were clearly distinct from avian strains (6) and that consumption or contact with poultry did not correlate with the colonization of antimicrobial-resistant *E. coli* (34).

Here, we seek to further test the hypothesis that APEC have zoonotic potential. Of particular interest are O18 strains, which are common in human NMEC but are also found amongst APEC (20, 26). In fact, it has been suggested that APEC O18:K1:H7 strains are potential human pathogens (27). Though it has been shown that human ExPEC can cause avian colibacillosis similar to that caused by APEC, suggesting that these ExPEC are not host specific (26), it has also been reported that *E. coli* from avian septicemia are more virulent to chicks than NMEC (33). However, the ability of APEC to cause disease in mammals has not yet been established.
The aim of this study was to explore the zoonotic potential of NMEC and APEC O18 strains by comparing their plasmid content, genotypes, phylogenetic group assignments, pulsed field gel electrophoresis (PFGE) patterns, sequence types (ST), as determined by multilocus sequence typing (MLST), and abilities to cause disease in the rat model of human neonatal meningitis and chicken models of avian colisepticemia.

Materials and Methods

Bacterial Strains. The *E. coli* strains used in this study were those of serogroup O18 obtained from a previously described collection of APEC and NMEC isolates (20), and are shown along with the control strains in Table 1.

Amplification of virulence plasmid-associated genes. PCR amplification was performed to determine the presence or absence of a selection of APEC virulence plasmid-associated genes as previously described (32).

Phylogenetic typing. Strains were assigned to phylogenetic groups according to the PCR amplification method described by Clermont *et al.* (2).

MLST. MLST was performed following the protocol for *E. coli* described by Wirth *et al.*, (39) on the MLST website (http://mlst.ucc.ie), with the exception of the primers for *mdh* which were replaced with those as described elsewhere (28).
addition, the annealing conditions for the *fumC* and *purA* reactions were altered
to 60 °C and 58 °C for 30 s respectively.

**PFGE.** PFGE was performed as previously described (31). *Salmonella enterica*
serotype Braenderup H9812 (ATCC #BAA-664) was used as the molecular
weight size standard. Restriction endonuclease digestion was carried out using
25 U *XbaI* (Invitrogen) in a final volume of 100 µl at 37°C for 3 h. DNA
macrorestriction fragments were resolved over 18 h on 1% SeaKem Gold
Agarose using the Chef Mapper XA system (Bio-Rad) auto algorithm function for
low molecular weight of 30 kb and high molecular weight of 600 kb. Gels were
stained in 1 µg ethidium bromide ml\(^{-1}\) reagent grade water for 30 min, and DNA
was visualized by UV transillumination.

Macrorestriction patterns were compared using the BioNumerics Fingerprinting II
Informatix software (Version 3.0, Bio-Rad). The similarity index of the isolates
was calculated using the Dice correlation coefficient option of the software with a
position tolerance of 1% and an optimization of 0.5%. The unweighted-pair group
method using average linkages (UPGMA) was used to construct the
dendrogram.

**Preparation of plasmid DNA.** Plasmid DNA was prepared using the Qiagen
Plasmid Mini Kit as recommended by the manufacturer. Plasmids were
separated by PFGE.
**PFGE for large plasmid separation.** PFGE was carried out using a CHEF Mapper XA System (Bio-Rad). Plasmids were separated in 1.0% (w/v) DNA grade agarose (SeaKem) as previously described (36). Plasmid DNA of known sizes from *E. coli* strains APEC O1 (17), APEC O2 (19) and χ7122 (23, 24) were used as molecular weight standards. Gels were stained in 1.2 µg ethidium bromide ml⁻¹ distilled water for 1 h, and DNA was visualized by UV transillumination.

**Pathogenicity testing.** All animal experiments were approved by the Iowa State University Institutional Animal Care and Use Committee and were performed in accordance with institutional guidelines.

**Rat neonatal meningitis model.** Groups of approximately 12 specific-pathogen-free (SPF), 5-day-old Sprague-Dawley rat pups were given approximately 200 (48-312) CFU of *E. coli* by the intraperitoneal (IP) route (8, 21). Pups were euthanized after 24 hours using Sleepaway (Fort Dodge). For bacterial enumeration, blood was collected from the jugular vein and plated on MacConkey agar to indicate septicemia, and cerebrospinal fluid (CSF), collected by cisternal puncture, was plated on MacConkey agar to indicate meningitis. The cerebrums were removed from the rats, fixed in 10% neutral buffered formalin, routinely processed for histopathology, stained with hemotoxylin and eosin, and examined for lesions consistent with bacterial meningitis.
**Chick embryo lethality assay (ELA).** Strains were assessed for lethality in chicken embryos by inoculation of overnight phosphate-buffered saline (PBS) washed cultures (~500 CFU) into the allantoic cavities of 12-day-old, embryonated, SPF eggs (29). PBS inoculated and uninoculated embryos were used as controls. Eggs were candled daily, and embryo deaths were recorded for a period of four days.

**Chick colisepticemia model.** Groups of 12 one-day-old Leghorn chicks were inoculated with approximately $10^7$ CFU by the intra-tracheal (IT) route. Birds were observed for 7 days, and mortalities recorded. The 8-day-old birds were euthanized by CO$_2$ inhalation and examined for airsacculitis, pericarditis, perihepatitis and peritonitis. The heart, brain and air sacs were swabbed and cultured on MacConkey agar at 37 °C overnight.

**Results**

**Isolation of plasmids from strains.** The method used here detects and predicts the size of plasmids greater than 50 kb with confidence and large plasmids ranging in size from 90 kb to 149 kb were identified in all strains except NMEC 4 (Figure 1). Multiple large plasmids were isolated from some strains, and the number of large plasmids isolated from each strain and their predicted sizes is indicated in Table 1. The relative size of the largest plasmid in all APEC strains
appeared to be identical (~133 kb), while the size of the plasmids in the NMEC strains varied.

**Amplification of virulence plasmid-associated genes.** The presence of APEC virulence plasmid-associated genes is shown in Table 1. APEC control strains carried all of the genes, while only the RepFIB replicon, *sitA* and *iroN* were amplified from the NMEC control strains, RS218 and C5. The majority of the genes were amplified from the O18 strains of interest in this study with the exception of NMEC 4, which carried only *iutA*, *sitA* and *iroN*.

**Phylogenetic typing.** All of the O18 strains examined belonged to the B2 phylogenetic group except for APEC 79, which was assigned to phylogenetic group A (Table 1).

**MLST.** MLST revealed that all of the O18 strains investigated belonged to the ST95 clonal complex, except APEC 79, which belonged to the ST23 clonal complex. All APEC O18 strains, except APEC 79 (ST23), were found to have identical allelic profiles (ST95), and while some variation occurred amongst the NMEC strains (NMEC 4 and NMEC 18:ST390; NMEC 58:ST416) most were ST95 (Table 1).

**PFGE.** Two major clusters were observed at approximately 52% identity after PFGE of XbaI digested genomic DNA (Figure 2). The seven NMEC strains including RS218 and C5 clustered together, while most of the APEC were found
in a separate cluster along with the lab adapted *E. coli* DH5α. However the
‘NMEC’ cluster also included the ELA positive control strain, APEC O2.
Additionally, two outliers were observed in this analysis: APEC 79 and χ7122.

Pathogenicity testing.

Rat neonatal meningitis model. Pathogenicity was based on the mortality rates, reisolation rates and bacterial enumeration observed for each strain (Table 2). No mortalities were observed for the rat pups inoculated with APEC 79, χ7122, or the negative controls, PBS and *E. coli* DH5α. The low number of mortalities observed in groups inoculated with APEC 353, APEC 370, NMEC 4 and the archetypal NMEC strain RS218 were not significantly different from the groups where no mortalities were observed. Inoculation with another well-studied NMEC strain, C5, resulted in 92% mortality, and mortalities observed in groups inoculated with APEC 380, NMEC 18, NMEC 38 and NMEC 58 were not significantly different from C5.

*E. coli* was not reisolated from the blood or CSF of rat pups inoculated with PBS or DH5α; however, it was reisolated from both the blood and CSF of all remaining rat pups in the groups inoculated with *E. coli* except for those in the groups inoculated with APEC 79, χ7122 and RS218.

The number of *E. coli* recovered from the blood and CSF of the rat pups was beyond the countable limits (4 × 10^3 CFU ml⁻¹ for an entire 50 µl blood sample,
1 × 10^4 CFU ml⁻¹ for an entire 20 µl CSF sample) for all groups except APEC 79 and χ7122. Histopathology of selected cerebrums showed bacteria in the meninges of rats infected with APEC 353 and NMEC 58, as well as low numbers of neutrophils in the meninges and outer cellular layer in the cerebrum (Figure 3). No lesions were observed in the cerebrum of rats infected with negative control strain DH5α.

Chick embryo lethality assay. The number of mortalities observed for uninoculated, PBS inoculated and APEC 79 inoculated eggs did not differ significantly from the eggs inoculated with the negative control, DH5α, while the number of mortalities observed for all the O18 strains, except APEC 79 and NMEC18, were not significantly different from the positive control strain APEC O2 (Table 3).

Chick colisepticemia model. The pathogenicity of the strains following IT inoculation was determined based on mortalities observed due to E. coli colonization, reisolation of the inoculated E. coli strain from the birds and the lesion scores attributed to the birds on post-mortem examination (Table 4). NMEC 58 was the only strain tested that did not cause mortality, while inoculation with the other three strains (APEC 353, APEC 380 and NMEC 15) resulted in a similar number of mortalities as that seen with the positive control APEC χ7122. The reisolation rate of NMEC 58 was comparable to that seen in
negative control birds that were inoculated with sterile PBS, while £7122, APEC 353, APEC 380 and NMEC 15 had significantly higher reisolation rates (P≤0.05) than the group inoculated with PBS. E. coli reisolated from the pericardial sac swab of two PBS inoculated birds lacked most of the virulence plasmid-associated genes, suggesting they were commensal E. coli contaminants.

NMEC 58 was the only strain tested that did not result in a significantly higher median lesion score than PBS, and NMEC 15 was the only strain that resulted in a significantly higher median lesion score than NMEC 58. The median lesion scores for all the O18 strains were not significantly different from that of the positive control strain APEC £7122.

While the mortality rates of each test strain appeared to be relatively consistent between different pathogenicity models (Figure 4), two APEC strains, APEC 79 (P=0.032) and APEC 353 (P=0.028) produced significantly lower mortality in neonatal rats than in chick embryos. Conversely, NMEC 58 produced significantly more mortalities (P<0.001) in both neonatal rats and chick embryos than in day-old chicks.

Discussion

Although it has previously been shown that strains isolated from cases of neonatal meningitis are similar to APEC (3, 20, 25, 27, 30) and were able to cause disease in models of avian colisepticemia (26), this is the first study to
demonstrate that strains isolated from cases of avian colibacillosis are able to cause disease in a rat model of human neonatal meningitis. These findings are supportive of the hypothesis that APEC may have zoonotic potential, and along with previous reports of APEC being isolated from retail poultry meat (12, 18), reflect a potential for APEC to act as a food-borne source of ExPEC causing disease in humans.

The presence of large plasmids, as seen in Figure 1, in conjunction with PCR amplification of the RepFIB replicon and plasmid-associated virulence genes, suggests that all test strains carry a variant of an APEC virulence plasmid with the exception of NMEC 4. In this strain, the genes iutA, sitA and iroN, may be located on the chromosome, which has previously been reported in both APEC and NMEC (15, 30, 36). However, while the other NMEC studied here, as well as NMEC strain S88 and many others (30), carry most of the genes present in the conserved region of the APEC virulence plasmid, the NMEC controls, RS218 and C5, do not, suggesting that they may be atypical NMEC.

The absence of plasmids carrying the conserved virulence region in archetypal NMEC strains RS218 and C5 might suggest that this region is not as important in the manifestation of neonatal meningitis as it is in avian colisepticemia. However, NMEC plasmid pS88, carrying the conserved virulence region, has been shown to be associated with high-level bacteremia in a neonatal rat model (30), and inoculation of neonatal rats with NMEC 4 and RS218, both of which lack the
typical APEC virulence plasmid, resulted in mortalities not significantly different to those of the avirulent controls. It has been shown that APEC plasmids contribute to the ability of *E. coli* to grow in human urine, cause urinary tract infections in mice (35), and meningitis in rats (16), suggesting that these plasmids contribute to survival within extraintestinal compartments. Certainly, further examination of the contribution of these plasmids to the pathogenesis of meningitis is warranted.

However, it is clear that ExPEC’s ability to cause meningitis cannot be completely attributed to virulence plasmids, as APEC 79, despite its virulence plasmid, was not lethal to rats, and C5, without a virulence plasmid, was lethal to rats. Indeed, it has previously been suggested that *E. coli* cause meningitis using different mechanisms (40) and that ‘so-called’ archetypal meningitis strains appear to be only partially representative of the pathogenic mechanisms harbored by NMEC isolates (1).

Of the O18 ExPEC examined, APEC 79 was the only one not belonging to the B2 phylogenetic group or the ST95 clonal complex. In addition, it was an outlier in the dendrogram constructed from PFGE profiles and appears to be unrelated to the remaining O18 strains and more closely related to the O78 APEC strain χ7122 (Phylogenetic group A and ST23). The atypical nature of APEC 79 was reflected in its lack of virulence in chick embryos and in rats, supporting a previous claim that B2 strains have enhanced virulence (9). Based on its unusual attributes, it is not surprising that APEC 79 was not found among the cluster of
isolates that were thought to represent potential zoonotic pathogens (20), while the other APEC O18 strains examined were.

APEC 79 was frequently depicted as an outlier suggesting the molecular based typing methods undertaken in this study discriminate similarly. While phylogenetic typing and MLST were unable to discriminate the human ExPEC strains from avian strains, they essentially clustered by host origin after cluster analysis of PFGE data, suggesting that in the case of ExPEC that PFGE has more discriminatory power than other typing methods, as seen previously for Salmonella (4). Although this group of APEC appears more related to each other than NMEC and vice versa, there was also a strong correlation between PFGE relatedness and their geographic site of isolation, making it difficult to determine which factor has more influence on PFGE relatedness.

Both APEC 79 and χ7122 appeared to be of low virulence in the neonatal meningitis model, causing no mortalities and resulting in a limited number of bacteria in the blood and CSF. Interestingly, both strains differed from the other ExPEC in this study in assignment to phylogenetic group and MLST (A and ST23, respectively), indicating they may be APEC-specific pathogens.

A one-way ANOVA using the Kruskal-Wallis test showed there was some significant differences in blood and CSF counts between groups (<0.0001); however, in some groups, where few pups survived, the sample size was too
small for Dunn’s multiple comparison post test to describe genuine differences. Still, the number of bacteria reisolated from the blood and CSF of rats inoculated with APEC 353, APEC 358 and APEC 370 was significantly higher than DH5α, suggesting these APEC, despite their lower mortality rates, were all able to cause septicemia and meningitis in neonatal rats. Indeed, histopathology performed on the cerebrums of selected rats, post inoculation with NMEC and APEC, showed lesions typical of meningitis and the presence of bacteria in the meninges. There appeared to be a high number of bacteria present in the cerebrums that did not elicit a strong immune response, which may be due to the short time period that had elapsed since inoculation. This, in addition to the high mortality rates observed with these isolates, suggest that they are highly virulent.

While the number of bacteria present in the blood and CSF were too numerous to count following inoculation of rats with APEC 353, APEC 370, NMEC 4 and RS218, the associated mortalities were low, suggesting that these isolates were of intermediate virulence. It has previously been suggested that RS218 has decreased in virulence over time (26) which could account for its lower virulence here. Intriguingly, RS218 appears to lack the virulence plasmid that other O18 strains contain, the loss of which might account for RS218’s atypical nature and relatively low virulence.

APEC 358 and NMEC 15 were significantly more virulent (P≤0.05) for neonatal rats than the groups with no mortalities, yet significantly less virulent than strain
C5. APEC 380 was the only APEC that appeared to be as virulent as the NMEC reference strain C5, while three other NMEC strains, NMEC 18, NMEC 38 and NMEC 58, were also highly virulent in the rat model.

While the number of mortalities caused by APEC 79 could not be differentiated from the negative controls in the ELA, mortalities caused by the rest of the strains were not significantly different from the positive control, APEC O2, except for those caused by NMEC 18. NMEC 18 was significantly different from both the negative and positive control, and thus, it could be considered to be of intermediate virulence according to this assay.

NMEC 58 was shown to be virulent in both the ELA and the rat neonatal meningitis model, but it appeared to be of low virulence in the chick colisepticemia pathogenicity assay. IT inoculation with NMEC 58 resulted in no mortalities and colonization of only three birds. These results showed that NMEC 58 was less capable of establishing an infection in day-old chicks, while the other strains (APEC 353, APEC 380 and NMEC 15) established infections as or more severe than those seen after inoculation with χ7122. Consistent with a previous study describing a ‘host-specific’ pathotype (25), this study appears to have identified a host-specific ExPEC strain of human origin. In contrast, another study by Moulin-Schouleur et al., was unable to find evidence of host specificity within the highly pathogenic subcluster B2-1 (26). Since NMEC 58 appears to possess the full complement of known APEC virulence plasmid-associated genes...
(Table 1), it does not appear that the virulence plasmid alone is responsible for a ‘broadening’ of an ExPEC’s host range from mammals to avian species or vice versa. Identifying the factors that are responsible for host specificity or the lack thereof will enhance our understanding of ExPEC pathogenesis and lead to improved efforts to control ExPEC diseases.

The results of this study suggest that ExPEC may have a core genome which is essential for their survival in extraintestinal environments and that individual strains possess additional genetic information that has been adapted to establish an infection after inoculation by a specific route in a particular host and tissue type. Similarly, another study found strains within the same clonal group did not necessarily have comparable virulence attributes and suggested acquisition of accessory genes may impart distinct niche-specific growth (38). This issue warrants further attention, and with an increase in the number of ExPEC genomes available, comparative genomics may help to identify the core ExPEC genome and distinguish host- or disease-specific regions.

The ExPEC strains described here were not easily differentiated despite being isolated from different hosts and varied disease manifestations, reinforcing previous claims of the zoonotic potential of ExPEC. This zoonotic potential was confirmed with the first report of APEC strains causing meningitis in a rat neonatal meningitis model. Many of the O18 strains examined in this study were shown to be highly virulent in avian and murine models, suggesting that they
were not host specific, and that they were capable of acting as zoonotic pathogens. By contrast, others caused disease in one host species, but not the other, indicating that they were host specific. An understanding of the factors contributing to ExPEC’s host specificity would better clarify this issue and could help in designing future ExPEC disease control strategies.

Acknowledgments

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6:285-95.


acquisition of antimicrobial resistant Escherichia coli causing urinary tract


contributes to virulence of avian pathogenic Escherichia coli. Microbiology 155:450-60.


TABLE 1. Characteristics of *E. coli* strains used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Plasmids (size(s) kb)</th>
<th>Serogroup</th>
<th>Phylogenetic group</th>
<th>MLST</th>
<th>Genes associated with the conserved virulence region of APEC plasmids</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>iutA sitA RepFIB hlyF ompT etsAB iss iroN</td>
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<td>DH5α⁺</td>
<td>-</td>
<td>0</td>
<td>NT</td>
<td>A</td>
<td>ST1060</td>
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<td>APEC 79</td>
<td>California</td>
<td>2 (133,90)</td>
<td>O18</td>
<td>A</td>
<td>ST23</td>
<td>-    +    +    +    +    +    +    +    +    +    +    +    +</td>
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<td>B2</td>
<td>ST95</td>
<td>+    +    +    +    +    +    +    +    +    +    +    +    +</td>
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<td>O18</td>
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<td>ST95</td>
<td>+    +    +    +    +    +    +    +    +    +    +    +    +</td>
</tr>
<tr>
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<td>1 (133)</td>
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<td>B2</td>
<td>ST95</td>
<td>+    +    +    +    +    +    +    +    +    +    +    +    +</td>
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<td>Nebraska</td>
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<td>B2</td>
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<td>B2</td>
<td>ST390</td>
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<td>NMEC RS218&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(40) 1 (123) O18 B2 ST95</td>
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<td>-</td>
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<tr>
<td>APEC χ7122&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(24) 3 (103,90,60) O78 A ST23</td>
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<tr>
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<td>B2</td>
<td>ST135</td>
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<sup>a</sup> Negative control for rat neonatal meningitis model and ELA

<sup>b</sup> Positive control for rat neonatal meningitis model

<sup>c</sup> Positive control for chick colisepticemia model

<sup>d</sup> Positive control for ELA
<table>
<thead>
<tr>
<th>Strain</th>
<th>Mortality Rate</th>
<th>Reisolation rate from blood of survivors</th>
<th>Reisolation rate from CSF of survivors</th>
<th>Inoculum (Log$_{10}$ CFU per animal)</th>
<th>Log$_{10}$ mean CFU ml$^{-1}$ blood</th>
<th>Log$_{10}$ mean CFU ml$^{-1}$ CSF</th>
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<td>PBS</td>
<td>0/12$^a$</td>
<td>0/12</td>
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<td>DH5α</td>
<td>0/12$^a$</td>
<td>0/12</td>
<td>0/12</td>
<td>2.68</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>APEC 79</td>
<td>0/12$^a$</td>
<td>7/12</td>
<td>2/12</td>
<td>3.36</td>
<td>2.25</td>
<td>1.62</td>
</tr>
<tr>
<td>APEC 353</td>
<td>2/12$^{ab}$</td>
<td>10/10</td>
<td>10/10</td>
<td>3.33</td>
<td>&gt;4.13</td>
<td>&gt;4.62</td>
</tr>
<tr>
<td>APEC 358</td>
<td>5/12$^{bcdf}$</td>
<td>7/7</td>
<td>7/7</td>
<td>3.37</td>
<td>&gt;4.51</td>
<td>&gt;4.78</td>
</tr>
<tr>
<td>APEC 370</td>
<td>1/12$^{ab}$</td>
<td>11/11</td>
<td>11/11</td>
<td>3.41</td>
<td>&gt;3.60</td>
<td>&gt;4.69</td>
</tr>
<tr>
<td>APEC 380</td>
<td>9/12$^{cd}$</td>
<td>3/3</td>
<td>3/3</td>
<td>3.39</td>
<td>&gt;3.60</td>
<td>&gt;4.77</td>
</tr>
<tr>
<td>NMEC 4</td>
<td>3/12$^{cd}$</td>
<td>9/9</td>
<td>9/9</td>
<td>3.35</td>
<td>&gt;3.90</td>
<td>&gt;4.58</td>
</tr>
</tbody>
</table>

$^a$ Mortality rate

$^b$ Reisolation rate from blood of survivors

$^c$ Reisolation rate from CSF of survivors

$^d$ Inoculum (Log$_{10}$ CFU per animal)

$^e$ Log$_{10}$ mean CFU ml$^{-1}$ blood

$^f$ Log$_{10}$ mean CFU ml$^{-1}$ CSF

TABLE 2. Pathogenicity of E. coli O18 strains in the neonatal rat meningitis model.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Passage</th>
<th>Mortality</th>
<th>ATP</th>
<th>Growth</th>
<th>Mortality</th>
<th>ATP</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMEC 15</td>
<td>5/12</td>
<td>7/7</td>
<td>7/7</td>
<td>3.42</td>
<td>&gt;3.96</td>
<td>&gt;4.74</td>
<td></td>
</tr>
<tr>
<td>NMEC 18</td>
<td>8/12</td>
<td>4/4</td>
<td>4/4</td>
<td>3.40</td>
<td>&gt;3.78</td>
<td>&gt;4.53</td>
<td></td>
</tr>
<tr>
<td>NMEC 38</td>
<td>9/10</td>
<td>1/1</td>
<td>1/1</td>
<td>3.32</td>
<td>&gt;3.90</td>
<td>&gt;5.00</td>
<td></td>
</tr>
<tr>
<td>NMEC 58</td>
<td>10/11</td>
<td>1/1</td>
<td>1/1</td>
<td>3.49</td>
<td>&gt;3.90</td>
<td>&gt;5.00</td>
<td></td>
</tr>
<tr>
<td>NMEC C5</td>
<td>12/13</td>
<td>1/1</td>
<td>1/1</td>
<td>3.43</td>
<td>&gt;4.30</td>
<td>&gt;4.40</td>
<td></td>
</tr>
<tr>
<td>NMEC RS218</td>
<td>2/13</td>
<td>9/11</td>
<td>8/11</td>
<td>3.18</td>
<td>&gt;3.55</td>
<td>&gt;4.09</td>
<td></td>
</tr>
<tr>
<td>APEC χ7122</td>
<td>0/13</td>
<td>12/13</td>
<td>4/13</td>
<td>3.14</td>
<td>3.02</td>
<td>3.73</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) In the mortality rate column, values with the same superscript lowercase letter(s) are not significantly different \((P≥0.05)\) by Fisher’s exact test.

\(^b\) Positive control strains for the neonatal rat meningitis model
TABLE 3. Mortality rates of chick embryos inoculated with APEC and NMEC isolates.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mortality Rate</th>
<th>P value vs DH5αa</th>
<th>P value vs APEC O2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>0/6</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PBS</td>
<td>3/10</td>
<td>0.300</td>
<td>0.001</td>
</tr>
<tr>
<td><em>E. coli</em> DH5α</td>
<td>2/20</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>APEC 79</td>
<td>7/20</td>
<td>0.127</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APEC 353</td>
<td>12/20</td>
<td>0.002</td>
<td>0.064</td>
</tr>
<tr>
<td>APEC 358</td>
<td>16/20</td>
<td>&lt;0.001</td>
<td>0.661</td>
</tr>
<tr>
<td>APEC 380</td>
<td>16/20</td>
<td>&lt;0.001</td>
<td>0.661</td>
</tr>
<tr>
<td>NMEC 15</td>
<td>14/20</td>
<td>&lt;0.001</td>
<td>0.235</td>
</tr>
<tr>
<td>NMEC 18</td>
<td>11/20</td>
<td>0.005</td>
<td>0.031</td>
</tr>
<tr>
<td>NMEC 38</td>
<td>19/20</td>
<td>&lt;0.001</td>
<td>1.000</td>
</tr>
<tr>
<td>NMEC 58</td>
<td>14/20</td>
<td>&lt;0.001</td>
<td>0.235</td>
</tr>
<tr>
<td>APEC O2b</td>
<td>32/40</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

a P value as determined by Fishers exact test
b Positive control for the ELA
TABLE 4. Pathogenicity of APEC and NMEC isolates in 1-day-old chicks inoculated by the intra-tracheal route.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mortality rate</th>
<th>Reisolation rate</th>
<th>Lesion score median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0-1)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>APEC 353</td>
<td>6/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5 (0-8)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>APEC 380</td>
<td>6/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 (0-8)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>NMEC 15</td>
<td>7/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 (1-8)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NMEC 58</td>
<td>0/12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/12&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0 (0-6)&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>APEC&lt;sub&gt;x7122&lt;/sub&gt;</td>
<td>6/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8/12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.5 (0-8)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In each column, values with the same superscript lowercase letter(s) are not significantly different (P≥0.05 by Kruskal-Wallis or Fisher’s exact test).
Figure 1. Plasmids purified from the test and control strains. Previously sequenced plasmids present in APEC O1, APEC O2 and APEC χ7122 were used as size standards. All strains except NMEC 4 contained at least one large plasmid which correlated 100% with the presence of the RepFIB amplicon. Note that a similar sized plasmid (~133 kb) is carried by all APEC O18 isolates.

Figure 2. PFGE of XbaI digested DNA from the O18 strains. Strain designation, phylogenetic group, MLST assignation and virulence genes associated with the conserved virulence region of APEC plasmids are shown at right. This unweighted pair-group method with arithmetic mean dendrogram was generated in BioNumerics software by using Dice coefficient with a 1.0% band position tolerance. The scale above the dendrogram indicates percent similarity.

Figure 3. (A) Histopathology of rat brain tissue 24 hours post inoculation with APEC 353 showed rod shaped bacteria (indicated by an arrow) in the meninges. (B) Histopathology of rat brain tissue 24 hours post inoculation with NMEC 58 with rod shaped bacteria in the meninges (indicated by arrows).

Figure 4. Column graph illustrating the percent mortality observed for each strain when examined using the murine and avian pathogenicity models. For each strain, the same lowercase letter on top of the column indicates the mortality was not significantly different (P≥0.05 by Fisher’s exact test) between pathogenicity
models. Note that not every strain was examined in all of the three pathogenicity models (marked by •).
Percent mortality

- Murine neonatal meningitis model
- Embryo lethality assay
- Chick colisepticemia model
- Was not done