Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny

J.A. Skyberg[1]*, K.E. Siek[1], C. Doetkott[2] and L.K. Nolan[1]

1 Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
2 Information Technology Services, North Dakota State University, Fargo, ND, USA

Keywords
avian pathogenic *Escherichia coli*, biofilm, *Escherichia coli*, phylogenetic typing, phylotype, poultry.

Correspondence
Lisa K. Nolan, Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA. E-mail: lknolan@iastate.edu

Present address
Jerod A. Skyberg, Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59718, USA.

*Done in partial fulfilment of the requirements for the PhD degree in Molecular Pathogenesis at North Dakota State University.

2006/0104: received 25 January 2006, revised 30 April 2006 and accepted 2 May 2006

doi:10.1111/j.1365-2672.2006.03076.x

Abstract

Aims: To assess the abilities of 105 avian pathogenic *Escherichia coli* (APEC) and 103 avian faecal commensal *E. coli* (AFEC) to form biofilms on a plastic surface and to investigate the possible association of biofilm formation with the phylotype of these isolates.

Methods and Results: Biofilm production was assessed in 96-well microtitre plates using three different media, namely, M63 minimal medium supplemented with glucose and casamino acids, brain–heart infusion broth, and diluted tryptic soy broth. Avian *E. coli* are highly variable in their ability to form biofilms. In fact, no strain produced a strong biofilm in all three types of media; however, most (75·7% AFEC and 55·2% APEC) were able to form a moderate or strong biofilm in at least one medium. Biofilm formation in APEC seems to be mostly limited to nutrient deplete media; whereas, AFEC are able to form biofilms in both nutrient deplete and replete media. Also, biofilm formation in *E. coli* from phylogenetic groups B2, D and B1 was induced by nutrient deplete conditions; whereas, biofilm formation by members of phylogenetic group A was strongest in a rich medium.

Conclusions: Biofilm formation by APEC and phylotypes B2, D and B1 is induced by nutrient deplete conditions, while AFEC are able to form biofilms in both nutrient rich and deplete media.

Significance and Impact of the Study: This is the first study to investigate biofilm formation by a large sample of avian *E. coli* isolates, and it provides insight into the conditions that induce biofilm formation in relation to the source (APEC or AFEC) and phylogenetic group (A, B1, B2 and D) of an isolate.

A biofilm is composed of surface-bound or -sessile microbes enclosed in an amorphous extracellular matrix (Donlan and Costerton 2002), often composed of exopolysaccharide, proteins and nucleic acids (Davey and O'Toole 2000). Residence in a biofilm community offers certain advantages to bacteria, one of which is the ability to acquire transmissible genetic elements, such as plasmids, at elevated rates (Davey and O'Toole 2000). This is of particular interest to the poultry industry as the virulence and antimicrobial resistance of avian pathogenic *Escherichia coli* (APEC), the aetiologic agent of avian colibacillosis (de Brito *et al.* 2003), may be largely mediated by conjugative plasmids (Dozois *et al.* 2000; Ginns *et al.* 2000; Rodriguez-Siek *et al.* 2005a). Plasmid-containing avian *E. coli* may also be transmitted to human beings, where they may cause extraintestinal infections or serve as sources of virulence or resistance genes for human pathogens (Levy *et al.* 1976; Caudry and Stanisich 1979). Therefore, residence in a biofilm could enhance avian *E. coli*'s ability to acquire plasmids, enabling it to better cause disease and resist therapy to the detriment of animal and public health.

Besides being better able to acquire transmissible genetic elements, bacteria living in biofilms also exhibit

enhanced resistance to cleansing and sanitation (Davey and O'Toole 2000). This characteristic is especially significant in poultry production and processing, where plastic surfaces on which biofilms develop, are used (Lindsay *et al.* 1996; Sainsbury 2000). For instance, in the poultry production environment, water systems are extensive and may be factors in transmission of many important avian pathogens, including *E. coli* (do Amaral 2004). In addition, plastic materials are also widely used in the food industry for the construction of tanks, pipework and cutting surfaces (Pompermayer and Gaylarde 2000).

It is likely that the poultry production and processing environments in which biofilms grow vary greatly in the availability of nutrients to support bacterial growth (Djordjevic *et al.* 2002). Therefore, the aims of this study were to determine the ability of avian *E. coli* to form biofilms on plastic surfaces in three different types of media. M63 supplemented with glucose and casamino acids was chosen, as it is a standard medium used to promote *E. coli* biofilm formation (O'Toole *et al.* 1999). Additionally, brain–heart infusion broth (BHI) and diluted tryptic soy broth (TSB) were chosen as nutrient replete and nutrient deplete media respectively.

In addition, we compared the abilities of APEC and avian faecal commensal *E. coli* (AFEC) of apparently healthy birds to form biofilms in order to determine if there is a relationship between biofilm formation and avian *E. coli* virulence. We also sought links between the ability to form biofilms and the phylotype of the strains, as phylogenetic typing has proved useful in predicting the pathogenic potential of extraintestinal *E. coli* (Picard *et al.* 1999).

Materials and methods

Bacterial strains

One hundred and five *E. coli* isolates incriminated in avian colibacillosis (APEC) and 103 *E. coli* isolates from the faeces of apparently healthy birds (AFEC) were used in this study. The APEC isolates were collected from various diagnostic laboratories throughout the USA and originated from different avian hosts, primarily chickens and turkeys, and sites within these hosts, including conjunctiva, sinus, trachea, air sacs, pericardium, spleen, liver, joint, ovary and yolk stalk. All isolates were stored at −80°C in BHI broth (Difco Laboratories, Detroit, MI, USA) with 10% glycerol until use (Sanderson and Zeigler 1991).

Quantification of biofilm formation

Biofilm formation was quantified in 96-well polystyrene microtitre plates (Falcon Microtest 353072; Becton Dickinson, Franklin Lakes, NJ, USA), based on previously described methods (O'Toole *et al.* 1999; Stepanovic *et al.* 2004). Overnight Luria–Bertani broth cultures of the strain to be tested were diluted 1 : 100 in either BHI (Difco), diluted (1/20) TSB (Difco) or M63 minimal media [12 g of KH_2PO_4 per litre, 28 g of K_2HPO_4 per litre, 8 g of $(NH_4)_2SO_4$ per litre supplemented with 1 mmol l^{-1} of $MgSO_4$, 0·2% glucose and 0·5% Casamino Acids] (Sturgill *et al.* 2004). A volume of 200-μl aliquots of each dilution were then dispensed into a microtitre plate well. Negative control wells contained uninoculated medium, and each strain was tested in triplicate. Plates were inoculated aerobically without shaking at 37°C for 24 h. The contents of the plates were then poured off, and the plates were washed with sterile double distilled water (ddH_2O). Microplates were then stained with 200 μl of 0·1% Crystal Violet for 30 min, washed four times with ddH_2O to remove excess stain, and air dried for 1 h. After drying, adherent cells were resolubilized with 200 μl of an 80 : 20 solution of ethanol and acetone. A volume of 150 μl of this solution was then transferred to a new microtitre plate, and the optical density (OD) of each well was measured at 600 nm (OD_{600} using an automated ELx808 Ultra MicroPlate Reader (Bio-Tek Instruments, Winooski, VT, USA). All tests were carried out in triplicate, and the results were averaged.

Based on the OD produced by bacterial biofilms, strains were classified into the following categories: non-biofilm producer, weak, moderate or strong, based on a previously described method (Stepanovic *et al.* 2004). Briefly, the cutoff OD (ODc) was defined as three standard deviations above the mean OD of the negative control. Strains were classified as follows: $OD < ODc$ = no biofilm production; $ODc < OD < (2 \times ODc)$ = weak biofilm producer $(2 \times ODc) < OD < (4 \times ODc)$ = moderate biofilm producer; and $(4 \times ODc) < OD$ = strong biofilm producer.

Phylogenetic typing

Isolates were assigned to phylogenetic groups according to the method of Clermont *et al.* (2000). The results of the APEC phylogenetic grouping were reported previously (Rodriguez-Siek *et al.* 2005b). Using this method, isolates are assigned to one of four groups (A, B1, B2 or D), based on their possession of two genes (*chuA* and *yjaA*) and a DNA fragment (TSPE4.C2), as determined by PCR. The following primer pairs were used: ChuA.1 (5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.2 (5′-TGCCGCCAGTACCAAAGACA-3′), YjaA.1 (5′-TGAAGTGTCAGGAGACGCT-3′) and YjaA.2 (5′-ATGGAGAATGCGTTCCTCAAC-3′) and TspE4C2.1 (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.2 (5′-CGCGCCAACAAAGTATTACG-3′), which generate 279-, 211- and 152-bp

fragments respectively. Amplification was performed in a 25-μl reaction mixtures, which included 18·3 μl of ddH_2O, 2·5 μl of 10X PCR buffer (Invitrogen, Carlsbad, CA, USA), 1·0 μl of 50 mmol l^{-1} $MgCl_2$, 0·5 μl of a 2·5 mmol l^{-1} dNTP mixture (USB, Cleveland, OH, USA), 0·075 μl of 0·1 mmol l^{-1} upper and lower primers (Integrated DNA Technologies, Coralville, IA, USA) (Table 2), 0·25 μl of (5 U/μl) *Taq* DNA polymerase (Invitrogen) and 2·0 μl of template DNA. Reaction mixtures were subjected to the following parameters in a Mastercycler Gradient thermocycler (Brinkmann Eppendorf, Westbury, NY, USA): 4 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 59°C and a final extension step of 5 min at 72°C, followed by a hold at 4°C.

Samples were subjected to horizontal gel electrophoresis in 1·5% (w/v) agarose, and the size of the amplicons was determined by comparison to the Hi-Lo DNA marker from Minnesota Molecular Inc. (Minneapolis, MN, USA). Strains known to possess or lack the genes of interest were examined with each amplification procedure. An isolate was considered to contain a gene of interest if it produced an amplicon of the expected size. Isolates were assigned to phylogenetic group 'A' if they exhibited the profile: (i) *chuA* (−), *yjaA* (+/−), and TSPE4.C2 (−); (ii) 'B1' by the profile of *chuA* (−), *yjaA* (+/−) and TSPE4.C2 (+); (iii) 'B2' by the genotype of *chuA* (+), *yjaA* (+) and TSPE4.C2 (+/−); or (iv) 'D' if they were *chuA* (+), *yjaA* (−) and TSPE4.C2 (+/−).

Biostatistics

The null hypothesis that the relative proportions of each of the four phylogenetic groups was equal across the APEC and AFEC isolates was tested with the chi-squared test of homogeneity (Dowdy *et al.* 2004). In addition, a three-way factorial analysis of variance (ANOVA) was conducted to determine the impacts of source, media and phylogenetic group on the ability of isolates to form a biofilm. Effects were deemed significant if the associated *P*-values were <0·05 (Dowdy *et al.* 2004). Due to the presence of significant interactions among independent variables, follow-up linear contrasts were specified to test effects of interest. A Bonferroni approach was used to adjust for multiple testing due to testing a variety of effects simultaneously (Dowdy *et al.* 2004). This involved dividing our familywise error of 0·05 by the number of tests run to get a pairwise error rate. Effects with *P*-values less than the new pairwise error were identified as significant.

Results

Most isolates (75·7% AFEC and 55·2% APEC) were able to form a moderate or strong biofilm (as defined in Materials and methods) in at least one type of medium. ANOVA suggests that the ability of isolates to form biofilms is related to source, media type and phylogenetic type (Table 1). Although the three-factor interaction is not significant, all of the two-factor interactions are significant using a Type I error of 0·05. APEC and AFEC differed in their abilities to form biofilms in the three media types (Table 2, Fig. 1). AFEC produced significantly

Table 1 ANOVA showing the interaction of biofilm formation with certain factor(s)

Factor	d.f.	*F*-value	*P*-value
Source	1	8·32	0·0041
Media	2	16·64	<0·0001
Source × media	2	3·98	0·0192
Phylotype	3	2·53	0·0566
Source × phylotype	3	5·51	0·0010
Media × phylotype	6	4·06	0·0005
Source × media × phylotype	6	1·95	0·0714

These results suggest that all the two-factor interactions are significant at the 95% confidence level. Also, the main effects for source and media are significant at the 95% confidence level, but due to the interactions, interpretations of the main effects must be performed with caution. Linear contrasts have been used to make specific comparisons of mean values within the interactions as discussed in Results.

Table 2 Biofilm formation by source

Group	None/weak (%)	Moderate (%)	Strong (%)	Total
In 1/20 TSB				
APEC	56 (53·3)	16 (15·2)	33 (31·4)	105
AFEC	58 (56·3)	13 (12·6)	31 (30·0)	103
Total	114 (54·8)	29 (13·9)	64 (30·8)	208
Chi-square (d.f. = 2) = 0·432, $P = 0·806$				
In BHI				
APEC	84 (80·0)	19 (18·1)	2 (1·9)	105
AFEC	63 (61·2)	14 (13·6)	26 (25·2)	103
Total	147 (70·7)	33 (15·9)	28 (13·5)	208
Chi-square (d.f. = 2) = 24·312, $P < 0·001$				
In M63 + 0·2% glucose/0·5% casamino acids				
APEC	103 (98·1)	2 (1·9)	0 (0·0)	105
AFEC	86 (83·4)	5 (4·9)	12 (11·7)	103
Total	189 (90·9)	7 (3·4)	12 (5·8)	208
Chi-square (d.f. = 2) = 14·797, $P < 0·001$				

Biofilm formation with respect to source of isolate (APEC or AFEC) in three different types of media. In BHI and M63, but not 1/20 TSB, there is strong evidence to reject the hypothesis of homogeneity of relative proportion for each of the biofilm classes in relation to source. Values in parentheses show the proportion of isolates in each group as a percentage of the total.

APEC, avian pathogenic *Escherichia coli*; AFEC, avian faecal commensal *E. coli*; BHI, brain–heart infusion broth; TSB, tryptic soy broth.

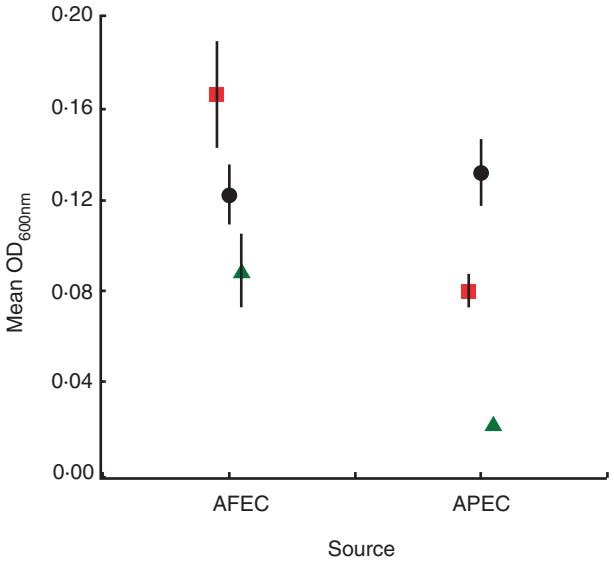

Figure 1 Biofilm formation in relation to source and type of media. The mean OD_{600} produced (plotted on the *y*-axis) by avian pathogenic *Escherichia coli* ($n = 105$) and avian faecal commensal *E. coli* ($n = 103$) biofilms in brain–heart infusion broth, 1/20 tryptic soy broth and M63 media. Error bars represent ±1 SEM. ■, BHI; ● 1/20 TSB; ▲, M63.

stronger biofilms in BHI and M63 than APEC; whereas, in 1/20 TSB, the groups were not significantly different in their ability to form biofilms. APEC biofilm formation in 1/20 TSB was significantly greater than in BHI or M63. AFEC produced stronger biofilms in BHI than in 1/20 TSB, although this difference was not significant. However, AFEC biofilm formation in BHI was significantly greater than in M63.

The results of the phylogenetic typing are in Table 3. A chi-squared analysis indicates that there is strong evidence to reject the hypothesis of homogeneity of relative proportions for each of the four phylogenetic groups across isolate source (APEC or AFEC).

Table 3 Phylogenetic typing

Group	A (%)	B1 (%)	B2 (%)	D (%)	Total
APEC	38 (36·2)	12 (11·4)	24 (22·9)	31 (29·5)	105
AFEC	38 (36·9)	32 (31·0)	20 (19·4)	13 (12·6)	103
Total	76 (36·5)	44 (21·2)	44 (21·2)	44 (21·2)	208
Chi-square (d.f. = 3) = 16·801, $P < 0·001$					

Analysis of these results show there is strong evidence to reject the hypothesis of homogeneity of relative proportions for each of the four phylogenetic groups across isolate source (APEC or AFEC). Values in parentheses show the proportion of isolates in each group as a percentage of the total.
APEC, avian pathogenic *Escherichia coli*; AFEC, avian faecal commensal *E. coli*.

Table 4 Biofilm formation by phylotype

Group	None/weak (%)	Moderate (%)	Strong (%)	Total
In 1/20 TSB				
A	50 (65·8)	11 (14·5)	14 (18·4)	76
B1	22 (50·0)	2 (4·5)	20 (45·5)	44
B2	20 (45·5)	12 (27·3)	12 (27·3)	44
D	22 (50·0)	4 (9·1)	18 (40·9)	44
Total	114 (54·8)	29 (13·9)	64 (30·8)	208
Chi-square (d.f. = 6) = 20·782, $P = 0·002$				
In BHI				
A	40 (52·6)	17 (22·4)	19 (25·0)	76
B1	32 (72·7)	7 (15·9)	5 (11·4)	44
B2	40 (90·9)	3 (6·8)	1 (2·3)	44
D	35 (79·5)	6 (13·6)	3 (6·8)	44
Total	147 (70·7)	33 (15·9)	28 (13·5)	208
Chi-square (d.f. = 6) = 22·609, $P < 0·001$				
In M63 + 0·2% glucose/0·5% casamino acids				
A	63 (82·9)	3 (3·9)	10 (13·2)	76
B1	43 (97·8)	1 (2·3)	0 (0·0)	44
B2	42 (95·5)	2 (4·5)	0 (0·0)	44
D	41 (93·2)	1 (2·3)	2 (4·5)	44
Total	189 (90·9)	7 (3·4)	12 (5·8)	208
Chi-square (d.f. = 6) = 13·841, $P = 0·032$				

In each medium there is strong evidence to reject the hypothesis of homogeneity of relative proportion for each of the biofilm classes in relation to phylotype.
TSB, tryptic soy broth; BHI, brain–heart infusion broth.

Associations were found in an isolate's ability to form a biofilm in relation to its phylogenetic group (Table 4, Fig. 2). Biofilm formation for *E. coli* of phylotypes B1, B2 and D was significantly greater in 1/20 TSB than in M63. The isolates of phylotypes B1, B2 and D also produced stronger biofilms in 1/20 TSB than in BHI. This difference was not statistically significant. Isolates of phylogenetic group A formed significantly stronger biofilms in BHI than in M63 or 1/20 TSB.

We also found that isolates of phylogenetic group A and those of groups B1, B2 and D varied in their abilities to form biofilms in certain media (Table 4, Fig. 2). The pooled mean biofilm production of isolates assigned to groups B1, B2 and D was significantly greater than those of group A in 1/20 TSB. However, in BHI and M63, biofilm formation by group A was significantly greater than that of the isolates from groups B1, B2 and D.

Discussion

APEC infections lead to multimillion dollar annual losses for the poultry industry as a result of increased morbidity, mortality and condemnations (Barnes *et al.* 2003; de Brito *et al.* 2003). APEC may also play a role in human disease as aetiologic agents of extraintestinal infections,

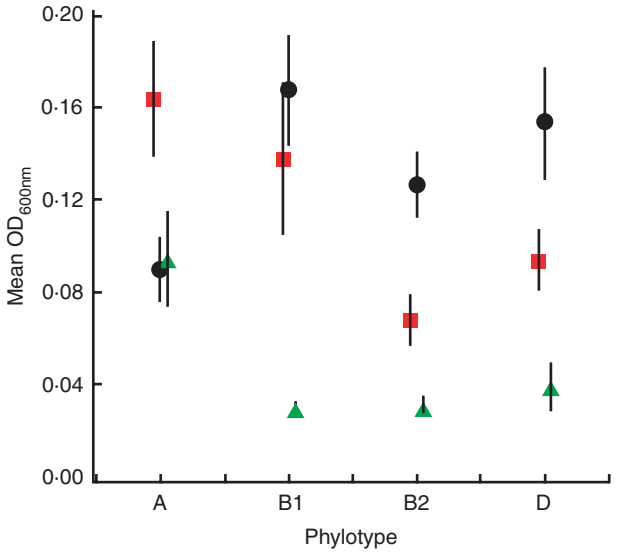

Figure 2 Biofilm formation in relation to phylotype and type of media. The mean OD_{600} produced (plotted on the *y*-axis) by the isolates of each of the four phylotypes, A ($n = 76$), B1 ($n = 44$), B2 ($n = 44$) and D ($n = 44$) in brain–heart infusion broth, 1/20 tryptic soy broth and M63 media. Error bars represent ±1 SEM. ■, BHI; ● 1/20 TSB; ▲, M63.

such as urinary tract infections, or as reservoirs of virulence and/or resistance genes for human bacterial pathogens (Johnson *et al.* 2003, 2005a; Johnson *et al.* 2005b; Rodriguez-Siek *et al.* 2005b). An important characteristic of APEC isolates is their possession of large conjugative R and/or virulence plasmids (Dozois *et al.* 2000; Ginns *et al.* 2000; Johnson *et al.* 2005c, 2006; Rodriguez-Siek *et al.* 2005a). The efficiency of conjugative transfer of these plasmids may be enhanced in biofilms (Davey and O'Toole 2000). Therefore, in this study, we wished to determine what *in vitro* conditions induce biofilm formation by avian *E. coli*, as similar conditions in the environment may better enable *E. coli* to acquire conjugative plasmids, rendering them more virulent and antibiotic resistant.

The microtitre plate method used here to assess biofilm formation does have some limitations. For example, cultures derived from a single species may not behave or react like the mixed population found in the natural environment (Pompermayer and Gaylarde 2000). Regardless, the microtitre plate method is a widely used tool in the study of biofilms (O'Toole *et al.* 1999; Stepanovic *et al.* 2004; Sturgill *et al.* 2004), allowing investigators to screen a large number of strains under several conditions (O'Toole *et al.* 1999).

The ability of 105 APEC and 103 AFEC to form biofilms on plastic microtitre plates in different types of media was assessed. We found that avian *E. coli* are highly variable in their ability to form biofilms and that an isolate's ability to form a biofilm in one type of medium is not necessarily indicative of its capability to form a biofilm in another. In fact, no isolate was found which produced a strong biofilm in all three media. APEC isolates produced the greatest amount of biofilm in nutrient deplete conditions (1/20 TSB); whereas, AFEC formed biofilms in 1/20 TSB but also were able to form biofilms in BHI and to a lesser extent in M63. Based on these results, it would appear the AFEC are generally better able to form biofilms than APEC. A parallel to this finding may be seen in studies of human extraintestinal pathogenic *E. coli*, where some antibiotic resistant strains appear less virulent than their antibiotic susceptible counterparts, the explanation being that success of these less virulent, antibiotic resistant isolates is due to their ability to resist antibiotics rather than their pathogenic capability (Johnson *et al.* 1994; Velasco *et al.* 2001; Johnson *et al.* 2002; Horcajada *et al.* 2005). So too, the success of AFEC in the environment may be due more to their biofilm forming ability than to their ability to cause disease.

We also investigated the possible association of biofilm formation and phylotype, as certain phylotypes are related to virulence among the *E. coli* of mammalian extraintestinal infections (Picard *et al.* 1999). Phylogenetic typing does not appear to discriminate between APEC and AFEC as well as it does between extraintestinal and commensal *E. coli* strains isolated from mammals. In the present study, *c.* 53% of the APEC was assigned to the pathogenic phylotypes, B2 and D; whereas, in a previous study conducted in our laboratory *c.* 85% of human ExPEC isolates were classified into the B2 or D phylotypes (Rodriguez-Siek *et al.* 2005b). However, phylotypes were not distributed evenly amongst APEC and AFEC. APEC contained a larger proportion of isolates assigned to group D than did the AFEC, and a larger proportion of AFEC belonged to the B1 phylotype than did APEC. There may be several explanations as to why phylogenetic typing does not definitively discriminate between APEC and AFEC, one of which is that APEC virulence may be largely plasmid mediated (Ginns *et al.* 2000; Rodriguez-Siek *et al.* 2005a). Thus, the chromosomal background on which phylogenetic typing is based might not be as important in determining APEC virulence as it is in human ExPEC. Another explanation may be that avian colibacillosis may be secondary to some predisposing condition (Dho-Moulin and Fairbrother 1999; Barnes *et al.* 2003), meaning that APEC may be opportunists rather than frank pathogens, a trait that could be reflected in their assignment to phylotype.

Regardless of the marginal utility of phylotyping in discriminating between APEC and AFEC, associations between biofilm formation and phylotype were observed.

Biofilm formation by isolates assigned to phylogenetic groups B2 and D was virtually limited to 1/20 TSB. Isolates of phylogenetic group B1 behaved similarly to groups B2 and D, as biofilm formation by isolates assigned to the B1 phylotype was strongest in 1/20 TSB. However, isolates of phylogenetic group A appeared to act quite differently. Phylogenetic group A isolates composed 37% of the total strains. However, they made up 10/12 (83%) and 19/27 (70%) of the strong biofilm formers in M63 and BHI respectively. In contrast, only 14/64 (22%) of phylogenetic group A strains were strong biofilm formers in 1/20 TSB. This disparity in the ability of group A strains to form biofilms is intriguing, and may be owed to the fact that they appear to be a phylogenetically distinct group, composed mostly of *E. coli* K-12-like strains as determined by a multilocus enzyme electrophoresis analysis of (Herzer *et al.* 1990).

The finding that APEC form their strongest biofilms in nutrient deplete conditions is interesting, as these are the conditions they would likely encounter in water systems at poultry production facilities (do Amaral 2004). As transfer of mobile genetic elements is enhanced in biofilms (Davey and O'Toole 2000), poultry waterers may be an origin of virulent, antibiotic-resistant avian *E. coli*, containing conjugative R and virulence plasmids. Therefore, the microbes of the biofilms found in poultry waterers and their possible role as reservoirs of virulent, antibiotic-resistant avian *E. coli* may warrant further investigation. Also, the fact that APEC biofilm production is induced in nutrient depleted conditions, thought to be similar to those that *E. coli* encounters during food processing (Leriche and Carpentier 2000), is also intriguing, as there is emerging evidence that APEC serve as aetiologic agents of human disease or reservoirs of virulence and/or resistance genes for the *E. coli* causing human infections (Johnson *et al.* 2003, 2005a, 2005b; Rodriguez-Siek *et al.* 2005b).

Acknowledgements

This work was supported by Iowa State University's Biotechnology Council, Provost's Office and Office of the Dean of the College of Veterinary Medicine.

References

do Amaral, L.A. (2004) Drinking water as a risk factor to poultry health. *Rev Bras Cienc Avic* **6**, 191–199.

Barnes, H.J., Vaillancourt, S.S. and Gross, W.G. (2003) Colibacillosis. In *Diseases of Poultry* ed. Saif, Y.M. pp. 631–652. Ames, IA: Iowa State University Press.

de Brito, B.G., Gaziri, L.C. and Vidotto, M.C. (2003) Virulence factors and clonal relationships among *Escherichia coli* strains isolated from broiler chickens with cellulitis. *Infect Immun* **71**, 4175–4177.

Caudry, S.D. and Stanisich, V.A. (1979) Incidence of antibiotic-resistant *Escherichia coli* associated with frozen chicken carcasses and characterization of conjugative R plasmids derived from such strains. *Antimicrob Agents Chemother* **16**, 701–709.

Clermont, O., Bonacorsi, S. and Bingen, E. (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* **66**, 4555–4558.

Davey, M.E. and O'Toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* **64**, 847–867.

Dho-Moulin, M. and Fairbrother, J.M. (1999) Avian pathogenic *Escherichia coli* (APEC). *Vet Res* **30**, 299–316.

Djordjevic, D., Wiedmann, M. and McLandsborough, L.A. (2002) Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Appl Environ Microbiol* **68**, 2950–2958.

Donlan, R.M. and Costerton, J.W. (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* **15**, 167–193.

Dowdy, S., Weardon, S. and Chilko, D. (2004) *Statistics for Research*. Hoboken: John Wiley and Sons.

Dozois, C.M., Dho-Moulin, M., Bree, A., Fairbrother, J.M., Desautels, C. and Curtiss, R. (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.

Ginns, C.A., Benham, M.L., Adams, L.M., Whithear, K.G., Bettelheim, K.A., Crabb, B.S. and Browning, G.F. (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.

Herzer, P.J., Inouye, S., Inouye, M. and Whittam, T.S. (1990) Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. *J Bacteriol* **172**, 6175–6181.

Horcajada, J.P., Soto, S., Gajewski, A., Smithson, A., Jimenez de Anta, M.T., Mensa, J., Vila, J. and Johnson, J.R. (2005) Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. *J Clin Microbiol* **43**, 2962–2964.

Johnson, J.R., Orskov, I., Orskov, F., Goullet, P., Picard, B., Moseley, S.L., Roberts, P.L. and Stamm, W.E. (1994) O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing urosepsis. *J Infect Dis* **169**, 119–126.

Johnson, J.R., van der Schee, C., Kuskowski, M.A., Goessens, W. and van Belkum, A. (2002) Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli isolates* from the Netherlands. *J Infect Dis* **186**, 1852–1856.

Johnson, J.R., Murray, A.C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M.A. and Smith, K.E. (2003) Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrob Agents Chemother* **47**, 2161–2168.

Johnson, J.R., Delavari, P., O'Bryan, T.T., Smith, K.E. and Tatini, S. (2005a) 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.

Johnson, J.R., Kuskowski, M.A., Smith, K., O'Bryan, T.T. and Tatini, S. (2005b) Antimicrobial-resistant and extra-intestinal pathogenic *Escherichia coli* in retail foods. *J Infect Dis* **191**, 1040–1049.

Johnson, T.J., Siek, K.E., Johnson, S.J. and Nolan, L.K. (2005c) DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic *Escherichia coli* transmissible R plasmid. *Antimicrob Agents Chemother* **49**, 4681–4688.

Johnson, T.J., Siek, K.E., Johnson, S.J. and Nolan, L.K. (2006) DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli*. *J Bacteriol* **188**, 745–758.

Leriche, V. and Carpentier, B. (2000) Limitation of adhesion and growth of *Listeria monocytogenes* on stainless steel surfaces by *Staphylococcus sciuri* biofilms. *J Appl Microbiol* **88**, 594–605.

Levy, S.B., FitzGerald, G.B. and Macone, A.B. (1976) Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature* **260**, 40–42.

Lindsay, D., Geornaras, I. and von Holy, A. (1996) Biofilms associated with poultry processing equipment. *Microbios* **86**, 105–116.

O'Toole, G.A., Pratt, L.A., Watnick, P.I., Newman, D.K., Weaver, V.B. and Kolter, R. (1999) Genetic approaches to study of biofilms. *Methods Enzymol* **310**, 91–109.

Picard, B., Garcia, J.S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J. and Denamur, E. (1999) The link between phylogeny and virulence in *Escherichia coli* extra-intestinal infection. *Infect Immun* **67**, 546–553.

Pompermayer, D.M.C. and Gaylarde, C.C. (2000) The influence of temperature on the adhesion of mixed cultures of *Staphylococcus aureus* and *Escherichia coli* to polypropylene. *Food Microbiol* **17**, 361–365.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. and Nolan, L.K. (2005a) Characterizing the APEC pathotype. *Vet Res* **36**, 241–256.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J., Fakhr, M.K. and Nolan, L.K. (2005b) Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology* **151**, 2097–2110.

Sainsbury, D. (2000) *Poultry Health and Management*. Ames, IA: Blackwell Science.

Sanderson, K.E. and Zeigler, D.R. (1991) Storing, shipping, and maintaining records on bacterial strains. *Methods Enzymol* **204**, 248–264.

Stepanovic, S., Cirkovic, I., Ranin, L. and Svabic-Vlahovic, M. (2004) Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. *Lett Appl Microbiol* **38**, 428–432.

Sturgill, G., Toutain, C.M., Komperda, J., O'Toole, G.A. and Rather, P.N. (2004) Role of CysE in production of an extracellular signaling molecule in *Providencia stuartii* and *Escherichia coli*: loss of *cysE* enhances biofilm formation in *Escherichia coli*. *J Bacteriol* **186**, 7610–7617.

Velasco, M., Horcajada, J.P., Mensa, J., Moreno-Martinez, A., Vila, J., Martinez, J.A., Ruiz, J., Barranco, M. *et al.* (2001) Decreased invasive capacity of quinolone-resistant *Escherichia coli* in patients with urinary tract infections. *Clin Infect Dis* **33**, 1682–1686.

