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Identification of F11 Fimbriae on Chicken *Escherichia coli* Strains

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Fimbriae were purified from *Escherichia coli* strains isolated from chickens with septicemia or colibacillosis. When grown on solid media, these strains expressed fimbriae with an apparent subunit molecular mass of 18 kDa. Morphological, biochemical, serological, functional, and molecular characterization revealed that these 18-kDa fimbriae are identical to F11 fimbriae, which were previously found to be involved in the pathogenesis of human urinary tract infection. Screening of a large strain collection showed that 78% of chicken *E. coli* strains expressed F11 fimbriae, whereas this percentage increased to 96% when the only strains taken into account were those with the serotypes most commonly encountered in avian colibacillosis (O1:K1, O2:K1, O35, and O78:K80). The prevalence of F11 fimbrial expression appeared to be independent of the country of isolation of the strains, except for the United States, where the prevalence seemed higher. Expression of F11 fimbriae by chicken *E. coli* strains could not be correlated with adherence to chicken tracheal or pharyngeal cells.

Escherichia coli septicemia or colibacillosis is a common disease in poultry, characterized by airsacculitis, pericarditis, perihepatitis, and occasionally salpingitis (21). Colibacillosis is usually a secondary infection, with *E. coli* entering via the respiratory tract after damage caused by respiratory pathogens like infectious bronchitis virus (IBV) or mycoplasmas (18–20, 42). A wide variety of *E. coli* serotypes are involved, but in most studies more than half of the infecting strains belong to one of the serotypes O1:K1, O2:K1, O78:K80, and O35.

With regard to the pathogenesis of avian colibacillosis, a correlation between virulence and adherence to tracheal or pharyngeal epithelial cells was suggested (12, 13, 31). Fimbriae are involved in mediating this adherence, but these fimbriae appeared to be type 1 or type 1-like fimbriae, which can be concluded from expression in broth cultures, mannose-sensitive adherence and hemagglutination, serological cross-reactions, and comparison of N-terminal amino acid sequences (1, 2, 14, 15, 22, 33–35).

The purpose of the present study was to investigate whether pathogenic avian *E. coli* strains also express fimbriae other than type 1 or type 1-like fimbriae.

(This work was presented in part at the Federation of European Microbiological Societies Workshop on Microbial Surface Components and Toxins in Relation to Pathogenesis, Mitzpeh Rachel, Israël, 15 through 19 May 1989.)

MATERIALS AND METHODS

Bacterial strains. A large collection of *E. coli* strains isolated from affected hearts of diseased chickens with *E. coli* septicemia, originating from various countries, was established. The serotypes of some strains were determined at the National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands. A number of strains with known serotypes were obtained from Ørskov (Copenhagen, Denmark) and from Achtman (Berlin, Germany). The *E. coli* fimbrial clones used are listed in Table 3. Strains were maintained at −70°C in Trypticase soy broth (TSB; BBL) supplemented with 30% glycerol.

Bacteria were grown on blood agar base no. 2 (Oxoid), TSB, or in brain heart infusion broth (BHI; Oxoid) for the fimbrial clones supplemented with the appropriate antibiotic.

Hemagglutination. For testing hemagglutination of strains, an assay on glass slides was used. Heparinized human blood was washed with phosphate-buffered saline (PBS; 0.04 M, pH 7.2), and a 2% erythrocyte suspension in PBS was prepared. Bacterial suspensions in PBS were mixed 1:1 with the 2% erythrocyte suspension on glass slides at room temperature. For testing mannose-resistant hemagglutination (MRHA), 1% D-mannose was added to the erythrocyte suspension. The strength of hemagglutination was scored from − to ++++.

Electron microscopy. Electron microscopy of bacterial and fimbrial preparations was carried out essentially as described previously (36). Briefly, Formalin-fixed bacterial or fimbrial preparations were allowed to attach to Formvar-carbon-coated copper grids and negatively stained with 1% phosphotungstic acid (pH 7.2) for 15 to 30 s.

Isolation of fimbriae. For wild-type *E. coli* strains, bacteria were grown overnight at 37°C on large numbers of blood agar base plates and harvested in 10 mM Tris-HCl buffer. Fimbriae were removed from the bacteria either by shearing for 15 min on ice in a Sorvall Omnimixer, by heating for 15 min at 65°C, or by a combination of these two treatments. Bacteria were removed by centrifugation for 30 min at 16,300 × *g*. The supernatant was washed in an Amicon ultrafiltration cell provided with a YM100 filter and is called the crude fimbrial preparation. Subsequently, fimbriae were purified by two methods.

Purification was first carried out essentially as described by Korhonen et al. (27), avoiding protein-denaturing conditions (6). Briefly, fimbriae were precipitated with 50% saturated ammonium sulfate, solubilized in 0.5% sodium deoxycholate, and subjected to 10 to 60% sucrose gradient centrifugation at 63,000 × *g* for 20 h. Final preparations were dialyzed extensively against saline (0.85% NaCl) and concentrated by ultrafiltration.

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The second purification method employed was direct precipitation with sodium dodecyl sulfate (SDS). Crude fimbrial preparations in 20 mM Tris-HCl buffer (pH 7.5) were adjusted to 2% SDS and heated for 30 min at 60°C.

Precipitated fimbriae were spun down by centrifugation for 30 min at 27,000 × *g*, dissolved in saline, and dialyzed against saline.

F11 fimbriae were purified from clone AM1727/pPIL291-15 expressing F11 fimbriae (7) by the modified Korhonen method described above. Bacteria were grown overnight in a fermentor in BHI containing 50 μg of ampicillin per ml at 37°C, pH 7.4, and 50% O_2 saturation. Bacteria were inactivated and fimbriae were extracted by heating for 15 min at 65°C. Bacteria were removed by centrifugation for 30 min at 16,300 × *g* and filtration through 0.45-μm-pore-size filters (Pellicon system; Millipore). The cell supernatant was then subjected to fimbrial purification.

Antisera. Antisera were raised in rabbits against fimbriae purified by the modified Korhonen method. When little material was available, the first injection was given subcutaneously in the armpits and groins with antigen emulsified in Freund complete adjuvant. Otherwise the priming was given intramuscularly. Six weeks after priming, the booster injection was given intramuscularly with antigen emulsified in a water-in-oil emulsion. The rabbits were bled 2 weeks after the booster injection.

Antisera against fimbriae were absorbed with the homologous strains grown for 2 days at 20°C on blood agar base. For each antiserum, bacteria from 10 large (15-cm) plates were harvested in saline and centrifuged for 15 min at 27,000 × *g*. The bacteria were resuspended in 10 ml of antiserum diluted 1:5 in saline with 0.25% Formalin. After incubation overnight at room temperature under gentle agitation, the bacteria were removed by centrifugation for 20 min at 27,000 × *g*. The absorbed antiserum was filtered through 0.2-μm-pore-size filters (Minisart; Sartorius) and preserved with 0.1% NaN_3.

Monoclonal antibodies (MAbs) specific for various *E. coli* fimbrial types were described before (5, 9, 10) and are listed in Table 4. All MAbs were used as mouse ascitic fluid.

Enzyme-linked immunosorbent assay (ELISA). Polystyrene flat-bottom microtiter plates (Greiner) were coated overnight at room temperature with 100 μl of purified fimbriae in 0.04 M PBS (pH 7.2) per well at a concentration of 2.5 μg of fimbriae per ml. The plates were then blocked for 20 min at 37°C with 200 μl of PBS per well with 5% bovine serum albumin, washed with tap water, and dried in the air.

Binding of antibodies was investigated by incubating 100 μl of serial dilutions of antiserum in 0.2 M PBS with 0.2 M NaCl, 0.1% bovine serum albumin, and 0.05% Tween 80 per well for 1 h at 37°C. After a wash with tap water, the plates were incubated for 30 min at 37°C with 100 μl of appropriately diluted conjugate (goat anti-rabbit or anti-mouse immunoglobulin G-peroxidase [PO] [heavy and light chains]; Nordic) per well and washed again.

Bound antibodies were determined colorimetrically by adding 100 μl of enzyme substrate solution, containing 15 parts of distilled water, 1.5 parts of 0.14% urea peroxide in sodium acetate-citric acid buffer (pH 5.5), and 0.2 parts of 0.6% 3,3′-5,5′-tetramethyl benzidine (TMB) in dimethyl sulfoxide per well. The reaction was carried out in the dark and stopped after 10 min by adding 50 μl of 2 M H_2SO_4 per well, and the A_{450} was measured in a micro-ELISA reader. The antibody titer was defined as the maximum antiserum dilution with an A_{450} of at least 1.5 times the mean A_{450} background.

Whole-bacteria ELISA. Bacteria were grown on blood agar base, for fimbrial clones supplemented with the appropriate antibiotic, and resuspended in coating buffer (1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$, and 0.2 g of NaN_3 per liter [pH 9.6]) to an A_{660} of 0.140 to 0.180. Polystyrene flat-bottom microtiter plates were seeded with 100 μl of these bacterial suspensions per well and allowed to dry at 55°C overnight. The plates were washed with tap water and blocked for 1 h at room temperature with 110 μl of PBS-T-S (0.04 M PBS, 0.5% Tween 80, 15% fetal calf serum [pH 7.2]) per well and washed again. Then 100 μl of serial dilutions of 1:100 prediluted absorbed rabbit antiserum KO7551 (raised against 18-kDa fimbriae purified from strain CH6) were added per well. Two wells with PBS-T-S per strain served as background controls. After 1 h of incubation at 37°C, the plates were washed, and 100 μl of peroxidase-conjugated goat anti-rabbit immunoglobulin G/PO (heavy and light chains) appropriately diluted in PBS-T-S, was added per well.

Bound antibodies were determined colorimetrically as described above for the fimbrial ELISA. Titers were determined as the highest antiserum dilution giving an A_{450} of at least 2 times the background A_{450}. All titers of ≥1:100 were considered positive. In each assay a positive strain and a negative control strain were included.

Protein assay, SDS-PAGE, and Western blotting. The protein content was measured by a modified Folin-Ciocalteu assay. SDS-polyacrylamide gel electrophoresis (PAGE) was performed essentially as described by Lugtenberg et al. (29); proteins were stained with Coomassie brilliant blue. Western immunoblotting was performed essentially as described by Muilerman et al. (30); preparations run in SDS-PAGE were transblotted to cellulose nitrate membrane filters, and antigens were visualized by successive incubation with antibodies, peroxidase-conjugated goat anti-rabbit immunoglobulin G/PO (heavy and light chains), and urea peroxide with 3,3′-diaminobenzidine–4HCl.

P-receptor binding test. Strains were tested for recognition of the P-receptor Galα1→4Galβ with latex beads coated with the receptor (BACH test; Swedish Sugar Co., Arlöv, Sweden) as described by De Man et al. (4). Bacteria were grown on blood agar base and suspended in a drop of saline on a glass slide. After addition of a drop of Galα1→4Galβ-latex, agglutination was recorded after 2 min while the slide was tilted back and forth.

Adherence to pharyngeal cells. Adherence of strains to pharyngeal epithelial cells was tested essentially as described by Dho and Lafont (13). Strains were grown on blood agar base and in TSB, and adherence was tested in the absence and presence of 1% D-mannose.

Amino acid analysis and N-terminal sequence analysis. The amino acid composition of 18-kDa fimbriae purified from strain CH4 was determined after hydrolysis of the protein in 6 M HCl and derivatization of the amino acids with *o*-phtalaldehyde and 2-mercaptoethanol in sodium borate buffer at pH 9.0. Amino acid derivatives were separated on a Nova-PAK C_{18} column and measured in a Perkin-Elmer fluorescence detector. The N-terminal amino acid sequence was determined by using a gas-phase sequencer.

RESULTS

Isolation of fimbriae from chicken *E. coli* isolates. In electron microscopy and when the bacteria were grown on solid media like blood agar base, rigid fimbriae could be observed on chicken *E. coli* isolates. Initially, eight strains were tested; three of these (CH2, CH4, and CH6) also

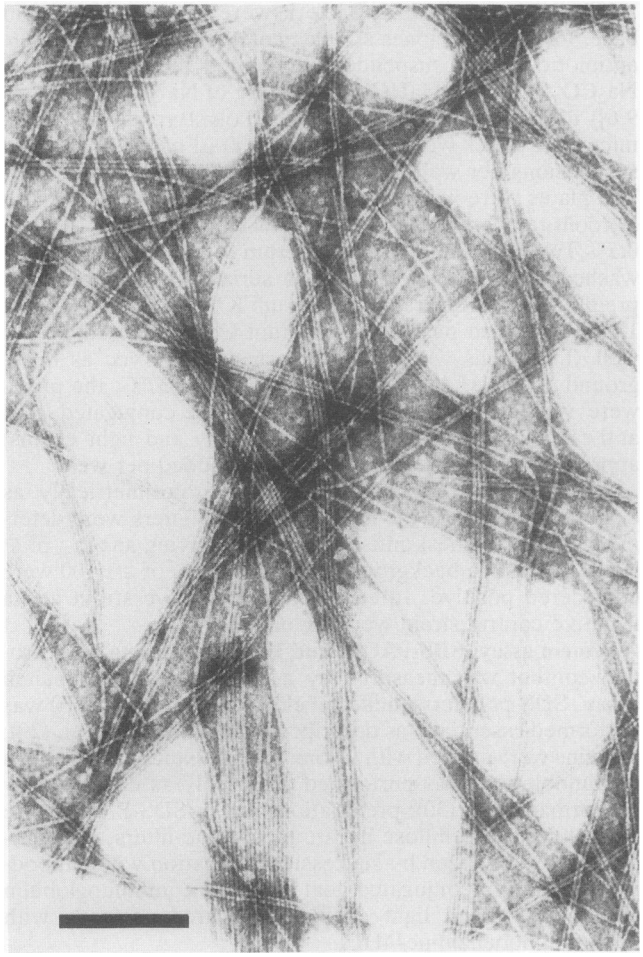

FIG. 1. Electron micrograph of purified 18-kDa fimbriae from *E. coli* CH4 (bar, 200 nm).

showed MRHA of human erythrocytes when grown at 37°C on blood agar base. MRHA was not observed when the bacteria were grown in broth media at 37°C or on blood agar base at 20°C.

Purification of fimbriae from strains CH2, CH4, and CH6 grown on blood agar base at 37°C resulted into preparations containing fimbriae with a subunit molecular mass in SDS-PAGE of approximately 18 kDa (Fig. 2, lanes a through c), as judged by electron microscopy (Fig. 1). The 18-kDa band seen in the preparation from strain CH2 (Fig. 2, lane a) was lost at reproduction but can be seen in Western blots of the same preparation (Fig. 3). Due to the very low yield of fimbriae, 0.5 to 2 mg of protein from the harvest of 40 large 15-cm agar plates, the level of purity was not very high, not even after two successive sucrose gradient centrifugations.

Therefore, as an alternative, CH4 fimbriae were purified by SDS precipitation (Fig. 2, lane e). With this method, fimbriae of high purity were obtained, with a yield of 1 to 1.5 mg from the harvest of 10 large 15-cm agar plates.

Cross-reactivity of chicken *E. coli* 18-kDa fimbriae and prevalence among chicken *E. coli* strains. Antisera were raised in rabbits against 18-kDa fimbriae purified from strains CH2, CH4, and CH6 and absorbed with the homologous strains grown at 20°C to make the antisera fimbriae specific. In Western blots of crude fimbriae preparations, all three

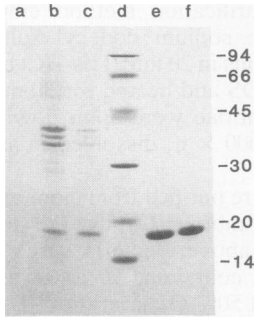

FIG. 2. SDS-PAGE of fimbrial preparations. Lanes: a, b, and c, fimbriae isolated by the modified Korhonen method from strains CH2, CH4, and CH6, respectively; d, molecular mass markers, as indicated in kilodaltons; e, fimbriae from strain CH4 purified by SDS precipitation; f, F11 fimbriae purified from clone pPIL291-15 by the modified Korhonen method.

antisera reacted with the 18-kDa band in all three fimbrial preparations. Figure 3 shows a reaction of antiserum raised against CH6 fimbriae with the 18-kDa band in fimbrial preparations of strains CH2, CH4, and CH6.

The same absorbed antiserum raised against CH6 fimbriae was used to screen a large collection of avian *E. coli* strains in an ELISA system with whole bacteria as the antigen. As shown in Table 1, 159 (78%) of the 203 strains tested cross-reacted with the 18-kDa fimbrial antiserum and were considered to express the same fimbriae. Expression of the 18-kDa fimbriae was confirmed by Western blotting on part of the collection (see Table 5). The prevalence of expression was the highest among strains from the United States and Canada (Table 1) and was 96% among strains with the common serotypes in colibacillosis (O1:K1, O2:K1, O35, and O78:K80) (Table 2).

Cross-reactivity of chicken *E. coli* 18-kDa fimbriae with other *E. coli* fimbriae. First, the absorbed antiserum raised against CH6 fimbriae was tested for reaction with various fimbrial clones in an ELISA with whole bacteria. A strong cross-reaction was seen with F11 and DC1 fimbriae, whereas no reaction or only a very weak reaction was observed with 1A, 1C, S, $F7_1$, $F7_2$, F8, F9, and F13 fimbriae (Table 3). A positive reaction was also observed with the F11 reference strain H291. Extensive cross-reaction between F11 and DC1 fimbriae was already shown previously with MAbs (8). No

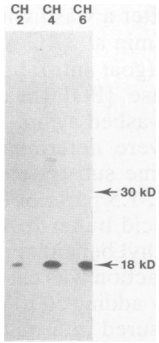

FIG. 3. Western blot of fimbrial preparations from strains CH2, CH4, and CH6 (Fig. 3, lanes a, b and c, respectively) with absorbed antiserum raised against CH6 fimbriae.

TABLE 1. Expression of F11 fimbriae by chicken *E. coli* strains in relation to country of origin

Country of origin	No. of strains		% of F11-positive strains
	Total	F11 positive	
Netherlands	33	21	64
Belgium	24	16	67
France	27	20	74
Denmark	4	4	*x*[a]
Germany	1	1	*x*
United States	20	19	95
Canada	13	13	100
Venezuela	41	26	63
China	2	2	*x*
Collection[b]	38	37	97
Total	203	159	78

[a] Number of strains too low for percentage calculation.
[b] Collections of strains with common serotypes (O1:K1, O2:K1, O78:K80) from various countries.

reaction was observed with K99 fimbriae in the ELISA (data not shown).

Second, 18-kDa fimbriae purified from strain CH4 were tested in the ELISA for recognition by MAbs specific for various fimbriae (Table 4). The 18-kDa fimbriae were recognized only by F11-specific MAbs and not by MAbs specific for 1A, 1C, $F7_1$, $F7_2$, F8, F9, F12, and F13. Various F11-specific MAbs, known to bind to different epitopes (38), showed the same reaction pattern in the ELISA when CH4 18-kDa fimbriae and F11 fimbriae were compared (Table 4). Polyclonal antisera raised against CH6 18-kDa fimbriae and F11 fimbriae reacted in the ELISA with 18-kDa fimbriae as well as with F11 fimbriae (Table 4).

Further identification of chicken *E. coli* 18-kDa fimbriae as F11 fimbriae. In SDS-PAGE, both the 18-kDa and F11 fimbriae had apparent molecular masses of 18 kDa (Fig. 2, lanes e and f). In Western blots of purified 18-kDa fimbriae and F11 fimbriae, complete cross-reaction was observed with the respective polyclonal antisera (Fig. 4).

The amino acid composition of purified 18-kDa fimbriae showed a high degree of similarity with the amino acid composition of F11 fimbriae as published by Van Die et al. (37). The first 17 N-terminal amino acids of 18-kDa fimbriae were identified as Ala-Pro-Thr-Ile-Pro-Gln-Gly-Gln-Gly-Lys-Val-Thr-Phe-Asn-Gly-Thr-Val, which are identical to the first 17 N-terminal amino acids of F11 fimbrial subunits (37).

TABLE 2. Expression of F11 fimbriae by chicken *E. coli* strains in relation to serotype of strains[a]

Serotype	No. of strains	
	Total	F11 positive
O1:K1	3	3
O2:K1	47	47
O78:K80	23	21
O35	8	7
Other	5	3
Unknown	117	78

[a] The percentages of F11-positive strains were as follows: 96% for O1:K1, O2:K1, O78:K80, and O35 serotypes; 66% for other serotypes and unknown serotypes.

TABLE 3. Cross-reactivity of antiserum raised against 18-kDa fimbriae from strain CH6 with various other fimbriae

Strain (clone)	Reference	Fimbrial type	ELISA titer[a]
pPIL38	26	1A	<2.0
pPIL110-51	41	1C	<2.0
pANN801-13	24	S	<2.0
pPIL110-70	39	$F7_1$	<2.0
pPIL110-37	40	$F7_2$	<2.0
pANN921	23	F8	2.0
pPIL288-10	6	F9	<2.0
pPIL291-15	7	F11	>4.7
pDC1	3	DC1	>4.7
pPAP5	28	F13	2.0
H291(C1976)	32	F11	>4.7

[a] Values are $\log_{10}$ titers in an ELISA with whole bacteria.

When the adhesive properties of the F11 reference strain and the F11-expressing clone were compared with those of a number of chicken *E. coli* strains, the chicken *E. coli* strains expressing 18-kDa fimbriae also caused MRHA of human erythrocytes and agglutination of P-receptor-coated latex particles (Table 5). The level of MRHA and P-receptor agglutination correlated with the level of 18-kDa or F11 fimbriae expression as determined in the ELISA with whole bacteria and Western blots.

Adherence of strains to chicken epithelial cells. Several strains were tested for adherence to chicken pharyngeal cells. Only a few strains, whether grown in broth or on agar plates, showed mannose-sensitive adherence to pharyngeal cells (Table 6). This adherence was not correlated with the expression of F11 fimbriae.

In addition, chicken *E. coli* strains did not adhere in vitro to (ciliated) cells isolated from chicken trachea, regardless of

TABLE 4. Cross-reactivity of MAbs and polyclonal antibodies with 18-kDa fimbriae purified from strain CH4 and with F11 fimbriae

Antibody	Antigen	ELISA titer[a] for:	
		CH4 18-kDa fimbriae	F11 fimbriae
MAbs			
M10-2	1A	<2.0	<2.0
M9-6	1C	<2.0	<2.0
M6-8	$F7_1$	<2.0	<2.0
M2-1	$F7_2$	<2.0	<2.0
M17-9	F8	<2.0	<2.0
M4-7	F9	<2.0	<2.0
M7-6	F11	3.8	4.1
M14-5	F12	<2.0	<2.0
20025-F2a	F12 related	<2.0	<2.0
M8-16	F13	<2.0	<2.0
M7-4	F11	4.1	4.1
M7-5	F11	2.9	2.6
M7-6	F11	4.4	4.7
M7-7	F11	3.2	3.5
M7-13	F11	4.1	4.1
Polyclonal antibodies			
KO7551	CH6 18-kDa fimbriae	3.2	3.5
KO84215	F11	2.9	3.5

[a] Values are $\log_{10}$ titers in an ELISA with purified fimbriae.

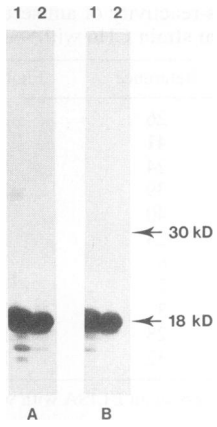

FIG. 4. Western blotting of F11 fimbriae purified from clone pPIL291-15 (lanes 1; see lane f of Fig. 3) and 18-kDa fimbriae purified from strain CH4 (lanes 2; see lane e of Fig. 3) with antisera raised against CH6 18-kDa fimbriae (A) and cloned F11 fimbriae (B).

whether tracheal cells were harvested from chickens pretreated with live IBV 3 days before cell harvesting (data not shown). No adherence to trachea in vivo was seen after *E. coli* aerosol challenge of chickens, with or without pretreatment with IBV 3 days before *E. coli* challenge, as judged from histological preparations made during the 5 days after *E. coli* challenge (data not shown).

DISCUSSION

In the present study it was shown that *E. coli* strains isolated from avian colibacillosis and grown on solid medium commonly express fimbriae with a subunit molecular mass of 18 kDa in SDS-PAGE. Strong evidence that these 18-kDa fimbriae on chicken *E. coli* strains are identical to F11 fimbriae was provided: (i) antiserum raised against the 18-kDa fimbriae cross-reacted with cloned F11 fimbriae and DC1 fimbriae, which were previously found to be closely related, if not identical (8), and not with cloned 1A, 1C, S, $F7_1$, $F7_2$, F8, F9, F13, and K99 fimbriae; (ii) migrations in SDS-PAGE of F11 fimbriae and 18-kDa fimbriae were similar; (iii) in Western blots, the 18-kDa and F11 fimbriae showed complete cross-reaction with each other's respective antiserum; (iv) The 18-kDa fimbriae were only recognized by F11-specific MAbs and not by MAbs specific for 1A, 1C, $F7_1$, $F7_2$, F8, F9, F12, or F13 fimbriae (also, a set of F11-specific MAbs known to recognize various epitopes [38] reacted similarly with 18-kDa and F11 fimbriae); (v) the amino acid compositions and the N-terminal sequences of 18-kDa and F11 fimbrial subunits were identical; (vi) the adhesive properties of 18-kDa fimbriae and F11 fimbriae were identical, with regard to both MRHA of human erythrocytes and binding to the P receptor.

TABLE 5. Expression of 18-kDa F11 fimbriae by strains and adhesive properties

Strain[a] (O:K serotype)	Expression of F11 fimbriae measured by:		Adherence measured by:	
	ELISA[b]	Blotting[c]	MRHA test[d]	P-receptor test[e]
H291 (O1:K1)	>4.7	++	+++	++
AM1727	–[f]	–	–	–
AM1727/pPIL291-15	>4.7	+++	++++	++++
CH2 (O78:K80)	3.0	+	+	+
CH4 (O2:K1)	>4.7	++	++	++
CH5 (O2:K1)	2.7	–	–	–
CH6 (O1:K1)	>4.7	++	++	++
CH7 (O15:K14)	–	–	–	–
CH96 (O78:K80)	3.2	+	++	+
CH139 (O2:K1)	4.7	++	+++	+++

[a] CH, chicken strains; H291, F11 reference strain C1976; AM1727, Fim$^-$ K-12 strain; pPIL291-15, F11 clone.
[b] Log titer in an ELISA with whole bacteria and F11 antiserum.
[c] Intensity of 18-kDa band in Western blots of crude fimbriae preparations with F11 antiserum.
[d] MRHA with human erythrocytes.
[e] Agglutination of Galα1→4Galβ-coated latex beads.
[f] –, no reaction seen.

TABLE 6. Adherence of strains to chicken pharyngeal cells

Strain[a]	F11 expression[b]	Adherence[c] of bacteria:			
		Grown on plates		Grown in broth	
		−M	+M	−M	+M
CH2	3.0	–	–	–	–
CH3	3.0	+	–	–	–
CH4	>4.7	++	–	+	–
CH5	2.7	+	–	–	–
CH6	>4.7	–	–	–	–
CH7	–[d]	–	–	–	–
CH96	3.2	–	–	NT	NT
CH139	>4.7	–	–	NT	NT
CH246(MT21)	–	+	–	++	–
CH247(MT99)	2.9	+	–	–	–
CH248(MT100)	3.2	–	–	–	–

[a] Strains CH246, CH247, and 248, provided by M. Dho, were included as positive and negative adherence controls.
[b] See footnote *b* to Table 5.
[c] Adherence tested in the absence (−M) or presence (+M) of 1% D-mannose; ++, >10 bacteria per cell; +, 5 to 10 bacteria per cell; –, <5 bacteria per cell; NT, not tested.
[d] –, no reaction seen.

The prevalence of F11 expression among a collection of 203 chicken *E. coli* strains isolated from affected internal organs of chickens with colibacillosis was 78%. Similar results were seen with *E. coli* strains isolated from turkeys and ducks (data not shown). The strains were collected in various countries all over the world, but the prevalence of F11 expression appeared independent of the origin of the strains, except for the United States and Canada, where the prevalence of F11 expression was as high as 95 and 100%, respectively (Table 1). Only a limited number of the strains were O:K serotyped by recognized reference laboratories. It is striking that strains with known serotypes belonging to one of the four serotypes most commonly encountered in avian colibacillosis (O1:K1, O2:K1, O78:K80, O35) had a very high prevalence of F11 expression (96%) (Table 2). Strains with other serotypes and strains that were not serotyped had a prevalence of 66% F11 expression.

The high prevalence of F11 expression among strains isolated from infected chickens, especially among strains with the most prevalent O:K serotypes, strongly suggests a role for F11 fimbriae in the pathogenesis of colibacillosis. However, the nature of this role is still obscure. F11 fimbriae belong to the class of P fimbriae, referring to the receptor structure Galα1→4Galβ to which these fimbriae can bind, and were previously found to be involved in the pathogene-

sis of human urinary tract infection by mediating adherence to human uroepithelial cells (4, 11, 32). But adherence to chicken tracheal and pharyngeal cells was not correlated with expression of F11 fimbriae. If strains were adhering, this adherence was inhibited by mannose, pointing at the involvement of type 1 fimbriae. Since avian colibacillosis is a secondary infection after respiratory infection by, e.g., IBV (18–20, 42), we tested adherence in vivo and in vitro to tracheal cells from chickens that were infected by IBV before; no adherence of strains to those damaged cells was observed. It is possible that the F11 fimbriae are especially equiped to adhere to the airsacs and other serous membranes, since avian colibacillosis starts as airsacculitis and progresses to pericarditis and perihepatitis (21), thus mainly affecting membranes before becoming septicemic, but this hypothesis was not yet investigated. Binding to fibronectin or collagen cannot be excluded as a possible explanation (17).

It is hard to understand at present why only F11 fimbriae, and not other P fimbriae as well, could be detected on chicken *E. coli* strains. No difference between the chicken *E. coli* F11 fimbriae and human uropathogenic *E. coli* F11 fimbriae has been found. A possible explanation might be found in the genetic organization of P fimbriae. It has been shown that the actual adhesin is not the P fimbria itself but is one of several minor components located at the tip of the fimbria (25). These minor components confer to the fimbriae their adherence specificity. For instance, F12 fimbriae, which are also P fimbriae, are found on both human and dog uropathogenic *E. coli* but differ in their receptor specificities because of differences in the minor components (16). Whether chicken *E. coli* F11 fimbriae and human *E. coli* F11 fimbriae differ from other P fimbriae with regard to their minor components will have to be determined.

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