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Original article

Sequence analysis demonstrates the conservation of *fimH* and variability of *fimA* throughout Avian Pathogenic *Escherichia coli* (APEC)

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Abstract – In this study we sequenced and analysed the *fimH* and *fimA* genes of 24 avian pathogenic *Escherichia coli* (APEC) isolates, in order to investigate their possible conserved nature. Additional parameters (serotype, presence of aerobactin receptor, expression of F1 pili and virulence for chickens) were investigated to look for correlations with the obtained sequences. The sequence analysis demonstrated that FimH is highly conserved among all investigated APEC strains (> 99% homology), whereas the major subunit FimA is less conserved, presenting 6 variable regions distributed along the protein. A hydrophilicity analysis suggested several variable domains of FimA to be potential epitopes. We were able to classify the investigated strains into three main groups, on the basis of the amino-acid sequences of the variable regions. This grouping was consistent throughout all variable regions and was independent of serotype, leading to an improved classification of the F1 pili. No correlation was found between the *fimH* and *fimA* sequences and the following parameters: avian species, organ of isolation, serotype, presence of aerobactin receptor and virulence for chickens. This study elucidated the molecular structure and the degree of conservation of FimH and FimA among various avian pathogenic *E. coli* strains.

***Escherichia coli* / avian / APEC / FimH / FimA**

1. INTRODUCTION

Escherichia coli infections are often diagnosed in animals and humans. In humans, pathogenic *E. coli* are responsible for both intestinal (diarrhoea) and extra-intestinal (urinary tract infection, meningitis) diseases, while in avian species *E. coli*

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infection only results in extra-intestinal diseases. Avian colibacillosis is initiated by an infection of the upper respiratory tract and often leads to a general infection of the internal organs: pericarditis, perihepatitis, peritonitis and salpingitis. This often results in loss of egg laying and death, causing substantial economic losses [4, 8].

An important step in the infection process is the adherence of APEC to the epithelium of the trachea. This could allow the bacteria to overcome resident defence mechanisms (tracheal mucus layer) and to perform a more successful colonisation. This adhesion phenomenon is mediated by the F1 pili, which are proteinaceous structures present on the outer membrane of most strains of *E. coli* and of several other members of the Enterobacteriaceae [8, 17]. The P pilus could also play an important role in avian colibacillosis. It is thought to act only in the later stages of the infection (internal organs), since P pili are not expressed in vivo on bacteria colonising the tracheal epithelium [17].

The *fim* gene cluster, which encodes the F1 pilus, consists of nine genes, seven of which are organised in a single operon [10]. The F1 pilus is composed of a major protein subunit, FimA (about 1000 subunits per pilus) and of several minor subunits, FimF, FimG and FimH [11]. The minor subunit FimH is comprised of 279 amino acids in its mature form and mediates adhesion to D-mannose residues, which are present on the tracheal epithelial cells, macrophages and many other host cells [9, 17].

The tracheal adhesion mediated by F1 pili has been proposed to play a role in the virulence of APEC [7, 18]. However, a debate still remains on the exact role of the F1 pilus and its FimH adhesin. A study by Marc et al. and Arné et al. [1, 12] demonstrated that neither the F1 pili nor FimH are strictly required for a successful colonisation of the respiratory tract in poultry, suggesting a more limited role in pathogenicity. However, FimH has been proven essential to human urinary tract infection, since deletion of FimH abolishes infection [14]. The FimH adhesin has been described to be conserved throughout several strains of *E. coli* and even other F1 fimbriated *Enterobacteriaceae* [2]. Several important amino acids in the protein have been identified and extensively analysed [16, 20, 21]. On the contrary, the FimA major protein has been reported to be variable between the different strains of *E. coli* [2].

The aim of this study was to elucidate the degree of conservation or variability of FimH and FimA in APEC strains, in order to allow construction and testing of an F1-based subunit vaccine. Although a vaccine currently exists against the F11 serotype of the P pilus (marketed by Intervet International, Boxmeer, The Netherlands), it will be interesting to investigate whether an F1-based subunit vaccine can offer a broader protection. An essential condition for the development of a broad range vaccine is the conserved nature of the antigen (i.e. FimH or FimA), which was analysed in this study. Moreover, a number of additional characteristics were determined (serotype, presence of an aerobactin receptor, expression of type 1 pili and virulence) to detect the possible correlations with FimH and FimA sequences.

2. MATERIALS AND METHODS

2.1. Bacterial strains and media

All *E. coli* strains from Belgium were isolated from animals showing distinct clinical symptoms of APEC infection (pericarditis, perihepatitis, peritonitis, salpingitis) and were collected on poultry farms from different parts of the country (Tab. I). The reference strains (MT203, MT512 and EC79) were extensively characterised and used in numerous studies [5–7, 13]. MT203 and MT512 were proven virulent in in vivo virulence studies. EC79

Table I. Analysed APEC strains.

APEC N° (1)	Date of collect	Place	Type of animal	Organ	Alive (2)	Serotype (3)	FimH accession N° (4)	FimA accession N° (4)
125	01/01	Belgium	Laying hen breeder	Peritoneum	No	NT	AF490846	AF490870
MT512	04/72	France	Laying hen	Trachea	Yes	O2	AF490867	AF490889
21	06/00	Belgium	Broiler chick	Pericard	No	O2	AF490854	AF490877
115	01/01	Belgium	Laying hen breeder	Pericard	No	O1	AF490844	AF490868
128	01/01	Belgium	Laying hen breeder	Pericard	No	NT	AF490847	AF490871
58	09/00	Belgium	Broiler chick	Pericard	No	O115	AF490859	AF490882
6	10/00	Belgium	Broiler chick	Pericard	No	NT	AF490862	AF490885
13	04/00	Belgium	Broiler chick	Pericard	No	NT	AF490850	AF490874
54	09/00	Belgium	Peacock chick	Pericard	No	O2	AF490856	AF490879
121	01/01	Belgium	Laying hen	Pericard	Yes	NT	AF490845	AF490869
60	09/00	Belgium	Laying hen	Pericard	No	O78	AF490861	AF490884
7	10/00	Belgium	Laying hen	Pericard	No	O78	AF490863	AF490886
5	10/00	Belgium	Broiler chick	Pericard	No	O78	AF490860	AF490883
57	09/00	Belgium	Laying hen	Peritoneum	No	O78	AF490858	AF490881
16	04/00	Belgium	Laying hen	Pericard	No	O78	AF490852	AF490876
15	04/00	Belgium	Broiler chick	Pericard	No	O1	AF490851	AF490875
133	01/01	Belgium	Laying hen	Pericard	No	NT	AF490848	AF490872
56	09/00	Belgium	Laying hen	Peritoneum	No	NT	AF490857	AF490880
53	09/00	Belgium	Laying hen breeder	Pericard	No	O78	AF490855	AF490878
137	01/01	Belgium	Laying hen breeder	Pericard	No	O83	AF490849	AF490873
MT203	04/82	France	Turkey chick	Liver	Yes	O1	AF490866	AF490888
17	04/00	Belgium	Laying hen	Peritoneum	No	NT	AF490853	NA
EC79	09/75	France	Broiler chicken	Feces	Yes	O2	AF490864	NA

(1) The strains are ordered according to Figure 1. (2) Alive means that the strain was isolated from an animal, still alive when handed to the laboratory (Animal Health Care, Flanders, Drongen). (3) NT: Non-Typable (serotype not belonging to the 28 serotypes tested). (4) GenBank accession numbers: NA means Not Applicable (no *fim*A was present).

is a non-virulent reference strain [7] (isolated from the faeces of a healthy chicken). For in vivo virulence tests, two additional strains were tested: an *E. coli* strain isolated from a pig and the TUNER strain (Novagen Inc., Madison, USA, laboratory strain for expression of proteins). The Struis strain was isolated from infected ostrich eggs (only the FimA sequence was determined, GenBank accession number AF490890). All strains were stored in a Luria-Bertani (LB) medium containing 15% glycerol at –80 °C and grown in a standard LB medium at 37 °C.

2.2. Serotyping and haemagglutination assay

The bacterial strains were isolated from affected organs. A swab was taken and transferred onto blood agar plates (Columbia sheep blood) and Mac Conkey agar plates. One single colony was selected for further characterisation and sequencing. The strains were classified as *E. coli* by biotyping (TSI, indole, citrate) and subsequently serotyped (Tab. I), using 28 antisera, specific for the 27 world-wide most frequently occurring APEC serotypes and the zoonotically important O157-serotype (antisera purchased at Sanofi, Pasteur, Chaska, USA). The investigated serotypes were O1, O2, O5, O6, O8, O9, O11, O12, O14, O15, O17, O18, O20, O35, O36, O45, O53, O78, O81, O83, O88, O102, O103, O115, O116, O132 (antisera purchased at the *E. coli* reference centre, University of Santiago de Compostela, Spain) and O109 (antisera produced by the VAR, department of Small Stock Diseases).

To investigate the F1 pili expression, an agglutination assay was performed as previously described [7] using blood collected from commercial laying hens and *E. coli* strains grown in Tryptic Soy Broth (TSB) at 37 °C.

2.3. PCR assays

Two PCR assays were performed for each strain to amplify the full-length *fimH* gene (903 bp) and part of the *fimA* gene (549–555 bp). Preparations of 100 ng of genomic DNA [3] were used as templates in 50 µL PCR mixtures containing 4 µL dNTP (2.5 mM each dNTP), 1.25 µL of forward and reverse primers (20 µM each), 2.5 µL DMSO, 34 µL Dnase free water, 5 µL buffer 10× (Stratagene) and 1 µL *Pfu* polymerase (2.5 U/µL, Stratagene, La Jolla, USA). The PCR reaction conditions were as follows: 95 °C for 2 min, then during 30 cycles: 95 °C for 30 s, 57 °C for 30 s and 72 °C for 2 min, followed by a final elongation 72 °C for 5 min. The following primers were used for the *fimH* gene: FimHF: 5' ATGAAACGAGTTATTACCCTGTTTG 3' and FimHR: 5' TTATTGATAAACAAAAGTCACGCC 3'.

For the *fimA* gene, the primers located in the constant intergenic regions flanking the *fimA* gene, as described by Peek et al. [15], were used:

97FimAF (forward primer, starts at position –97, relative to the start codon):

5' ACTGTGCAGTGTTGGCAG 3';

63FimAR (reverse primer, starts at position + 63 relative to the stop codon):

5' GTTATTTTTATCGCACAAGG 3'.

The PCR products were subsequently purified using the Qiagen PCR Purification Kit and concentrated using a standard ethanol-Na-acetate precipitation.

2.4. Sequencing and DNA analysis

Sequencing reactions were performed on the *fimH* and *fimA* PCR products using the ABI PRISM Bigdye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (ABI, Foster City, USA), following the manufacturers manual. After the cycle sequencing reaction, the mixtures were purified and concentrated by ethanol/Na-Acetate precipitation. The pellet was redissolved in 2 µL red loading buffer (Microstop, Microzone LTD, Lewes, UK). The products were sequenced on a 377 ABI-PRISM automated DNA sequencer according to the manufacturers manual.

The *fimH* was sequenced using the FimHF and FimHR amplification primers as well as two internal primers: $FimHF_2$ and $FimHR_2$.

$FimHF_2$: 5' TTGCCGTGCTTATTTTGCGAC 3' (439–459).

$FimHR_2$: 5 CAGCTTTAATCGCCACCCCGC 3' (409–429).

The *fimA* was sequenced using the 97AF and 63 AR amplification primers. Due to the limited length of *fimA* (549 to 555 bp), no internal primers were needed. The chromatograms of the sequences were

visualised using the CHROMAS 2.0 software (Technelysium Ltd., Helensvale, Australia). The DNA sequences were analysed (aligned) using DNAMAN version 5.0 (Lynnon Biosoft, Quebec, Canada).

2.5. Presence of the aerobactin receptor gene

A PCR assay was performed to detect the *iutA* gene (encoding the aerobactin receptor) using the following primers:

IutAF: 5'ATGAGCATATCTCCGGACG 3',
IutAR: 5'CAGGTCGAAGAACATCTGG 3'.

1.5 µL of *E. coli* overnight cultures were used as templates in 20 µL PCR mixtures containing 2 µL buffer 10×, 1.6 µL dNTP (2.5mM), 1 µL of forward and reverse primers (20 µM), 1.2 µL $MgCl_2$ (1.5 mM), 12.12 µL Dnase free water and 0.08 µL *Taq* DNA polymerase (2.5 U/µL, Promega, Madison, USA). The following PCR conditions were used: 93 °C for 10 min, followed by 30 cycles: 93 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, concluded by a final elongation of 72 °C for 5 min.

2.6. Testing for in vivo virulence

All 23 strains (20 strains from Belgium and the 3 reference strains from France) were tested for virulence. Each APEC strain was cultured for two periods of 24 h each in TSB at 37 °C and 0.5 mL of the last culture was injected subcutaneously in the necks of five 1-day-old Specific Pathogen Free (SPF) chicks (Lohmann, Cuxhaven, Germany). Mortality was monitored for 5 days. As described in Dho and Lafont [6], if more than 1 out of the 5 chicks died during the 5-day-period, the strain was considered virulent. If no chicks died, the strain was considered non-virulent. Control groups received 0.5 mL of sterile TSB medium. All experiments on animals were approved by the Ethical Commission for Experimental Use of Animals of the Catholic University Leuven (project number 01059).

3. RESULTS

3.1. Analysis of the FimH protein sequences

The protein sequences, deduced from the obtained nucleotide sequences, gave a homology ≥99% between the analysed strains. Analysis of the multiple protein alignment indicated that 17 out of the 24 investigated strains presented the amino-acid N^{70}-S^{78} motif, 3 presented the S^{70}-N^{78} motif and 4 the N^{70}-N^{78} motif.

A multiple alignment was performed between the FimH sequences from avian *E. coli* strains and available FimH sequences from human *E. coli* strains (GenBank accession numbers AF288194 and AF317710). The FimH sequences from the human strains were identical to FimH from the APEC 15, 121 and MT512 strains and a large homology with the other strains was found. Both FimH sequences from human strains presented the S^{70}-N^{78} motif. Sequence AF317710 presented an alanine at position 62 (of the mature protein), while all other sequences had a serine. No association of the FimH sequence was observed with the avian species of origin, organ of isolation, serotype, presence of the aerobactin receptor nor with virulence for chicks.

3.2. Analysis of the FimA protein sequences

The protein sequences, deduced from the obtained nucleotide sequences, are shown in Figures 1 and 2. No *fimA* gene was detected in the APEC 17 and EC79 strains, although the *fimH* gene was present, indicating an incomplete *fim* operon. Both strains were unable to agglutinate chicken erythrocytes, which was in accordance with their lack of *fimA*.

Based on the multiple protein alignment (Fig. 2), six variable regions were detected in the FimA sequences: V1 (AA 24-27),

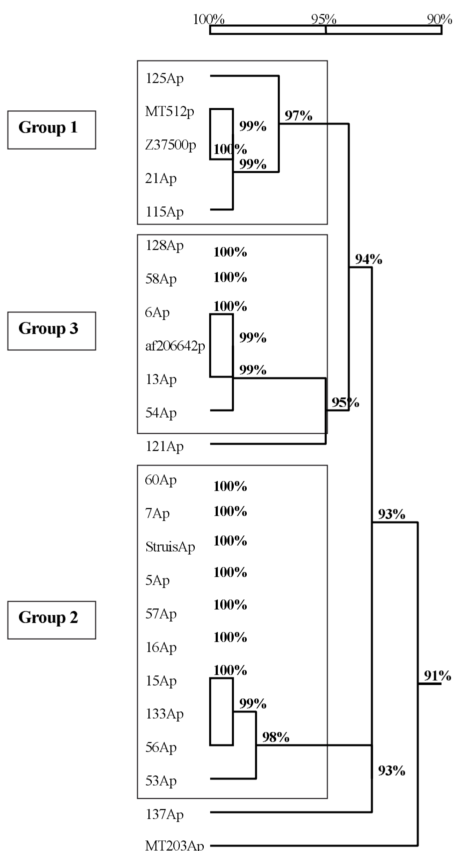

Figure 1. Homology tree of the multiple FimA alignment at the protein level. The three groups (based on the variable domains) are indicated. The percentages indicate the degree of homology. p or Ap indicate that the sequence is a protein sequence.

V2 (AA 65-69), V3 (AA 89-92), V4 (AA 104-109), V5 (AA 134-137) and V6 (AA 145-146). The first variable domain (V1) is characterised by an insertion of two amino acids. Based on the sequences of all variable regions, the analysed APEC strains could be associated in 3 major groups, as indicated in Figures 1 and 2, each presenting a unique consensus sequence. This grouping is consistent throughout the six variable regions. The last two variable regions (V5 and V6) are identical for groups 2 and 3, which can only be distinguished by the other variable regions. Region AA 163–167 could be considered as a seventh variable domain. The motif of this region, as shown in the APEC 125 strain (Fig. 2), is however only present in the APEC 121 and APEC 137 strains and not in the other strains. The latest two strains do not fall entirely in the proposed grouping, as shown in Figure 1. At position 19, there is an additional variable position (A ↔ T), which is, however, independent of the proposed grouping.

Group 1 includes the APEC 21, 115, 125 and MT512 strains, group 2 includes the APEC 6, 13, 54, 58 and 128 strains, and group 3 includes the APEC 5, 7, 15, 16, 53, 56, 57, 60, 133 and StruisA (isolated from ostrich) strains. Strain 137 could not be grouped precisely. It is closely related to group 2, but shows a number of additional variations. In the same manner, strain 121 is related to group 3. The homology of the groups was in the range of 92–94%, which is lower than the overall homology in FimH (98–100%).

Two additional strains, whose *fimA* sequences had already been determined, were compared to the sequenced strains of this study: strain MT78 is an APEC strain (GenBank accession number Z37500) and strain AF206642 (GenBank accession number) is from mammalian origin. The APEC MT78 strain could be classified in group 1, while the AF206642 strain of mammalian origin belonged to group 3. When additional sequences of mammalian origin [15] were incorporated into the multiple alignment, all of them fell within the proposed groups and the established variable domains (data not shown).

We studied the hydrophilic or hydrophobic nature of the variable regions of FimA in order to look for potential epitopes (Fig. 3). Region V1 (AA 24–27) was shown to be hydrophobic, while regions V2 (AA 65–69) and V3 (AA 89–92) were strictly hydrophilic among all strains and this could indicate possible epitopes. Region V4 (AA 104–109) was

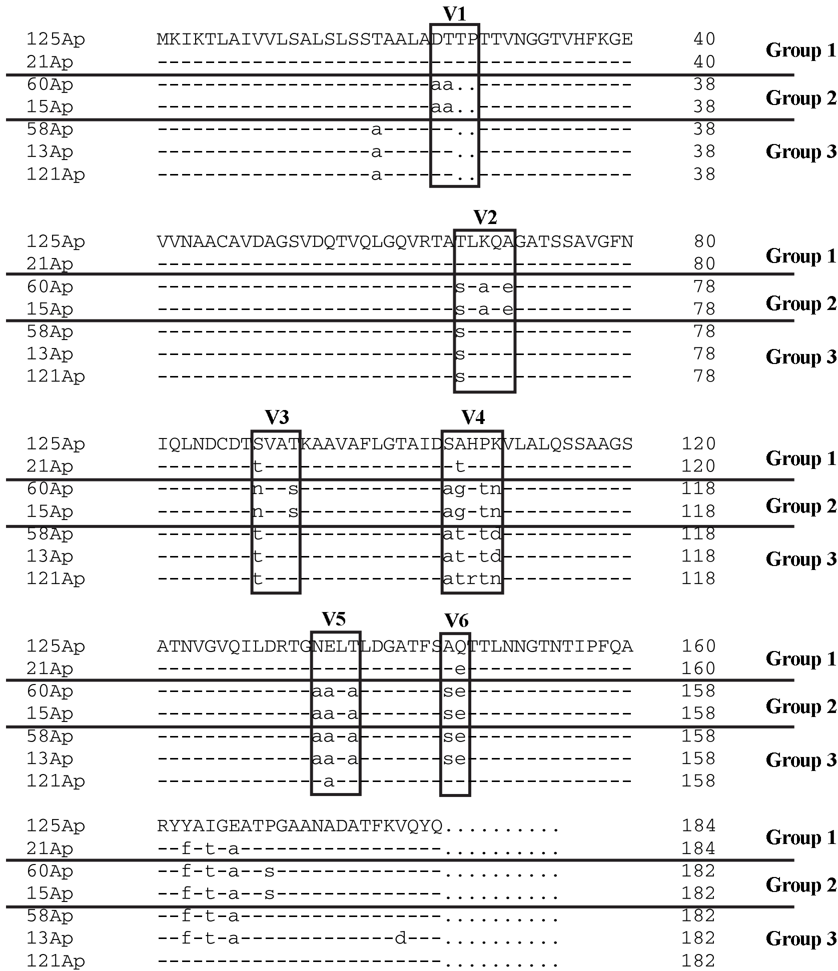

Figure 2. Multiple protein alignment of the FimA sequences. The three groups are indicated and separated by horizontal lines. From each group two or three sequences have been selected to represent the entire group (to limit the alignment). The variable domains are indicated by the black rectangles (V1 to V6).

shown to be partially hydrophilic and partially hydrophobic. Region V5 (AA 134–137) was hydrophobic, except in the APEC 125 and APEC 21 strains (see also Fig. 2). Region V6 (AA 145–146) was shown to be hydrophilic for all strains, except for the APEC 121 and APEC 125 strains (AQ instead of SE).

The studied characteristics of the strains (serotype, avian species of origin, organ of isolation, presence of the aerobactin receptor, and virulence for chicks) did not show any association with the grouping of the strains based on the FimA sequence. The serotypes were randomly distributed over the three groups (Tab. I).

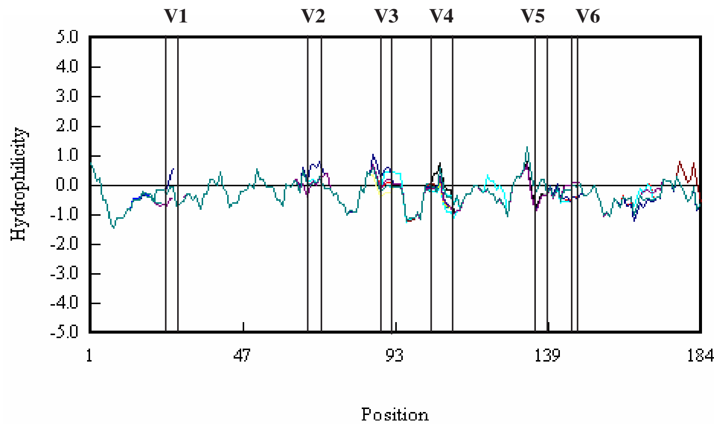

Figure 3. Hydrophilicity plot of FimA. The different curves indicate the different APEC strains. The vertical lines delineate the variable domains, as labelled on top by V1 to V6.

3.3. In vivo virulence

All strains from Belgium were shown to be virulent by the lethality test used. The virulent nature of the MT203 and MT512 strains of French origin was confirmed in this experiment. Strain EC79 has been proven non-virulent. The Tuner strain and the pig-isolated strain were both proven to be non-virulent to chickens.

3.4. Presence of aerobactin receptor gene and F1 pili

All strains, except APEC 17 and EC79, possessed the aerobactin receptor gene *iutA*. All strains except two, showed mannose-sensitive haemagglutination, corresponding to the presence of F1 fimbriae. EC79 and APEC 17 did not show any agglutination, in accordance with their lack of *fimA*. APEC 133 showed mannose resistant-haemagglutination, which could indicate the presence of P pili, but impaired the detection of the F1 pili.

4. DISCUSSION

The goal of this study was to investigate the molecular variability of *fimH* and *fimA* in *E. coli* from avian species and to look for similarities and differences with the current knowledge. Numerous studies have investigated the molecular structure of the *fimH* gene. However, these studies focussed mainly on *fimH* isolated from human *E. coli.* (uropathogenic *E. coli*). Notably the work of Sokurenko et al. [20–22] gives us in-depth insights on the FimH molecular structure, the implications of several amino acid positions and the differences between FimH from commensal and from uropathogenic strains. However, until now, no detailed study of FimH isolated from avian pathogenic *E. coli* was performed.

Our data clearly demonstrate that FimH is highly conserved (protein homology $\geq 99\%$). A closer investigation of the amino acid sequence reveals some interesting positions. Sokurenko et al. [20] found two consensus sequences, namely N^{70}-S^{78} and S^{70}-N^{78}, both associated with a low adhesion to D-mannose and reported that these phenotypes are characteristic for commensal *E. coli* strains. In our study, 20 out of the 24 investigated avian strains presented one of both consensus sequences (17 presented N^{70}-S^{78} and 3 S^{70}-N^{78}). However, these strains were of extra-intestinal origin and their virulence was checked in the present study. Thus the correlation between these

consensus sequences and the non-virulent, commensal nature of the strains may thus not be valid in avian *E. coli*. In four strains we found a new motif, N^{70}-N^{78}. It could be that this motif confers to FimH, an altered specificity to D-mannose. However this hypothesis requires further study.

Pouttu et al. [16] demonstrated that the presence of a serine at position 62 of the mature FimH (instead of alanine) completely abolishes binding of *E. coli* to collagen. All investigated APEC strains and the human AF288194 strain showed a serine at position 62 that could correspond to a lack of collagen binding. Adhesiveness to collagens is an important factor for the penetration of enteric bacteria through tissue barriers in the bloodstream and subsequently to secondary infection sites [19]. The transition of APEC from the air sacs and lungs to the bloodstream occurs through gas-exchange regions and is, in view of the FimH sequence results, probably not mediated by collagen binding. The observed FimH sequences did not show any association with the avian species from which the strains had been isolated. In the same manner, comparison of the FimH sequence from APEC strains with available FimH sequences of *E. coli* of human origin (Crohn disease, meningitis) revealed a 100% identity with APEC 15, 121 and MT512 strains and a homology ≥ 98% with the other APEC strains (alignment not shown), confirming that FimH is not specific.

We also investigated some validation criteria for the characterisation of APEC strains (virulence, presence of the aerobactin receptor, presence of F1 pili). No association was found between these criteria and the FimH sequence. Moreover, there appears to be no association between the FimH sequence and the following parameters: avian species of origin, organ of isolation and serotype.

The *fimA* gene of the avian strains, encoding the major subunit of the F1 pili, was also sequenced and analysed. The APEC 17 and EC79 strains did not have the *fimA* gene, although they did have the *fimH*, indicating an incomplete *fim* operon. It is likely that in the absence of *fimA* the F1 fimbriae are not formed, explaining the lack of haemagglutination. The fact that a *fimA*$^-$ strain, such as APEC 17, is virulent, does not exclude the possible role of type 1 pili in infection. In the virulence test used in this study, the bacteria were directly inoculated into the chicken body, where it has been shown that pili other than type 1 are activated (P pili) and that F1 pili are repressed [17]. Type 1 pili are mainly expressed in the respiratory tract, which was completely bypassed in the virulence test used. Another test, using a more natural way of infection, would be necessary to investigate whether the *fimA*$^-$ strain remains virulent.

Marc and Dho-Moulin [13] sequenced the *fimA* gene from avian pathogenic *E. coli* and compared it with other available sequences (*fimA* from a uropathogenic strain, two avian strains and two *Klebsiella pneumoniae* strains). Upon comparison, four regions with considerable variation were found. Our *fimA* analysis of a larger collection of APEC strains confirmed the same four variable regions, but detected two additional variable domains (AA 89–92 and 145–146). We were able to group the investigated strains into three groups (or families), based on the sequences of the variable domains. The grouping was consistent throughout the six domains, although the last two variable domains are identical for groups two and three. Our comparison also included the original sequenced *fimA* by Marc and Dho-Moulin [13] (GenBank accession number Z37500) and a *fimA* from *E. coli* of mammalian origin, from Peek et al. [15] (GenBank accession number AF206642). We also included a *fimA* sequence, amplified from an *E. coli* strain isolated from infected ostrich eggs. These strains were included to broaden the alignment to a wide variety of strains. We noticed that the *E. coli* strains of mammalian origin fell into one of the established

groups and did not take an exclusive position. Even additional *fimA* sequences of mammalian origin from the study of Peek et al. [15] were incorporated into the multiple alignment: all of them fell within the proposed groups and the established variable domains (data not shown).

The grouping and the variable domains were independent of the serotype. Marc and Dho-Moulin [13] suggested that there could be an association between the variable domains and the O2 serotype. We found that the serotypes were distributed at random throughout the different groups and that consequently no association between serotype and *fimA* sequence could be found. Strains from the same serotype (e.g. the most frequent occurring O78 serotype) can be classified in different groups, with distinctly different FimA. The variable regions were also independent of the animal species of isolation (chicken, turkey, ostrich, peacock or mammalian origin), animal line (chickens layers, broilers or breeders), the organ of isolation (peritoneum, pericardium or trachea), the presence of the aerobactin receptor and the virulence for chicks.

These *fimA* variable domains allow the design of new, specific primers to detect the three *fimA* variants and to perform a more rapid classification of the F1 pili. Since there is one (conserved) adhesin, FimH, and three variants of the main FimA subunit, the nomenclature of F pili may be revised, allowing several classes of F1 pili. Hydrophilicity analysis of the FimA sequences shows that several variable domains harbour a hydrophilic nature. Notably region V2 and V3 are hydrophilic in all strains and, except in two strains, region 6 is also hydrophilic. These hydrophilic domains constitute potential epitopes at the surface of the pilus. This could allow an alternative approach to vaccine development based on partial *fimA* sequences (containing one or more variable domains).

In conclusion, this study elucidated the FimH and FimA molecular situation in *E. coli* from avian origin. Sequence analysis indicated the conserved nature of the FimH adhesin and the more variable nature of FimA. Future studies could investigate the adhesive properties of the different FimH variants to look for further correlations between protein sequences and specific phenotypes. Finally the molecular structure of FimA allows a non-serotype based grouping of $F1^+$ APEC strains.

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