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Common Virulence Factors and Genetic Relationships between O18:K1:H7 *Escherichia coli* Isolates of Human and Avian Origin

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Extraintestinal pathogenic (ExPEC) *Escherichia coli* strains of serotype O18:K1:H7 are mainly responsible for neonatal meningitis and sepsis in humans and belong to a limited number of closely related clones. The same serotype is also frequently isolated from the extraintestinal lesions of colibacillosis in poultry, but it is not well known to what extent human and avian strains of this particular serotype are related. Twenty-two ExPEC isolates of human origin and 33 isolates of avian origin were compared on the basis of their virulence determinants, lethality for chicks, pulsed-field gel electrophoresis (PFGE) patterns, and classification in the main phylogenetic groups. Both avian and human isolates were lethal for chicks and harbored similar virulence genotypes. A major virulence pattern, identified in 75% of the isolates, was characterized by the presence of F1 variant fimbriae; S fimbriae; IbeA; the aerobactin system; and genomic fragments A9, A12, D1, D7, D10, and D11 and by the absence of P fimbriae, F1C fimbriae, Afa adhesin, and CNF1. All but one of the avian and human isolates also belonged to major phylogenetic group B2. However, various subclonal populations could be distinguished by PFGE in relation to animal species and geographical origin. These results demonstrate that very closely related clones can be recovered from extraintestinal infections in humans and chickens and suggest that avian pathogenic *E. coli* isolates of serotype O18:K1:H7 are potential human pathogens.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains may cause various infections in humans and animals. These strains are implicated in a large range of extraintestinal infections in humans, such as neonatal meningitis, septicemia, urinary tract infections, and pneumonia, as well as in animals, such as urinary tract infections in cats and dogs, septicemia in calves and pigs, and systemic colibacillosis in birds (28, 41, 52, 53). ExPEC strains are characterized by virulence factors that may be present in various combinations. They include adhesins (F1, P, and S fimbriae), iron-sequestering systems (aerobactin and *iro* gene clusters), hemolysin, capsules (K1, K5), and various factors whose functions are not yet completely elucidated (Tsh, IbeA, CNF1, CDT, TraT), (25, 28, 41, 54, 67). These virulence factors are preferentially present in particular genetic backgrounds, and it has been shown that ExPEC strains mainly belong to the phylogenetic group ECOR B2 and, to a lesser extent, to group D (20, 48).

Avian pathogenic *E. coli* (APEC) strains are responsible for avian colibacillosis, a systemic infection in poultry that starts in the respiratory tract (4, 16, 34). APEC strains show similarities with human ExPEC strains. Even though no specific set of virulence factors can be associated with APEC strains, most of the virulence genes that they possess are similar to those identified in human ExPEC strains (13, 16, 21, 33, 39, 42, 50, 52, 63). Various genomic islands have also been identified to be possibly involved in virulence (11, 18, 36, 47, 58). Furthermore, several studies have demonstrated that some APEC strains could belong to the same clones as human ExPEC strains (1, 70, 71).

Among ExPEC strains, O18 strains are frequently isolated from neonatal meningitis and septicemia (2, 9, 27, 32). They belong to a limited number of related clones and possess common virulence factors, among which the K1 capsule plays a major role in circumventing host defenses (2, 5, 25, 27, 44, 60). The O18:K1 serotype may also be isolated from avian colibacillosis (6, 7, 51, 63); and the virulence factor IbeA, which is involved in human neonatal meningitis, has been shown to be preferentially associated with O18:K1 APEC strains (21). Even though no extensive study has been devoted to the comparison of O18 APEC strains with human O18 ExPEC strains, the genetic properties of O18:K1 strains of avian origin allow the hypothesis that they could represent a source for human contamination.

In order to assess this hypothesis, we compared O18:K1 ExPEC strains of human and avian origin on the basis of the presence of virulence determinants and pulsed-field gel electrophoresis (PFGE) patterns.

MATERIALS AND METHODS

Bacterial strains. A total of 55 extraintestinal clinical isolates of *E. coli* O18 were used in this study: 22 strains of human origin and 33 strains of avian origin.

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TABLE 1. Primers used for PCR amplifications

Gene	Primers	Primer sequence (5′–3′)	Size of PCR product (bp)	Annealing temp (°C)	Reference
fimA$_{J96}$	fimA1 fimA2	CGGCTCTGTCCCTSAGT GTCGCATCCGCATTAGC	500	52	This study
fimAv$_{MT78}$	fimA201 fimA215	TCTGGCTGATACTACACC ACTTTAGGATGAGTACTG	266	52	37
fimH	fimH2 fimH17	GATCTTTCGACGCAAATC CGAGCAGAAACATCGCAG	389	52	3
neuC	neu1 neu2	AGGTGAAAAGCCTGGTAGTGTG GGTGGTACATCCCGGGATGTC	676	61	This study
felA	fel1 fel2	GGTCAASCAGCTAAAAACGGTAAGG CCTTCAGAAACAGTACCGCCATTCG	239	61	This study
papC	pap1 pap2	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	61	35
papGI	papGIF papGIR	TCGTGCTCAGGTCCGGAATTT TGGCATCCCCCAACATTATCG	461	63	40
papGII	papGIIF papGIIR	GGGATGAGCGGGCCTTTGAT CGGGCCCCCAAGTAACTCG	490	63	24
papGIII	papGIIIF papGIIIR	GGCCTGCAATGGATTTACCTGG CCACCAAATGACCATGCCAGAC	258	63	24
sfaS	sfaSF sfaSR	GTGGATACGACGATTACTGTG CCGCCAGCATTCCCTGTATTC	242	63	29
focG	focGF focGR	CAGCACAGGCAGTGGATACGA GAATGTCGCCTGCCCATTGCT	362	63	29
afa	afa1 afa2	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	65	7, 35
iutA	iutA1 iutA15	ATGAGCATATCTCCGGACG CAGGTCGAAGAACATCTGG	587	55	This study
tsh	tsh03 tsh15	GGTGGTGCACTGGAGTGG AGTCCAGCGTGATAGTGG	640	55	19
ibeA	ibeAF ibeAR	TGAACGTTTCGGTTGTTTTG TGTTCAAATCCTGGCTGGAA	814	55	21
cdt	cdt-s1 cdt-s2	GAAAGTAAATGGAATATAAATGTCCG GAAAATAAATGGAACACACATGTCCG	466	55	65
	cdt-as1 cdt-as2	AAATCACCAAGAATCATCCAGTTA AAATCTCCTGCAATCATCCAGTTA	466	55	65
cnfI	cnf-1S cnf-1R	GGGGGAAGTACAGAAGAATTA TTGCCGTCCACTCTCACCAGT	1,111	55	65
A9	13F 13R	TTTCGACTGCTGGATGAAC AATCATGATTGACCGTGC	934	50	58
A12	16F 16R	ATGCACTCGATAAAAAAAGT TTAAGAAGGTCGATATACGT	860	50	58
D1	17F 17R	ATGAATTCACAATTACTGGC TTAGCTGTTCAGTAGCTCAC	1,998	50	58
D7	cat31 cat32	TCAGTAAGAACGAAAGTGTG ACAGGAACAATCCCGTGGAT	565	50	This study
D10	D10F2 D10R2	ATCTTTACCGTCCTCACC CGTACCGCCTTCATTATC	135	50	This study
D11	20F 20R	ATGCTGAACATGCAACAACA TCAACCCTGTAGTAAACCAAT	1,230	50	58
chuA	chuA.1 chuA.2	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	59	12
yjaA	yjaA.1 yjaA.2	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	59	12
tspE4	tspE4C2.1 tspE4C2.2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	59	12

All strains had been isolated between 1989 and 2001 from various European countries and, with the exception of strains BEN2782 and BEN2783, were epidemiologically unrelated; strains BEN2782 and BEN2783 were isolated from the same patient on different dates.

The human isolates included 15 strains isolated in Spain from the blood of septicemic adult patients 38 to 90 years old and identified as O18:H7 (this study) and 7 strains from neonatal meningitis cases in The Netherlands previously serotyped as O18:K1:H7 and named SP1, SP3, SP15, SP28, SP38, SP44, and SP58, according to their references in the work of Johnson et al. (27). The 33 avian isolates had been recovered from the heart blood or liver of chickens or turkeys with clinical signs of colibacillosis in Spain (24 isolates), France (6 isolates), and Belgium (3 isolates). They were selected on the basis of their O18:H7 serotype from a collection of 1,601 avian *E. coli* strains collected in Europe (63).

Furthermore, various *E. coli* strains were used as positive controls in PCR assays: strain BEN2908 (58) for genes *neuC*, *fimA*, *fimH*, and *ibeA*; strain BEN2908 or strain CFT073 (69) for genomic fragments A9, A12, D1, D7, D10, and D11; strain MT189 (17) for genes *felA* and *papC*; strains J96 (43), CFT073, and 536 (22) for alleles *papGI*, *papGII*, and *papGIII*, respectively; strain χ7122 (19) for *tsh* and strain KH576 (72) for *iutA*; strains 536 and CFT073 for *sfaS* and *focG*, respectively; strain BM2-1 (14) for *cnfI*; strain A30 (35) for *afa*; and strain E6468/62 (59) for *cdt*. Strains from the ECOR collection (45) were a kind gift from Thomas Whittam. They were used as controls for phylogenetic grouping: ECOR26 (B1 group), ECOR50 (D group), and ECOR62 (B2 group). *E. coli* strain MG1655 (8) was used as a negative control for virulence typing and as a positive control for phylogenetic ECOR group A. The *E. coli* strains were routinely grown in Luria-Bertani broth at 37°C with aeration and stored at −70°C in 20% glycerol until they were used.

Serotyping. The determination of O and H antigens was carried out by agglutination by the method described by Blanco et al. (6) and with all available O (O1 to O181) and H (H1 to H56) antisera. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the nonspecific agglutinins. The O antisera were produced in the Laboratorio de Referencia de *Escherichia coli* (Lugo, Spain), and the H antisera were obtained from the Statens Serum Institut (Copenhagen, Denmark).

The presence of the capsular antigen K1 was detected by amplification of the *neuC* gene, as described below, and was checked phenotypically with a monoclonal antibody by using the Wellcogen *Neisseria meningitidis* B/*E. coli* K1 kit (Oxoid).

ECOR grouping. The strains were classified into the four main phylogenetic groups of the ECOR collection by PCR, as described by Clermont et al. (12), by using three primers pairs: chuA1 and chuA2, yjaA1 and yjaA2, and tspE4C2.1 and tspE4C2.2 (Table 1). Classification into phylogenetic ECOR groups A, B1, B2, and D was based on the amplification of the genes *chuA* and *yjaA* and of fragment tspE4C2.1, as described by Clermont et al. (12).

Detection of cytotoxic activities of bacterial lysates and supernatants. Experiments and the preparation of bacterial lysates were conducted as described previously (65). Briefly, nonconfluent HeLa cell monolayers in 96-well plates were infected with culture supernatants and with sonic lysates obtained from 48-h bacterial cultures. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 4 days. Morphological changes in the HeLa cells characteristic of the activity of cytolethal distending toxin were determined. *E. coli* strain DH5α (56) was used as a negative control, and all experiments were conducted in triplicate.

Virulence for chicks. The virulence of each *E. coli* isolate was determined by using a test of lethality for 1-day-old chicks, as described previously (15). Briefly, groups of five 1-day-old specific-pathogen-free chickens were inoculated subcutaneously with a 24 h Luria-Bertani broth culture (about 10^8 CFU), and the mortality was recorded 4 days postinoculation.

Virulence genotyping. Virulence genes were detected by PCR amplification carried out in a Perkin-Elmer temperature cycler 9700 (Applied Biosystems). The primers used are listed in Table 1. DNA crude extracts were prepared by a rapid boiling method. Six multiplex PCR assays were designed to detect simultaneously (i) *fimA*, the *fimA* variant (*fimAv*$_{MT78}$), and *fimH*; (ii) *neuC*, *felA*, and *papC*; (iii) *papGI*, *papGII*, and *papGIII*; (iv) *sfaS* and *focG*; (v) *tsh* and *iutA*; and (vi) *cdt*. The other genes were detected in simple PCR assays. DNA fragments were amplified in a 25-μl PCR mixture with 1 U of *Taq* DNA polymerase (Promega), 25 pmol of the forward and reverse primers, and 5 nmol of each deoxynucleoside triphosphate (Promega) in 1× buffer. PCR conditions were as follows: 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, the annealing temperature for 1 min, and 72°C for at least 30 s, according to the size of the amplified fragment (1 kbp/min), and then a final extension at 72°C for 10 min. Data were statistically analyzed by the chi-square test.

PFGE. PFGE was conducted as described previously (58). Briefly, agarose plugs were prepared from a bacterial culture grown in brain heart infusion broth to an optical density at 600 nm of 1.0. Following incubation in a lysozyme solution (10 mM Tris HCl, pH 9, 100 mM EDTA, 5 mg ml^{-1} lysozyme, 0.05% Sarkosyl), they were then incubated overnight at 55°C (without shaking) in a lysis solution (10 mM Tris HCl, pH 9, 100 mM EDTA, 1 mg ml^{-1} proteinase K, 1% sodium dodecyl sulfate) and washed three times for 1 h each time in TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA). After equilibration in the appropriate restriction enzyme incubation buffer, half plugs were digested overnight with 10 units of the enzyme XbaI (Takara Bio Europe). Pulsed-field gel electrophoresis was conducted in a CHEF-DRIII apparatus (Bio-Rad). The gels (1% agarose) were run at 14°C for 24 h in TBE buffer (Tris, 4 mM; borate, 4 mM; EDTA, 1 mM; pH 8.3) at 6 V cm^{-1}. The pulse times were increased from 10 to 30 s. A bacteriophage lambda ladder CHEF DNA size standard (Bio-Rad) was used as a molecular size marker. The PFGE profiles of the strains were compared by using the Pearson similarity coefficient. The resulting dendrogram was calculated by using the unweighted pair group method with arithmetic averages (UPGMA) (62) included in the GelCompar software (Applied-Maths, St-Martens-Latem, Belgium).

PLS. Partial least-squares (PLS) regression was also used, as described previously (38), to investigate the relationships between the PFGE profiles (variables *X*) and the classification of isolates as a function of their origins (avian strains versus human strains and strains from Spain versus strains from other countries for avian strains) (variables *Y*). PLS regression was established with SIMCA-P software, version 9.0 (UMETRI, Umea, Sweden).

The principle of this analysis is to search for regions of the PFGE profiles that could explain the origins of the strains (animal versus human or Spain versus other countries). Briefly, PLS components $t[1], t[2], \ldots, t[n]$, which are linear combinations of variables *X*, are calculated as follows: $t[1] = w^*_{11}X_1 + w^*_{12}X_2 + \cdots + w^*_{1n}X_n$, $t[2] = w^*_{21}X_1 + w^*_{22}X_2 + \cdots + w^*_{2n}X_n$, $\ldots$, $t[n] = w^*_{n1}X_1 + w^*_{n2}X_2 + \cdots + w^*_{nn}X_n$. They describe the variables *X* and explain the variables *Y*. The w^* terms denote the *X* loadings. The number of useful PLS components is determined by cross-validation (61): some of the PFGE profiles are not used for model development, and their values are then predicted by the model and compared with the actual values. The prediction error sum of squares (PRESS) is the squared differences between the observed and the predicted values for the data kept out of the model-fitting process. This procedure is repeated several times until each PFGE profile has been kept out once and only once. The final PRESS then has contributions from all data. For each component, SIMCA computes the overall PRESS/SS, where SS is the residual sum of squares of the previous component. A component is considered significant if PRESS/SS is statistically smaller than 1.0. Then, regressions relating the variables *Y* to the PLS components $t[1], t[2], \ldots, t[n]$ are built as follows: $Y1 = c_{11}t[1] + c_{12}t[2] + \cdots + c_{1n}t[n]$ + residuals, $Y2 = c_{21}t[1] + c_{22}t[2] + \cdots + c_{2n}t[n]$ + residuals, $\ldots$, $Yn = c_{n1}t[1] + c_{n2}t[2] + \cdots + c_{nn}t[n]$ + residuals. The *c* terms denote the *Y* loadings. The results of the analysis are visualized as groups of strains located in a plane defined by the PLS components. The explanatory performance of the models (expressed in percent) is evaluated by using the R^2Y coefficient, which corresponds to the part of the variation of the *Y* matrix, as explained by the PLS components.

RESULTS

Avian and human isolates harbored similar virulence genotypes. It was confirmed that all 55 strains expressed the O18 and the H7 antigens. All but one *E. coli* strain (human isolate BEN2794) possessed the *neuC* gene and also expressed the K1 capsular antigen. PCR was used to search for the presence of various virulence genes and of genomic fragments previously identified in APEC strain BEN2908 to be putatively associated with the virulence of ExPEC isolates (58).

The prevalence of virulence genes and genomic fragments was similar in both populations of human and avian isolates, and no statistically significant difference in their prevalence could be shown by the chi-square test (Table 2).

More than 97% of strains in each group possessed genes of the *fim* operon and the *sfa* operon, but genes encoding other adhesins (P fimbriae, F1C fimbriae, or Afa) could not be de-

TABLE 2. Prevalence of virulence genes and of phylogenetic groups in avian and human ExPEC isolates

Gene, sequence, or group	% Positive	
	Human isolates (n = 22)	Avian isolates (n = 33)
Genes		
neuC	95.5	100
$fimA_{MG1655}$	0	0
$fimAv_{MT78}$	100	100
fimH	100	100
felA	0	0
papC	0	0
papG	0	3[a]
sfaS	100	97
focG	0	0
afa	0	3
iutA	86.3	84.8
ibeA	95.5	100
tsh	45.5	42.4
cdt	63.6	63.6
cnf1	4.5	3
Sequences		
A9	100	84.8
A12 (*sitA*)	100	97
D1	100	93.9
D7	100	97
D10	100	100
D11 (*iroD*)	100	97
Phylogenetic groups		
ECOR B2	95.5	100
ECOR B1	4.5	0

[a] *papGII*.

tected. Only one avian strain (strain BEN806) possessed the *papGII* gene.

Interestingly, all isolates harbored a particular allele of *fimA* ($fimAv_{MT78}$) that had previously been described in APEC strains (37, 66). The sequence of this *fimA* variant differs from the *fimA* sequence in strains MG1655 (K-12) and J96 by six variable domains (31, 46). It was detected by using the specific primers fimA201 and fimA215 (Table 1), whose sequences correspond to those of the first variable domain (amino acids 24 to 28, the characteristic N-terminal region of mature FimA) and the fourth variable domain (amino acids 104 to 109) of the FimA precursor, respectively. The complete sequences of the *fimA* genes of APEC strains BEN79 and BEN374 were obtained (data not shown), and it was confirmed that they were identical to that of $fimAv_{MT78}$ (GenBank accession no. Z37500).

Gene *ibeA*, which is involved in penetration across the blood-brain barrier, was present in all but one strain (human strain SP1).

The aerobactin system was also present in more than 85% of the strains, as shown by the amplification of the *iutA* gene, which encodes the aerobactin receptor.

All sequences corresponding to genomic fragments identified in APEC strain BEN2908 following a subtractive hybridization (58) showed a high prevalence in avian isolates (85%) and were even present in 100% of isolates of human origin.

Genes *tsh* (which codes for an autotransporter) and *cdt* (which codes for a cytolethal distending toxin) showed a significantly lower prevalence compared with the prevalences of the other genes, and the *cnf1* gene could be detected in only one avian isolate (BEN79) and one human isolate (BEN2794).

Thus, the major virulence pattern of avian and human ExPEC isolates of serotype O18:K1:H7 could be characterized by the presence of F1 variant fimbriae; S fimbriae; IbeA; the aerobactin system; and genomic fragments A9, A12, D1, D7, D10, and D11, as well as by the absence of P fimbriae, F1C fimbriae, the Afa adhesin, and CNF1. This pattern was present in 75% of the strains studied.

Two virulence genes, *tsh* and *cdt*, were not regularly present in human strains or in avian strains. The presence of the *cdt* gene was perfectly correlated with the expression of the cytolethal distending toxin, as demonstrated by the cytotoxic effect on HeLa cells (data not shown).

Both avian and human isolates were virulent for chicks. All human isolates were lethal for 1-day-old chicks (at least one of five chicks inoculated was dead), and most of them (77.2%) killed five of five inoculated chicks. Among the avian strains, 72.7% of isolates killed five of five inoculated chicks, but four isolates (12%) did not kill any chicks. The last result was checked in a second test.

Two control strains were inoculated in the same experiment: virulent APEC strain MT512 (58), which killed five of five chicks, and avirulent strain EC79 (58), which did not kill any chicks.

Both avian and human isolates belonged to the same phylogenetic major ECOR group, group B2, but various subclonal populations were discriminated by PFGE. As determined by the PCR assay of Clermont et al. (12), all but one strain (BEN2779) were assigned to phylogenetic ECOR group B2 (Table 2), which includes a majority of strains involved in extraintestinal infections (48).

In order to get a better discrimination of the strains, we compared the PFGE profiles of the 22 ExPEC strains of human origin and the 16 avian ExPEC strains. The 38 strains studied showed 37 different electrophoretic patterns (strains BEN2782 and BEN2783, isolated from the same patient, showed identical PFGE profiles), and their similarities were calculated. The dendrogram obtained (Fig. 1) showed a group of seven APEC strains (strains BEN1245, BEN1343, BEN382, BEN381, BEN384, BEN374, and BEN380) with more than 80% similarity. All these strains had been isolated from diseased chickens in Spain. Other avian strains showing more than 80% similarity were BEN30 and BEN134 (isolated from turkeys in France), BEN687 and BEN835 (isolated from chickens in Belgium), and BEN806 and BEN1158 (isolated from chickens in Spain). Moreover, two groups of human ExPEC strains with similarities higher than 80% were identified: strains BEN2782, BEN2783, and BEN2785 and strains BEN2778, BEN2792, and BEN2787. All these strains had been isolated in Spain. No relationships with higher than 80% similarity were observed between avian and human ExPEC strains.

A PLS regression analysis based on the PFGE profiles confirmed that the avian ExPEC strains could be clearly differentiated from the human ExPEC strains (including nonpathogenic strain MG1655) in the 95% probability region (Fig. 2). However, these major groups were not homogeneous; and among the human strains three subgroups, groups 1a, 1b, and

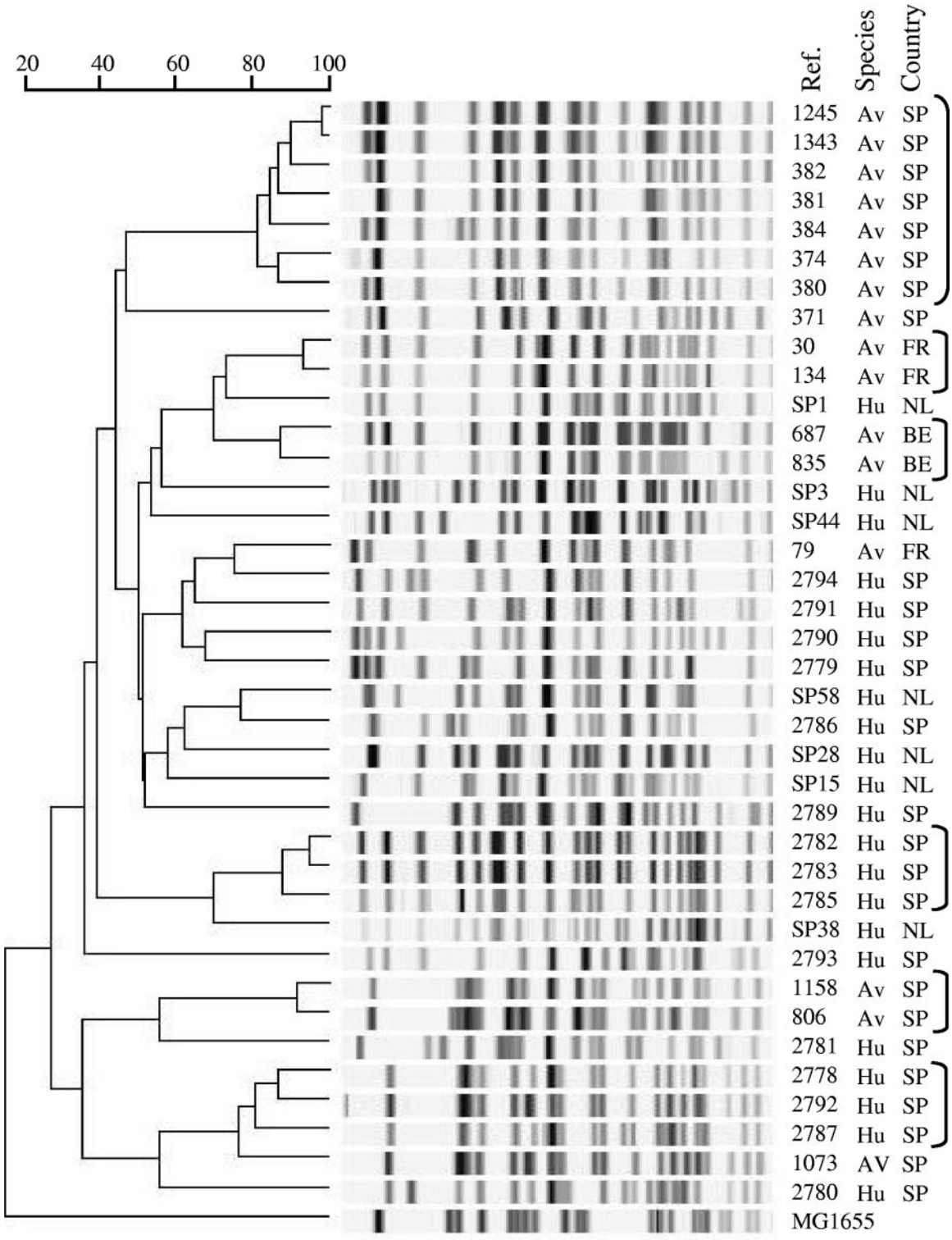

FIG. 1. Genetic relationships among 38 ExPEC isolates of human and avian origin. The PFGE profiles obtained by XbaI restriction were compared by using the Pearson similarity coefficient, and the resulting dendrogram was calculated by the UPGMA method. The origins of the isolates are indicated: Av, avian; Hu, human; FR, France; SP, Spain; NL, The Netherlands. Groups of strains showing the highest similarity are indicated with brackets.

1c (Fig. 2), that were related by both the geographical origin of the strains and the presence and expression of the *cdt* gene could be differentiated. No other correlation with virulence genes could be detected. All seven strains belonging to group 1a were from The Netherlands, and all but one (strain SP15) were *cdt* positive. The four strains in group 1c were from Spain and were *cdt* negative. All 11 strains in group 1b (except for strain MG1655) were also from Spain but showed heterogene-

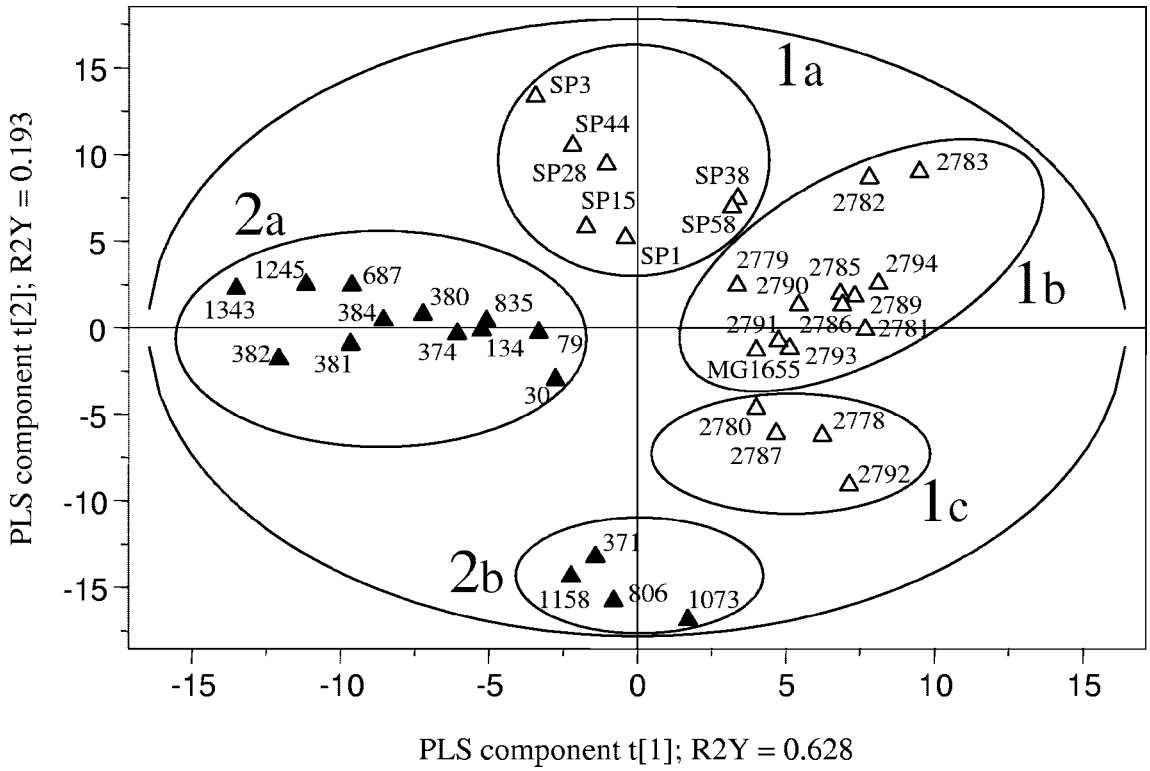

FIG. 2. PLS discrimination between PFGE profiles of *E. coli* strains of human origin (open triangles; $n = 23$) and of avian origin (black triangles; $n = 16$). The R^2Y coefficients correspond to the part of the variation of the Y matrix, as explained by the PLS components, and the explanatory performance of the model is evaluated by adding the R^2Y coefficients. The cross-validation led to three PLS components (only the results for $t[1]$ and $t[2]$ are represented here), and the corresponding PLS model explained 91.6% of the variation of the Y matrix. The 95% probability region defined by the model is delimited by the ellipse. Five groups of strains could be distinguished and are delimited by circles: 1a, human *cdt*-positive strains (except SP15); 1b, human *cdt*-positive and *cdt*-negative strains; 1c, human *cdt*-negative strains; 2a, avian *cdt*-positive strains; and 2b, avian *cdt*-negative strains.

ity for the presence of the *cdt* gene. The PLS regression analysis also revealed the heterogeneity of the avian ExPEC strains according to the presence and the expression of the *cdt* gene. In Fig. 2, groups 2a and 2b include *cdt*-positive and *cdt*-negative avian ExPEC strains, respectively. Moreover, a PLS regression analysis conducted with the avian ExPEC strains only (Fig. 3) showed that group 2a was not homogeneous, and *cdt*-positive APEC strains could be discriminated, according to their geographical origins, into subgroup 2a1 (seven strains from Spain) and subgroup 2a2 (five strains from France and Belgium). Group 2b was homogeneous and included only four *cdt*-negative APEC strains from Spain.

DISCUSSION

As shown by previous studies, some APEC strains are closely related to a recognized widespread clone that includes human ExPEC strains isolated from meningitis and septicemia (2, 71). Most of these studies included strains of various serogroups; thus, it is not clear to what extent APEC and human strains are identical and whether APEC strains could represent a zoonotic risk. In order to answer these questions more accurately, we focused our work on a collection of avian and human ExPEC strains belonging to the same serotype, serotype O18:K1:H7, which is one of the predominant serotypes both in neonatal septicemia and meningitis and in avian colibacillosis.

Our results confirm previous findings showing that ExPEC strains of human and avian origin share several virulence factors (26, 50). However, as pointed out by Mokady et al., only a few common virulence factors are present in nearly all the strains and thus can be considered important or even essential for the infectious process (41).

In the present study no statistically significant difference was observed in the prevalence of the 22 virulence genes and genomic fragments searched for in the human and the avian strains tested. Furthermore, 75% of the strains tested harbored the same virulence genotype, characterized by $fimAv_{MT78}$; *fimH*; *neuC*; *sfaS*; *ibeA*; *iutA*; and genomic fragments A9, A12, D1, D7, D10, and D11. With the exception of the *fim* genes, all these genes usually belong to genomic regions that are absent from the genome of *E. coli* MG1655.

The corresponding virulence factors have been clearly associated with the pathogenicities of ExPEC strains for humans and/or chickens, as demonstrated for K1 (30, 39), F1 fimbriae (64), S fimbriae (10, 57), IbeA (21, 23), and the aerobactin-sequestering system (10, 33). The translated sequences of genomic fragments A12 and D11 from APEC strain BEN2908 are both homologous with proteins SitA and IroD, respec-

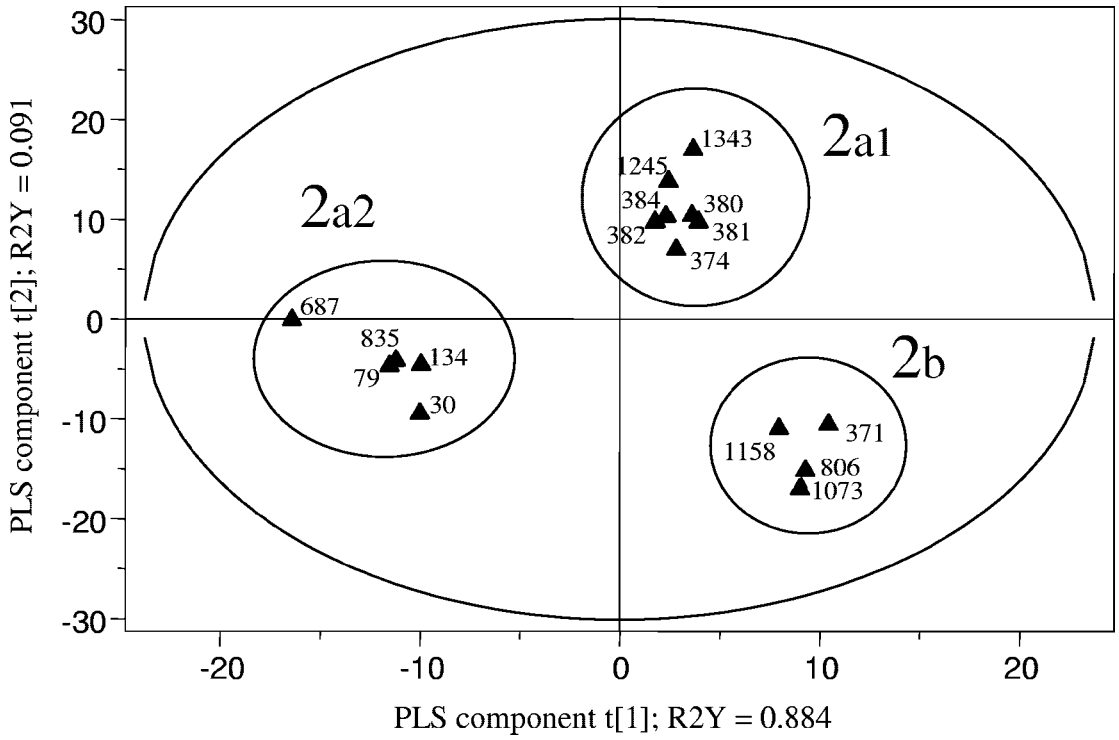

FIG. 3. PLS discrimination between PFGE profiles of avian *E. coli* strains of different geographical origins. The R^2Y coefficients correspond to the part of the variation of the *Y* matrix, as explained by the PLS components, and the explanatory performance of the model is evaluated by adding the R^2Y coefficients. The cross-validation led to two PLS components, represented here as *t*[1] and *t*[2]. The corresponding PLS model explained 97.5% of the variation of the *Y* matrix. The 95% probability region defined by the model is delimited by the ellipse. The 2a group of *cdt*-positive strains could be subdivided into two subgroups, according to the geographical origins of the strain: 2a1, avian *cdt*-positive strains from Spain, and 2a2, *cdt*-positive strains from France and Belgium. The 2b group comprised *cdt*-negative strains from Spain only.

tively, which are involved in iron acquisition (58). These proteins are considered potential virulence factors in the human strain *E. coli* CFT073 and in APEC strains (18, 55, 69). The putative role in virulence of the other genomic fragments (A9, D7, and D10) is determined on the basis of their higher incidence in pathogenic avian *E. coli* strains than in nonpathogenic strains and on their presence in several human ExPEC strains (58).

It is noticeable that all strains tested possessed a particular variant of *fimA* that was first described in APEC strain MT78 (GenBank accession no. Z37500) and then in various APEC strains (37, 66). This allele is identical to the *fimA* allele of the archetypal strains RS218 and IHE3034 that was recently specified by Weissman et al. as the *fimA* C_0 allele (68). The presence of this particular allele indicates phylogenetic relationship between the strains; indeed, it was shown to be present in K1 ExPEC strains of the phylogenetic ECOR group B2 belonging to the sequence type 95 complex (68).

The possession of a set of homologous virulence genes by avian and human strains suggests that they have similar virulence mechanisms. Moreover, all human strains were lethal when they were inoculated subcutaneously into 1-day-old chicks. However, when the physiopathologies of both infections are compared, the portals of entry of *E. coli* are different between chickens and human newborns. Avian colibacillosis initiates in the upper respiratory tract, following injury of the tracheal epithelium by viruses or mycoplasma, and then APEC cells colonize the air sacs and lungs and invade the bloodstream (4). In the case of neonatal meningitis, *E. coli* first colonizes the intestinal tract and then translocates from the intestinal lumen to the bloodstream (9, 49). As specific colonization factors have not yet been identified in human isolates or in APEC isolates, inoculation of a human strain into chickens via the intratracheal route could bring a first answer to the question of possible cross-contamination with O18:K1:H7 strains.

The results obtained here suggest strong genetic relationships between human ExPEC strains and APEC strains of serotype O18:K1:H7. All but one of the strains tested belonged to phylogenetic ECOR group B2 and had the classic meningitis-associated traits (*sfa* and *ibeA*). White et al. (71), comparing avian and human ExPEC strains by multilocus enzyme electrophoresis, showed that O18:H7 isolates of both origins were located in the same "cluster D" and were closely related to the O18:K1 clone originally identified by Achtman et al. (2). As demonstrated by Achtman et al. (2), the O18:K1 clone can be subdivided into two subclones that correspond to outer membrane protein patterns 6 and 9 (OMP6 and OMP9, respectively) and that are related to the geographical origins of the strains. In our study, the European origin of the isolates is consistent with their belonging to the OMP9 subclone. Moreover, the lack of the *pap* genes is another characteristic of OMP9 *E. coli* isolates, as pointed out by Johnson et al. (27).

Belonging to this widespread clone are also human strains

SP1, SP3, SP15, SP28, SP38, SP44, and SP58, as demonstrated by Johnson et al. (27), who identified a large "cluster II" that comprised predominantly O18:K1 neonatal meningitis-associated isolates.

Thus, our results demonstrate that avian and human ExPEC strains of serotype O18:K1:H7 show strong similarities according to their virulence genotypes and phylogenetic groups. However, by using PFGE, which is considered a highly discriminating method, 37 different PFGE profiles were obtained; and cluster analysis by UPGMA revealed that only a few subclusters that had more than 80% similarity were observed, and the subclusters never comprised both human and avian isolates. PLS regression analysis of the PFGE profiles showed that the strains could be distinguished according to their human or avian origin. The five groups resulting from PLS regression analysis (Fig. 2) clearly showed that overlap between human and avian strains does not exist, thus demonstrating some diversity among isolates of clone O18:K1:H7. Moreover, strains were grouped according to their geographical origin (Spain, France and Belgium, or The Netherlands), showing the local dissemination of closely related clones. It is noticeable that groups defined by PLS regression analysis included *cdt*-positive isolates only or *cdt*-negative isolates only, with the exception of group 1b, which included both types of isolates. This observation is in favor of the parallel evolution of different clones with close genetic relationships.

Studying a collection of O18:K1:H7 *E. coli* isolates from women with acute cystitis, healthy control patients, and infants with neonatal meningitis, Johnson et al. (25) demonstrated a high degree of commonality between these strains on the basis of randomly amplified polymorphic DNA analysis, nicotinamide auxotrophy, outer membrane protein patterns, and virulence factor profiles. Our results show that even though O18:K1:H7 *E. coli* strains seem to be very closely related according to their virulence genotypes and their phylogenetic groups, they can be differentiated by a highly discriminating method such as PFGE. The results obtained with the set of strains that we have studied here show that various but closely related clones can be recovered from extraintestinal infections in humans and chickens. Epidemiological studies are required to demonstrate if they have evolved independently or if cross-contamination between human and avian communities is possible, leading to the hypothesis that avian colibacillosis due to O18:K1:H7 strains could be considered a zoonosis.

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