INFECTION AND IMMUNITY, Aug. 1996, p. 3118–3126 Vol. 64, No. 8
0019-9567/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Escherichia coli Cellulitis in Broiler Chickens: Clonal Relationships among Strains and Analysis of Virulence-Associated Factors of Isolates from Diseased Birds†

MUSANGU NGELEKA,[1] JACOB K. P. KWAGA,[1] DAVID G. WHITE,[2] THOMAS S. WHITTAM,[3] CRAIGMYLE RIDDELL,[4] ROBERT GOODHOPE,[4] ANDREW A. POTTER,[1,5] AND BRENDA ALLAN[1]*

Veterinary Infectious Disease Organization[1] and Canadian Bacterial Diseases Network,[5] University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E3; Department of Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B4[4]; Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, North Dakota 58105[2]; and Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, Pennsylvania 16802[3]

Received 25 January 1996/Returned for modification 29 February 1996/Accepted 2 May 1996

Thirty-nine *Escherichia coli* isolates from broiler chickens with cellulitis were serotyped and analyzed for clonal relationships by multilocus enzyme electrophoresis. The isolates were further characterized with respect to hemagglutination (HA); serum resistance; antibiotic susceptibility; production of aerobactin, colicin V, and hemolysin; expression of K1 or K5 capsule; sensitivity to cloacin DF13 after treatment with diphenylamine; expression of iron-regulated outer membrane proteins; and virulence in 1-day-old chickens. In addition, the isolates were examined for the presence of DNA sequences related to F1A (*fim*) and P (*pap*) fimbriae, aerobactin synthesis (*iuc*) and transport (*iut*), hemolysin operon *hly*, and TraT lipoprotein-induced serum resistance (*traT*). Only 38.4% of the isolates were typeable with standard O antisera, and of these, serogroups O25 and O78 were the most frequently observed. Multilocus enzyme electrophoresis, based on 20 enzymes, resolved 17 electrophoretic types, forming seven clusters. Isolates from four of these clusters fell into *E. coli* clone complexes that have been previously reported to be commonly associated with avian colibacillosis. All isolates expressed two to five iron-regulated outer membrane proteins, were resistant to serum and cloacin DF13, and possessed DNA sequences homologous to *fim* and *iuc/iut*. Most isolates (72%) were positive for *traT*, and a majority produced colicin V and aerobactin (92 and 82%, respectively). Assays for the presence of *fim* and *pap* DNA sequences, for HA, and for virulence gave variable results but suggest that cellulitis isolates may express F1A and/or other mannose-resistant HA fimbriae different from P and may be virulent in 1-day-old chickens. Our results support the hypothesis that cellulitis in broilers in many cases is caused by *E. coli* clones identical to other pathogenic avian *E. coli* strains. Certain clones may be specific to cellulitis, because 25% of the isolates tested belong to clusters not related to known clone complexes.

Certain *Escherichia coli* clones may cause intestinal or extraintestinal infections in humans and in domestic animals (29). In poultry, pathogenic *E. coli* strains usually induce extraintestinal infections which are manifested as generalized infection, swollen-head syndrome, and cellulitis (14). In generalized infection, the air sacs may be the first organs affected, and extension of the infection can result in pericardial lesions, characterized by a fibrinous exudate covering the heart, and in fibrinous perihepatitis (14). Cellulitis, also referred to as necrotic dermatitis, infectious process, or inflammatory process, causes an inflammatory exudate under the abdominal skin of broiler chickens (14, 23, 25). No clinical signs are associated with cellulitis in living birds, but the presence of lesions results in the condemnation of part or all of the carcasses at processing. *E. coli* is the principal infectious agent isolated from cases of cellulitis, and serogroups O2, O71, and O78 are the most prevalent (11, 23, 31). Strains isolated from birds with cellulitis can induce the disease when swabbed on skin that has been scratched or on feather follicles of young chickens, whereas random isolates from the environment do not cause the disease (11, 25, 30). It has been suggested that cellulitis is associated with health problems in some flocks, since flocks with few health problems tend to have few condemnations due to cellulitis (25). In addition, some of the serogroups isolated from birds with cellulitis (e.g., O2 and O78) are frequently reported in other *E. coli* poultry diseases such as airsacculitis and swollen-head syndrome (1, 7, 43). These observations suggest that *E. coli* strains that causes cellulitis and other syndrome may be clonally related.

The virulence factors associated with extraintestinal *E. coli* in human and domestic animals include fimbrial antigens (e.g., F1A, P, S, and F165), outer membrane proteins (e.g., TraT, Iss), lipopolysaccharide (e.g., O7, O18), capsules (e.g., K1, K5), and O-antigen capsule (e.g., K″V165″) (15–18, 24, 28, 39, 40). Furthermore, the same bacterial strains may possess high-molecular-weight plasmids, such as ColV plasmids, and produce aerobactin and various cytotoxins such as α-hemolysin (18, 27). Pathogenic avian *E. coli* strains typically express F1A or F1A-like and/or P or P-related fimbriae (7). These adhesins, in association with other virulence factors, allow the bacteria to attach to eukaryotic cells, invade the host tissue, and produce disease. The discovery and characterization of virulence factors possessed by pathogenic clones will lead to the understanding of pathogenesis of infectious diseases and provide tools for efficient therapy and prevention of these diseases.

* Corresponding author. Mailing address: VIDO, University of Saskatchewan, 120 Veterinary Rd., Saskatoon SK S7N 5E3, Canada. Phone: (306) 966-7465. Fax: (306) 966-7478. Electronic mail address: AllanB@sask.usask.ca.

† Published with the permission of the director of VIDO as journal series 190.

TABLE 1. Relevant characteristics of DNA probes used for genotypic analysis of cellulitis-derived *E. coli*

DNA probe specificity (gene)	*E. coli* positive control	Plasmid	Restriction endonuclease fragment(s) and probe size (kbp)	Reference
Aerobactin synthesis (*iuc*)	HB101/pABN5	pABN5	*Eco*RI-*Hin*dIII (6.8), *Ava*I (1.8)	2
Aerobactin receptor (*iut*)	HB101/pABN1	pABN1	*Pvu*II (2.2)	2
Type 1 fimbriae (*fim*)	HB101/pBP7	pBP7	*Eco*RI-*Sph*I (2.0)	32
P fimbriae (*papC*)	HB101/pPILL1006	pPILL1006	*Pst*I (0.3)	8
Hemolysin (*hly*)	HB101/pSF4000	pSF4000	*Pvu*II (ca. 1.8), *Ava*I (6.5)	16
TraT outer membrane lipoprotein (*traT*)	K-12/pKT107	pKT107	*Bst*EII (ca. 0.6)	24

A study of *E. coli* strains associated with avian cellulitis showed that these bacteria often were of the same O serogroups as those associated with respiratory and septicemic diseases in poultry, although 25% of the strains were untypeable. These strains usually produced aerobactin and colicin, two virulence factors implicated in generalized infection in poultry (31). Because gene expression is influenced by environmental conditions, bacterial virulence factors active in vivo may not be expressed in vitro. Therefore, it is useful to determine if cellulitis-derived isolates possess the genes required for biosynthesis of known virulence factors of pathogenic avian *E. coli*. The purpose of this study was twofold: (i) to characterize isolates from broiler chickens with cellulitis in western Canada by examination of virulence-associated factors at the genotypic and phenotypic levels, and (ii) to determine the genetic relationships, if any, between these isolates and other pathogenic avian *E. coli* strains by multilocus enzyme electrophoresis.

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophages, and culture condition. Thirty-nine *E. coli* strains were isolated in pure culture from broiler chickens originating from 10 different flocks in Saskatchewan, Canada. Carcasses with thickened, discolored skin ventral to the vent were selected at the slaughter plant after feather removal but prior to evisceration. Four isolates were obtained per flock, except for one flock, from which three strains were obtained.

The isolates were stored at −70°C in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, Mich.) supplemented with 25% glycerol. Isolates were grown on brain heart infusion (BHI) agar (Difco) for 18 h at 37°C for phenotypic or genotypic analysis.

The recombinant plasmids, containing virulence-associated genes, which served as the source of DNA fragments for preparation of probes are listed in Table 1. Strains harboring these plasmids were subcultured on LB agar (Difco), and selective pressure against loss of the plasmids was imposed by adding appropriate antibiotics to the culture medium. Ampicillin, tetracycline, and chloramphenicol (Sigma Chemical Co., St. Louis, Mo.) were used, when appropriate, at final concentrations of 100, 15, and 30 μg ml^{-1}, respectively.

Bacteriophages specific for K1 or K5 capsule and strains CA1 pKT274 (Ap^r) and MS101 (St^r) expressing these capsules, respectively, were obtained from I. Roberts, University of Leicester, Leicester, England. *E. coli* K12-Row, 1107, N3024, N5160, and KH576 were obtained from J. M. Fairbrother, University of Montreal, Montreal, Quebec, Canada, and strain LG1522 was obtained from J. B. Neilands, University of California, Berkeley, Calif.

Preparation of DNA and probes and colony hybridization. Total cellular DNA was prepared by the sodium dodecyl sulfate-facilitated freeze-thawing-induced lysis technique of Galan and Curtis (10). Plasmid DNA was isolated by the small-scale alkali lysis method (34). DNA was digested with restriction enzymes under conditions specified by the manufacturers (Pharmacia LKB Biotechnology, New England BioLabs, Inc., Mississauga, Ontario Canada). Restriction endonuclease fragments from cloned genes (Table 1) were extracted from agarose gels (Sigma) with the Geneclean kit (BIO 101 Inc., La Jolla, Calif.). The fragments were randomly labeled with [α-^{32}P]dCTP (DuPont Canada Inc., Mississauga, Ontario, Canada) with a DNA-labeling kit (Pharmacia) and purified through a Nick Column (Sephadex G-50 DNA grade) (Pharmacia) for use as DNA probes. Bacterial isolates were grown in LB broth for 6 h, spotted onto LB agar, and incubated at 37°C for 12 h. Colonies were transferred to nitrocellulose membranes (Bio-Rad Laboratories, Richmond, Calif.) and were prehybridized, hybridized, and washed under high-stringency conditions (34).

Multilocus enzyme electrophoresis. Protein extracts from disrupted cells of overnight cultures were prepared and subjected to horizontal starch gel electrophoresis and selective enzyme staining as described elsewhere (35, 44). Electromorphs, the distinct mobility variants of an enzyme, were resolved by measuring differences in the migration rates of specifically stained protein bands. A total of 20 enzymes were individually assayed for electrophoretic variation and are listed in Table 2. Electromorphs for each enzyme were ranked and numbered by anodal migration. Isolates that lacked detectable enzyme activity were assigned a null allelic state at the locus in question.

Clonal analyses. Electromorphs of an enzyme were equated with alleles at the corresponding gene locus so that each bacterial strain was characterized by its allele combination or multilocus genotype for the enzyme-encoding loci assayed (35). Distinctive multilocus genotypes were designated electrophoretic types (ETs) and were numbered by their inferred relationships from a cluster analysis based on the average linking algorithim (35). The ET designations are cognate with those used elsewhere (41, 42, 44). For cluster analysis, a matrix of genetic distances between all pairs of ETs was calculated from comparisons of electrophoretic profiles. Each entry in the distance matrix was equal to the proportion of mismatches (the number of enzyme loci with different alleles divided by the number of loci compared) between two ETs. For each comparison, loci with null states were not included.

Genetic diversity for each locus within a group of ETs was calculated as $h = n(1 - \Sigma p_i^2)/(n - 1)$ where p_i is the frequency of the *i*th allele in a group and n is the number of ETs in a group (26).

Serotyping. Preliminary serotyping of isolates was done by standard slide and tube agglutination tests with antisera against somatic antigens O1, O2, and O78. Further O serogroup analysis and determination of the H-flagellar antigen groups were performed at the *E. coli* Reference Center, Pennsylvania State University, University Park, Pa., with *E. coli* standard antisera.

HA assays. Mannose-sensitive and mannose-resistant hemagglutination (MSHA and MRHA) were assayed by the microhemagglutination test with 96-well U-bottom microtiter plates (Falcon 3911; Becton Dickinson Labware, Oxnard, Calif.) (33), with chicken, guinea pig, and human OP$_1$ erythrocytes. OP$_1$ erythrocytes are from human blood group O, and they express P$_1$ antigen, which has been identified as receptor for fimbriae of the P family of classes I and II (16, 37). Erythrocytes were collected from heparinized blood, washed three times, and diluted in cold phosphate-buffered saline (PBS; pH 7.2) to a final concentration of 3% (vol/vol). For MSHA, bacteria were subjected to four consecutive passages of 48 h each in BHI broth (Difco) at 37°C under static conditions. They were washed in PBS (pH 7.2) and tested with chicken or guinea pig erythrocytes, in the presence or absence of 5% D-mannose. For MRHA, the isolates were grown for 18 h at 37°C on minimal Davis agar plus Casamino Acids (MD-1 agar; Difco) or on sheep blood agar (PLM Microbiologicals, Mississauga, Ontario, Canada) and tested with chicken or human OP$_1$ erythrocytes in the presence of 5% D-mannose (9). The isolates were also tested for MRHA with human OP$_1$ erythrocytes after 10 consecutive passages on sheep blood agar.

Serum bactericidal assay. Bacterial survival in fresh nonimmune chicken serum was examined by the bactericidal assay described by Taylor (38). Fresh serum was obtained from blood collected by wing vein puncture of 4- to 5-week-old chickens. Bacteria were grown for 18 h at 37°C in BHI broth, washed twice in gelatin-Veronal-buffered saline plus magnesium and calcium ions (GVB^{2+}; pH 7.35), and resuspended in GVB^{2+} to a concentration of approximately 10^6 CFU ml^{-1}. A volume of 0.1 ml of the bacterial suspension was added to 0.2 ml of normal chicken serum and incubated at 37°C. Viable-cell counting was done just before incubation (time zero) and at 1 h and 3 h after incubation. Bacterial serum sensitivity was defined as a 2-log-unit decrease in the number of viable bacteria. Serum-resistant (EC317, from our *E. coli* collection) and serum-sensitive (HB101) *E. coli* strains were used as positive and negative controls, respectively.

Colicin V and aerobactin production. The production of colicin V was determined by overlaying chloroform-killed colonies of the test organism with *E. coli* K-12-Row, as previously described (16). Isolates were stabbed onto blood agar plates, incubated for 48 h at 37°C, and killed by exposure to chloroform vapor for 30 min. After 10 min, the plates were overlaid with 5 ml of tryptic soy agar (0.75% agar; Difco) containing approximately 5×10^7 CFU of the colicin indicator strain *E. coli* K-12-Row. The plates were incubated for 18 h at 37°C, and the zones of inhibition of growth were recorded. *E. coli* 1107 was used as the positive control for colicin V production.

Aerobactin production was determined by cross-feeding *E. coli* K-12 LG1522 (1). Lawns of LG1522 (approximately 10^7 CFU per plate) were spread on M9 minimal agar containing 0.5% Casamino Acids (Difco), 40 μg of tryptophan (Sigma) ml^{-1}, and 200 μM 2,2′-dipyridyl (Sigma). The isolates to be tested were

TABLE 2. Allele combinations defining 17 ETs among 39 avian cellulitis-derived isolates

Cluster	ET[a]	Clone complex[b]	n[c]	Allele at locus for enzyme[d]													
				PGI	ACO	PE2	AK	MDH	PGD	M1P	GOT	BGA	ADH	MPI	G6P	SKD	GLU
I	1	B3	7	4	6	7	2	4	6.5	8	6	4	0	4	4	2	2
	2	B3	4	4	6	7	2	4	6.5	8	6	4	0	4	4	2	3
II	3	—[e]	1	6	6	2	4	4	6	4	8	4	6	4	4	8	2
	4	—	1	6	6	2	4	4	6	4	6	0	0	4	4	8	2
III	5	D	2	6	6	4	4	4	8	2	6	4	4	4	4	6	2
	6	D	1	6	6	4	4	4	6	2	6	4	4	6	4	6	2
	7	D	1	6	6	4	4	4	6	8	6	4	0	3	4	12	2
IV	8	—	7	6	6	2	2	4	6.5	4	6	6	6	6	4	11	2
V	9	A1	1	5	5	2	2	4	6.9	4	6	4	4	10	4	6	2
	10	A1	3	5	5	2	2	4	12	4	6	4	4	10	4	6	2
	11	A1	1	5	5	2	2	4	5	4	6	4	0	10	4	6	2
	12	A1	1	5	5	3	2	4	7	4	5	4	4	10	6	6	2
	13	A1	3	5	5	3	2	4	7	4	6	4	4	10	4	6	2
VI	14	B1	1	5	6	5	2	4	6	6	6	8	4	5	2	4	2
	15	B1	3	5	6	5	2	4	6	6	6	4	4	5	2	4	2
	16	B1	1	5	6	5	2	4	7	6	6	4	4	5	2	4	2
VII	17	—	1	6.5	6	5	2	2	8	8	6	8	0	5	6	11	2

[a] ETs were based on distinct allele combinations for 14 polymorphic enzymes detected in the total sample of 39 isolates.

[b] Clone complexes (B3, D, A1, and B1) were identified on the basis of clonal relationships among 17 ETs from cellulitis-derived *E. coli* isolates and analysis of other pathogenic avian *E. coli* strains (41–44).

[c] Number of isolates.

[d] Abbreviations for enzyme loci: PGI, phosphoglucose isomerase; ACO, aconitase; PE2, phenylalanyl-leucine peptidase; AK, adenylate kinase; MDH, malate dehydrogenase; PGD, gluconate-6-phosphate dehydrogenase; M1P, mannitol-1-phosphate dehydrogenase; GOT, aspartate aminotransferase; BGA, β-galactosidase; ADH, alcohol dehydrogenase; MPI, mannose phosphate isomerase; G6P, glucose-6-phosphate dehydrogenase; SKD, shikimate dehydrogenase; GLU, glutamate dehydrogenase. All isolates were monomorphic for isocitrate dehydrogenase, glyceraldehyde-3-phosophate dehydrogenase, indophenol oxidase, carbamate kinase, nucleoside phosphorylase, and threonine dehydrogenase.

[e] Unassigned to clone complex.

grown for 18 h at 37°C in BHI broth, washed once, and diluted in normal saline (0.85% NaCl) to approximately 10^7 CFU ml^{-1}. The isolates were spotted onto the lawn of the aerobactin indicator LG1522 strain and incubated for 18 h at 37°C. A halo of satellite growth around the isolates indicated aerobactin production. *E. coli* EC317 (from our *E. coli* collection) was used as the positive control for aerobactin production.

Cloacin DF13 susceptibility assay. The susceptibility of bacteria to cloacin DF13 was tested by the technique described by Lafont et al. (21). *E. coli* isolates were grown for 18 h at 37°C in M9 minimal salts medium with or without 50 μg of diphenylamine (Sigma) ml^{-1}. Diphenylamine increases the susceptibility of *E. coli* to cloacin DF13 by reducing the amount of bacterial O-polysaccharide which can interfere with the receptor of this compound on the cell wall (3, 22). LB agar plates containing 200 μM of 2,2′-dipyridyl were seeded with a lawn of test bacteria (10^5 CFU). Plates were dried for 15 min at 37°C, and 25 μl of cloacin DF13 preparation was added. Following incubation for 18 h at 37°C, inhibition of bacterial growth within the cloacin DF13 spot was evaluated. *E. coli* N5160, N3024, and KH576 were used for cloacin DF13 production, as the cloacin DF13-resistant control, and as the cloacin DF13-sensitive control, respectively.

Production of IROMPS. Bacteria were grown in BHI broth with or without 200 μM 2,2′-dipyridyl to an A_{660} of 1.5. The cells were washed twice in normal saline (0.85% NaCl) and suspended in 10 μM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid buffer (HEPES [pH 7.4]; BDH Inc., Toronto, Ontario, Canada), and lysed with a Vibra cell sonicator (Sonics and Materials Inc., Danbury, Conn.). Iron-regulated outer membrane proteins (IROMPs) were extracted with Sarkosyl (*N*-lauroylsarcosine sodium salt) (Sigma) as previously described (4). They were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 12% polyacrylamide gels and the buffer system of Laemmli (20). Proteins were stained with Coomassie brilliant blue R-250.

Hemolysin assay and detection of K1 and K5 capsules. Overnight bacterial cultures were streaked on sheep blood agar and incubated for 18 h at 37°C. The appearance of a zone of erythrocyte lysis around or under bacterial colonies indicated hemolysis. *E. coli* EC49 and EC50 (from our *E. coli* collection) were used as positive and negative controls, respectively. The presence of K1 or K5 capsule was determined by using K1 or K5 specific bacteriophages as previously described (13).

Antibiotic susceptibility. In vitro susceptibility of isolates to 14 antimicrobial agents that are frequently used in the poultry industry was determined by the disk standard procedure. Selection of disk concentrations and zone diameter interpretations were done as recommended by the manufacturers (Difco and BBL Microbiology Systems, Cockeysville, Md.) and the National Committee for Clinical Laboratory Standards. The following antibiotics and amounts per disk were used: ampicillin (10 μg), cephalothin (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), lincomycin (2 μg), neomycin (30 μg), penicillin (10 μg), spectinomycin (100 μg), streptomycin (10 μg), sulfamethoxazole-trimethroprim (23.75 and 1.25 μg, respectively), tetracycline (30 μg), and triple sulfa (1,000 μg).

In vivo virulence. The virulence of 20 selected cellulitis-derived isolates was tested by subcutaneous injection of 1-day-old chickens, obtained from a local hatchery. The isolates were grown overnight at 37°C in BHI broth to an A_{660} of 1.5 and diluted in the same medium to a concentration of approximately 2×10^6 CFU ml^{-1}. Chickens in groups of 10 or 11 were inoculated with 0.25 ml of culture ($\sim 5 \times 10^5$ CFU). The dose chosen ($\leq 10^6$ CFU) represents the 50% lethal dose (LD_{50}) that has been considered highly virulent in the young-chicken model (5). The actual infecting doses were determined by viable-cell counts of the inoculum. Chickens were observed for clinical symptoms and mortality 6 h postinoculation and then every 12 h for 7 days. At the time of necropsy, the birds were examined for pericardial lesions and samples were collected aseptically, with a sterile swab, from the pericardium for bacterial culture. *E. coli* EC317, a highly virulent strain (LD_{50}, 5×10^2 CFU) isolated from a generalized infection (19) was used as a positive control. Negative controls, chosen from our culture collection, included a nonpathogenic environmental *E. coli* strain (EC160) and an avian isolate of *E. coli* (EC106) that had previously been shown to be avirulent. Isolates that killed more than 50% of birds were considered highly virulent, whereas those that killed 20 to 50% and 0 to 10% of birds were classified as moderately virulent and avirulent, respectively (5). At the end of the experiment, all surviving birds were killed and examined for pericardial lesions and for the presence of *E. coli*. Birds were handled according to the guidelines outlined in the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care.

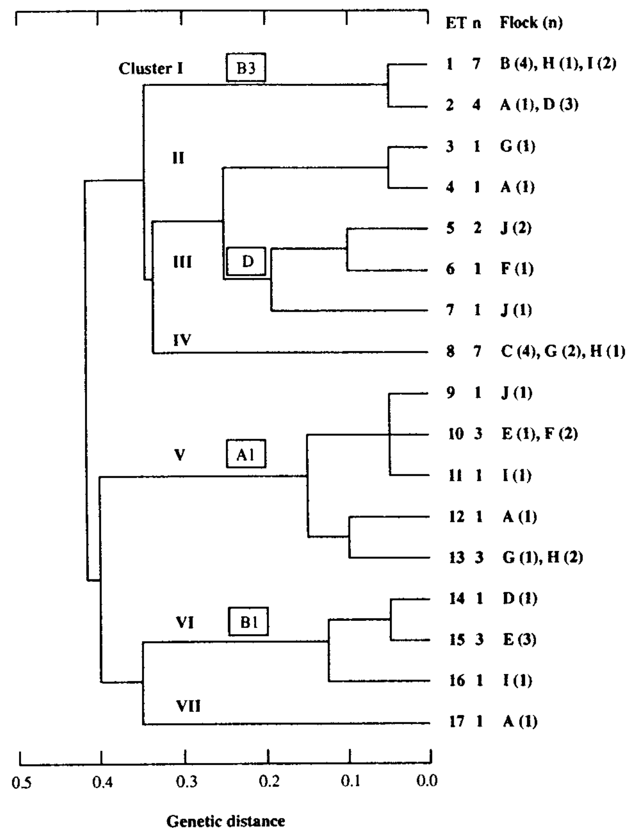

FIG. 1. Dendrogram of the genetic relationships among 17 ETs of cellulitis-derived *E. coli* isolates based on the average linkage cluster analysis. The genetic distance between clusters was measured on the basis of allelic variation detected by multilocus enzyme electrophoresis at 20 enzyme-encoding loci. The total number of isolates in each ET and flock (*n*) is marked on the right. Four distinctive clusters of ETs, B3 (including ET-1 and ET-2), D (ET-5, ET-6, and ET-7), A1 (ET-9, ET-10, ET-11, ET-12, and ET-13), and B1 (ET-14, ET-15, and ET-16) were identified on the basis of the clonal relationships among 17 ETs from the 39 cellulitis-derived isolates and analysis of other pathogenic avian *E. coli* strains (41–44).

RESULTS

Enzyme polymorphisms, ETs, and serotyping. Of the 20 enzymes tested, 14 were polymorphic, i.e., had more than one allele (electromorph) detected at the corresponding enzyme loci. The remaining six enzymes were monomorphic at their respective loci (Table 2). On the basis of distinct allele combinations for the 14 polymorphic enzymes, the 39 cellulitis-derived isolates formed a total of 7 clusters containing 17 ETs (Table 2). Average linkage cluster analysis showed that the 17 ETs could be divided into seven clusters grouped at a distance of 0.3 (Fig. 1). Seven of the ETs had more than one isolate, with each of ET-1 and ET-8 represented by seven isolates and ET-2 represented by four isolates. Cluster I was the largest and represented ~30% of the isolates. This cluster included two ETs (ET-1 and ET-2) that are very closely related and differ only by a single enzyme allele, glutamate dehydrogenase (Table 2). In some cases, isolates from different flocks belonged to the same ET. For example, isolates of ET-1 were from three different flocks (B, H, and I) (Fig. 1; Table 3).

Among the isolates, 15 (38%) were typeable with standard antisera (Table 3). The most frequently observed serogroups were O25 (7 isolates) and O78 (3 isolates), which belonged to ET-8, and ET-1, respectively. Isolates of serogroup O25 were found in three different flocks, whereas isolates of serogroup O78 were from the same flock (Table 3). Serogroup O20, O29, O83, O32/O83, and O(NT):H49 were each represented by a single isolate. The remaining 24 isolates (62%) were untypeable.

Relationships between cellulitis-derived isolates and major groups of ETs from pathogenic avian *E. coli*. On the basis of the clonal relationships among the 17 ETs and previous analysis of pathogenic avian *E. coli* (41–44), there are four groups of ETs, which define clusters I, II, V, and VI (clone complexes previously referred to as B3, D, A1, and B1) (Fig. 1). These clusters accounted for the majority (~75%) of our cellulitis isolates (Table 2). Cluster I was the largest and included 11 isolates of ET-1 and ET-2 (Table 2). Three of the cluster I (ET-1) isolates expressed O78 antigen and were highly virulent in 1-day-old chickens (Table 3). Clusters V and VI represented eight distinct ETs and are composed of nine and five isolates, respectively. Most of the isolates in clusters V and VI were nontypeable. Ten (~25%) of the isolates belonging to clusters II (ET-3, ET-4), IV (ET-8), and VII (ET-17) could not be assigned to any previous known clone complex and may represent *E. coli* strains that are specific to cellulitis in broilers. Of these isolates, seven belong to cluster IV (ET-8) and expressed O25 (Table 3).

HA, expression of K1 or K5 capsule, and hemolysin production. Although all the isolates possess *fim*-related DNA sequences responsible for the expression of F1A fimbriae, only 15 and 22 isolates (38 and 56%, respectively) expressed MSHA of chicken or of guinea pig erythrocytes, respectively (Table 3). Using a probe specific for *papC*-related DNA sequences responsible for biosynthesis of MRHA associated with fimbriae of the P family, we observed that 20 isolates (51%) were *papC*$^+$. Several isolates, lacking *papC* DNA sequences in their genome, showed MRHA of chicken or of human OP_1 erythrocytes after growth on MD-1 agar. However, after the isolates negative for *papC* were passaged 10 times on sheep blood agar, they did not hemagglutinate chicken or human OP_1 erythrocytes. No correlation was found between the presence of *papC* gene in cellulitis isolates and MRHA of human OP_1 erythrocytes.

Three isolates (8%), belonging to cluster III (ET-5 and ET-6), expressed the K1 capsule as detected with a bacteriophage specific for this capsule (Table 3). All isolates were negative for K5 capsule expression. None of the isolates produced detectable hemolysis of sheep blood agar or hybridized with *hly* probe related to DNA sequences for hemolysin biosynthesis and expression (results not shown).

Bactericidal effect of normal serum, and colicin V and aerobactin production. All 39 isolates examined were resistant to killing by normal chicken serum, and most isolates multiplied in this serum (results not shown). Twenty-eight isolates (72%) were positive when probed with *traT* DNA sequences responsible for the expression of TraT lipoprotein-induced serum resistance (Table 3).

Colicin V was detected in 36 isolates (92%). The *iuc* DNA sequence associated with aerobactin synthesis was present in all 39 isolates, but aerobactin production was detected in only 32 isolates (82%). All seven aerobactin-negative isolates belonged to electrophoretic cluster IV (ET-8) and expressed the O25 antigen (Table 3).

Sensitivity to cloacin DF13 and expression of IROMPs. To determine the presence of a functional aerobactin transport system, we tested the sensitivity of the isolates to cloacin DF13. This compound is a bacteriocin that attaches to the outer membrane protein involved in the transport of aerobactin. The assay showed that all the cellulitis-derived isolates were resis-

TABLE 3. Serotypes and presence of specific DNA-related sequence in the genome of cellulitis-derived *E. coli* isolates in association with their phenotypic characteristics

Cluster	ET	Serotype[a]	n[b]	Flock	No. of isolates positive for:									
					Specific DNA-related sequence[c]		Colicin V	Aerobactin	K1 capsule	HA[d]				NHA[e]
										MSHA		MRHA		
					papC	*traT*				Ch	Gp	Ch	$HuOP_1$	
I	1	O78:HN	3	B	3	1	3	3	0	0	3	3	3	0
		ON:HN	4	B, H, I	4	1	2	4	0	0	2	4	3	0
	2	ON:HN	4	A, D	0	3	4	4	0	1	1	0	0	3
II	3	O83:HN	1	G	1	1	1	1	0	0	0	0	0	1
	4	ON:HN	1	A	0	1	1	1	0	1	1	0	0	0
III	5	ON:HN	2	J	2	2	2	2	2	2	1	0	0	0
	6	ON:HN	1	F	1	1	0	1	1	1	1	0	0	0
	7	ON:HN	1	J	0	0	1	1	0	1	0	0	0	0
IV	8	O25:HN	7	C, G, H	2	7	7	0	0	6	3	4	3	0
V	9	ON:HN	1	J	0	0	1	1	0	0	0	1	1	0
	10	O29:HN	1	F	1	1	1	1	0	0	1	1	1	0
		ON:HN	2	E, F	2	2	2	2	0	2	2	0	0	0
	11	ON:HN	1	I	1	1	1	1	0	0	0	1	1	0
	12	O32/O83:HN	1	A	0	1	1	1	0	0	0	0	0	1
	13	ON:HN	3	G, H	1	1	3	3	0	0	2	2	0	1
VI	14	ON:H49	1	D	1	1	1	1	0	0	1	1	0	0
	15	ON:HN	3	E	0	2	3	3	0	0	3	3	0	0
	16	ON:HN	1	I	1	1	1	1	0	0	0	1	1	0
VII	17	O20:HN	1	A	0	1	1	1	0	1	1	0	0	0
Total	17		39	A–J	20	28	36	32	3	15	22	21	13	6

[a] N, nontypeable.
[b] *n*, number of isolates.
[c] All isolates contained *iuc*, *iut*, and *fim* sequences.
[d] MS, mannose sensitive; MR, mannose resistant; Ch, chicken erythrocytes; Gp, guinea pig erythrocytes; $HuOP_1$, human erythrocytes of blood group OP_1.
[e] NHA, nonhemagglutinating.

tant to the bacteriolytic activity of cloacin DF13. Treatment of bacteria with 50 μg of diphenylamine ml^{-1} had no effect on the sensitivity of isolates to cloacin DF13 (results not shown). Moreover, incubation of bacteria with concentrations of diphenylamine above 50 μg ml^{-1} was bactericidal for 50% of the isolates and could not be used.

Most isolates produced at least four IROMPs ranging from 70 to 83 kDa when grown under iron-restricted conditions (i.e., in the presence of 200 mM 2,2′-dipyridyl). Some isolates expressed five IROMPs, whereas others expressed only two or three. All isolates that expressed two or three IROMPs of 70 to 72 or 75 to 83 kDa belong to serogroup O25 (results not shown).

Antibiotic susceptibility. The pattern of antibiotic resistance for all isolates is given in Table 4. All the isolates were resistant to penicillin and lyncomycin but sensitive to ciprofloxacin, and less than 10% of the isolates were resistant to ampicillin, cephalothin, and sulfamethoxazole-trimethoprim (results not shown). High levels of resistance (more than 50% of isolates) to erythromycin, triple sulfa, streptomycin, tetracycline, spectinomycin, and doxycycline were observed. Twenty isolates were of intermediate sensitivity to cephalothin (results not shown). Among the ETs represented by more than two isolates, ET-1, ET-2, and ET-10 were relatively homogeneous in their antibiotic susceptibility pattern.

Virulence in chickens. Twenty selected isolates were screened for virulence by subcutaneous inoculation of 1-day-old chickens. The virulence of these isolates was compared with that of the virulent *E. coli* EC317, which induces generalized infection in chickens (19). In this experiment, strain EC317 killed 100% of inoculated chickens. Pericardial lesions were observed in some of the birds that died 24 h postinoculation or later. Chickens that were inoculated with EC317 or cellulitis-derived isolates and died within the first 24 h seldom had pericardial lesions (results not shown). Of the 20 cellulitis isolates tested for lethality, 8, including all of cluster III, were highly virulent on the basis of their ability to kill 50% or more of the tested birds (Table 5). Death usually occurred within the first 2 days postinoculation, and *E. coli* could be isolated in pure culture from the blood of the chickens at necropsy. Most of the birds that died later than 48 h postinoculation showed pericardial lesions similar to those observed in chickens inoculated with *E. coli* EC317. Some of the selected cellulitis-derived isolates, as well as our negative control *E. coli* EC106, did not kill any birds up to 7 days postinoculation (Table 5). However, in contrast to the negative control, birds inoculated with cellulitis-derived isolates that were avirulent at the time of autopsy had mild pericardial lesions in some cases (Table 5). *E. coli* could be isolated in small numbers from these lesions.

TABLE 4. Antibiotic resistance patterns of cellulitis-derived *E. coli* isolates

Cluster	ET	n[a]	No. of isolates resistant to[b]:							
			E (15)	T3 (100)	S (10)	T (30)	SPT (1,000)	D (30)	GM (10)	N (30)
I	1	7	7	7	7	7	7	7	5	7
	2	4	4	4	4	0	4	0	0	0
II	3	1	0	1	1	1	1	1	1	0
	4	1	1	1	1	1	0	1	0	0
III	5	2	2	1	0	2	0	2	0	0
	6	1	1	0	0	0	0	0	0	0
	7	1	1	0	1	0	0	1	0	0
IV	8	7	6	5	5	2	5	0	5	0
V	9	1	1	1	1	1	0	1	0	1
	10	3	3	3	3	3	3	3	3	0
	11	1	1	1	1	1	1	1	0	1
	12	1	0	1	0	1	0	1	0	0
	13	3	3	1	2	3	3	3	0	0
VI	14	1	1	1	1	1	1	1	1	0
	15	3	1	2	0	0	0	0	0	0
	16	1	0	1	1	1	0	1	0	1
VII	17	1	0	1	1	1	0	1	0	1
Total	17	39	32	31	29	25	25	24	15	11

[a] *n*, number of isolates.
[b] Abbreviations for antibiotics: E, erythromycin; T3, triple sulfa; S, streptomycin; T, tetracycline; SPT, spectinomycin; D, doxycycline; GM, gentamicin; N, neomycin. Numbers in parentheses give the disk potency in micrograms.

DISCUSSION

Our results indicate that *E. coli* strains causing cellulitis possess, both at the phenotypic and at the genotypic levels, characteristics and virulence-associated factors similar to those found in *E. coli* strains that induce generalized infection of avian species (1, 8, 21, 31). The majority (~75%) of isolates belonged to clone complexes A1, B1, B3, and D (clusters I, III, V, and VI, respectively), which have been previously described to contain strains from cases of avian colibacillosis and swollen-head syndrome (42–44). Serogroups O25 and O78 were frequently isolated, but the majority of isolates remained untypeable. Serogroup O78, one of the most common serogroups reported in avian colibacillosis, was characteristic of strains of the B3 clone complex. This clone complex has previously been found to be most common among isolates recovered from turkeys with septicemia (42). ET-1 and ET-2 cellulitis isolates were identical in multilocus enzyme genotype to two predominant clones of the B3 complex that have been recovered from turkeys with colisepticemia (42). In contrast, ET-8, consisting of seven isolates which express O25 antigen, could not be assigned a clone complex. The isolates from ET-8 tested in the young-chicken model were of low virulence (Table 5) and did not produce aerobactin (Table 3). This suggested that the production of aerobactin may be important in causing mortality in the young-chicken model but may not be necessary for the production of cellulitis. ET-8 appeared to be relatively widespread, as isolates originated in 3 of the 10 flocks sampled. These isolates may represent, along with isolates included in ET-3, ET-4, and ET-17, clones which are specific for cellulitis in broilers.

All three of the isolates that produced K1 capsular antigen belong to cluster III (Table 3) and are part of the clone complex D, which has also been associated with extraintestinal infections in humans and animals (43, 44). Expression of K1 capsule is a characteristic of *E. coli* isolates from clone complex D and has been highly associated with virulence of *E. coli* causing septicemia in humans and other animals (15, 43, 44). Isolates expressing the K1 capsular antigen, including the positive control strain EC317, were highly virulent and caused 100% mortality (Table 5), usually within 24 h of inoculation (data not shown). The only isolate in cluster III that did not express K1 capsule was also highly virulent but did not produce mortality as rapidly as did isolates expressing the K1 capsule. The small number of cellulitis-derived *E. coli* isolates expressing K1 capsule in this study suggests that this factor may not be important in the development of cellulitis. However, isolation of K1 capsule-positive strains from broilers with cellulitis indicated that *E. coli* from cluster III can cause both septicemia and cellulitis.

Bacterial adhesins play an important role in adherence of bacterial to host epithelial cells (15). Pathogenic avian *E. coli* strains may produce F1A or F1A-like and/or P or P-related fimbrial antigens (7). The role of F1A fimbriae in poultry colisepticemia has been well documented (6), whereas the role of P-related adhesins in *E. coli* pathogenicity has not been ascertained. In this study, all isolates tested contain the *fim* gene but only 57% of these isolates exhibited MSHA of guinea pig erythrocytes typical of type 1 fimbriae. Many isolates (51%) contain *pap*-related DNA sequences associated with Pap (pyelonephritis-associated pili) fimbriae of the P family. However, only 50% of the pap^+ isolates exhibited MRHA of human erythrocytes, indicating that in many cases, type I or II P fimbriae were not expressed. The occurrence of MRHA-negative, pap^+ isolates of *E. coli* pathogenic for poultry has been described by others (7, 8). One cannot exclude the role of P fimbriae in infection, because the expression of P fimbriae

TABLE 5. Virulence of selected cellulitis-derived *E. coli* isolates in 1-day-old chickens

Cluster	ET	Serogroup[a]	Reference isolate[b]	No. of chickens affected/total no.		% of birds affected after 7 days
				Mortality after 7 days	Morbidity after 7 days[c]	
I	1	O78	EC421	5/11	1/6	55
		O78	EC422	5/10	1/5	60
		O78	EC423	6/11	0/5	55
		ON	EC471	4/10	1/6	50
		ON	EC475	1/10	2/9	30
	2	ON	EC420	2/11	5/9	64
		ON	EC435	5/10	0/5	50
II	3	O83	EC447	4/10	1/6	50
III	5	ON*	EC477	10/10	—	100
		ON*	EC479	11/11	—	100
	6	ON*	EC443	10/10	—	100
	7	ON	EC480	10/10	—	100
IV	8	O25	EC427	3/10	2/7	50
		O25	EC448	4/10	3/6	70
		O25	EC470	1/10	1/9	20
V	10	ON	EC441	2/10	2/8	40
		O29	EC444	3/10	2/7	50
	12	O32/O83	EC429	7/10	3/3	100
VI	14	ON	EC438	0/10	2/10	20
VII	17	O20	EC419	4/10	0/6	40
—[d]	—[d]	O2*	EC317	10/10	—	100
		O2*	EC317	10/10	—	100
—[d]	—[d]	ON	EC106	0/10	0/10	0
		O20	EC160	1/10	0/9	10

[a] Asterisks indicate isolates positive for K1 capsule.
[b] Birds were challenged with 5×10^5 to 1×10^6 CFU of bacteria.
[c] Number of animals with obvious lesions at time of autopsy when the experiment terminated.
[d] Multilocus enzyme electrophoresis was not done on control strains.

could be stimulated by conditions that occur in vivo. An alternative explanation may be that the *pap* operon may code for adhesions lacking MRHA of human erythrocytes, as would be the case for class III variants of PapG (37). Lastly, DNA rearrangements or deletions may have occurred in some *pap* genes, which may result in *pap*$^+$ P fimbria-negative isolates, as has been previously described in isolates from urinary tract infections (7).

Resistance to normal serum has been associated with *E. coli* causing generalized infection in poultry and extraintestinal infections in other species (1, 6, 16, 28). All 39 cellulitis-derived isolates were resistant to killing by normal chicken serum, suggesting that serum resistance is advantageous during the production of cellulitis. We found that DNA sequences responsible for the expression of the outer membrane TraT lipoprotein associated with bacterial serum resistance (24) were detected in 72% of the isolates. The recovery of *traT*-negative isolates suggests that factors other than TraT are involved in resistance to serum. Serum resistance is clearly a multifactorial characteristic involving OMPs, cell surface lipopolysaccharide, capsule and O-antigen capsule, and production of aerobactin (1, 17, 18, 28).

Although the role of colicin V in *E. coli* pathogenicity is controversial, production of this bacteriocin may indicate that an organism possesses a battery of properties associated with ColV plasmids, including serum resistance and an aerobactin iron uptake system (40). Previous reports indicate that the majority of *E. coli* isolates causing generalized infection in poultry produced colicin V and the siderophore aerobactin (21, 36), and our results suggest that production of colicin V and production of aerobactin are also common characteristics of *E. coli* strains that cause cellulitis. Iron is required for growth of microorganisms, and the ability to scavenge iron is often critical in the pathogenesis of infectious diseases. *E. coli* strains, including those pathogenic for poultry, overexpress high-molecular-weight IROMPs under conditions where the concentration of iron is limiting. These IROMPs may be potential receptors for aerobactin and enterobactin (1, 12). In our study, under iron-restricted conditions, all the isolates expressed four or five IROMPs, except those of serogroup O25, which expressed only two or three IROMPs. The expression of such IROMPs by cellulitis isolates, along with the production of aerobactin, points to a complex iron-sequestering system. The presence of the aerobactin receptor in these isolates has not yet been confirmed because of problems encountered with the cloacin DF13 assay. Proof of the uptake of aerobactin will require the use of an aerobactin receptor assay. Expression of the IROMPs and production of aerobactin may not be sufficient to confirm the presence of a functional iron transport system in these bacteria, since the IROMPs may require addi-

tional proteins to complete the process of transporting iron into the bacterial cell (12).

A high rate of resistance to several of the antimicrobial agents frequently used in the poultry industry was exhibited by the *E. coli* isolates from cellulitis lesions. Similar results have been observed in other pathogenic avian *E. coli* strains and may reflect the selection of resistant populations owing to extensive use of antibiotics (1). Antibiotic resistance profiles appear to be conserved within ET-1, ET-2, ET-5, and ET-10 and provide further evidence of the relatedness of the isolates within these groups.

The cellulitis-derived isolates tested in 1-day-old-chicken model exhibited a wide range of virulence. It will be interesting to determine if isolates classified as virulent and avirulent with the young-chicken model can induce cellulitis in chickens infected experimentally.

Inoculation of chickens with cellulitis-derived isolates resulted in pericardial lesions in some birds in each of the infected groups. Similar lesions were previously observed when isolates of *E. coli* that cause airsacculitis and associated generalized infection were used in the same model of infection (6, 14). To confirm that *E. coli* isolates from birds with cellulitis are capable of producing generalized infection, it will be necessary to use a more natural route of infection to experimentally inoculate chickens.

None of the cellulitis isolates produced hemolysin or hybridized with *hly* DNA probe. Similar observations have been made in other pathogenic avian *E. coli* and suggest that hemolysin production is not an important factor in poultry colibacillosis.

We have demonstrated that cellulitis-derived *E. coli* isolates belong to the same clone complexes and possess, at both the phenotypic and the genotypic levels, characteristics of pathogenic *E. coli* recovered from avian colibacillosis (1, 7, 31, 43, 44). Furthermore, when inoculated into 1-day-old chickens, these isolates induced pericardial lesions similar to that observed with *E. coli* that causes generalized infection in poultry. We conclude that in many cases, *E. coli* strains isolated from cases of cellulitis, airsacculitis, and associated generalized infection in poultry are clonally related. However, the 25% of the isolates characterized here that belong to clone complexes not previously described may represent *E. coli* strains unique to cellulitis.

Our laboratory has been involved in the development of strategies for the treatment and prevention of colibacillosis in poultry (19). We believe that by reducing the occurrence of *E. coli* causing generalized infection in poultry, we may be able to reduce the prevalence of cellulitis in broilers.

ACKNOWLEDGMENTS

This work was supported by grants from the Alberta Agricultural Research Institute, the Natural Sciences and Engineering Research Council of Canada, and the Canadian Bacterial Diseases Network. Musangu Ngeleka was the recipient of a postdoctoral fellowship from the Saskatchewan Health Services Utilization and Research Commission.

We thank the VIDO animal care unit for technical assistance.

REFERENCES

1. **Allan, B. J., J. V. van den Hurk, and A. A. Potter.** 1993. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. Can. J. Vet. Res. **57:**146–151.
2. **Bindereif, A., and J. B. Neilands.** 1983. Cloning of the aerobactin-mediated iron assimilation system of plasmid ColV. J. Bacteriol. **153:**1111–1113.
3. **Bouchet, A., M. A. Valvano, M. Dho-Moulin, D. Le Roy, and A. Andremont.** 1994. Immunological variants of the aerobactin-cloacin DF13 outer membrane protein receptor IutA among enteric bacteria. Infect. Immun. **62:** 3017–3021.
4. **Deneer, H. G., and A. A. Potter.** 1989. Effect of iron restriction on the outer membrane proteins of *Actinobacillus* (*Haemophilus*) *pleuropneumoniae*. Infect. Immun. **57:**798–804.
5. **Dho, M., and J. P. Lafont.** 1984. Adhesive properties and iron uptake ability in *Escherichia coli* lethal and nonlethal for chicks. Avian Dis. **28:**1016–1025.
6. **Dozois, C. M., N. Chanteloup, M. Dho-Moulin, A. Bree, C. Desautels, and J. M. Fairbrother.** 1994. Bacterial colonization and *in vivo* expression of F1 (type 1) fimbrial antigens in chickens experimentally infected with pathogenic *Escherichia coli*. Avian Dis. **38:**231–239.
7. **Dozois, C. M., J. M. Fairbrother, J. Harel, and M. Bosse.** 1992. *pap*- and *pil*-related DNA sequences and other virulence determinants associated with *Escherichia coli* isolated from septicemic chickens and turkeys. Infect. Immun. **60:**2648–2656.
8. **Dozois, C. M., S. A. Pourbakhsh, and J. M. Fairbrother.** 1995. Expression of P and type 1 (F1) fimbriae in pathogenic *Escherichia coli* from poultry. Vet. Microbiol. **45:**297–309.
9. **Fairbrother, J. M., A. Broes, M. Jacques, and S. Lariviere.** 1989. Pathogenicity of *Escherichia coli* O115K″V165″ strains isolated from pigs with diarrhea. Am. J. Vet. Res. **50:**1029–1036.
10. **Galan, J. E., and R. Curtiss III.** 1991. Distribution of the *invA*, *-B*, *-C*, and *-D* genes of *Salmonella typhimurium* among other *Salmonella* serovars: *invA* mutants of *Salmonella typhi* are deficient for entry into mammalian cells. Infect. Immun. **59:**2901–2908.
11. **Glunder, G.** 1990. Dermatitis in broilers caused by *Escherichia coli*: isolation of *Escherichia coli* from field cases, reproduction of the disease with *Escherichia coli* O78:K80 and conclusions under consideration of predisposing factors. J. Vet. Med. **B37:**383–391.
12. **Griffiths, E.** 1994. Iron acquisition systems in *Escherichia coli*, p. 533–566. *In* C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom.
13. **Gross, R. J., T. Cheasty, and B. Rowe.** 1977. Isolation of bacteriophages specific for K1 polysaccharide antigen of *Escherichia coli*. J. Clin. Microbiol. **6:**548–550.
14. **Gross, W. G.** 1994. Diseases due to *Escherichia coli* in poultry, p. 237–259. *In* C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom.
15. **Hacker, J.** 1992. Role of fimbrial adhesins in the pathogenesis of *Escherichia coli* infections. Can. J. Microbiol. **38:**720–727.
16. **Harel, J., J. M. Fairbrother, C. Forget, C. Desautels, and J. Moore.** 1993. Virulence factors associated with F165-positive *Escherichia coli* strains isolated from piglets and calves. Vet. Microbiol. **38:**139–155.
17. **Jann, K., and B. Jann.** 1992. Capsules of *Escherichia coli*, expression and biological significance. Can. J. Microbiol. **38:**705–710.
18. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
19. **Kwaga, J. K. P., B. J. Allan, J. van den Hurk, H. Seida, and A. A. Potter.** 1994. A *carAB* mutant of avian pathogenic *Escherichia coli* serogroup O2 is attenuated and effective as a live oral vaccine against colibacillosis in turkeys. Infect. Immun. **62:**3766–3772.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
21. **Lafont, J. P., M. Dho, H. M. D'Hauteville, A. Bree, and P. J. Sansonnetti.** 1987. Presence and expression of aerobactin genes in virulent avian strains of *Escherichia coli*. Infect. Immun. **55:**193–197.
22. **Marolda, C. L., M. A. Valvano, and J. H. Crosa.** 1991. Polymorphism in the aerobactin-cloacin DF13 receptor genes from an enteroinvasive strain of *Escherichia coli* and pColV-K30 is associated only with a decrease in cloacin susceptibility. Infect. Immun. **59:**357–364.
23. **Messier, S., S. Quessy, Y. Robinson, L. A. Devriese, J. Hommez, and J. M. Fairbrother.** 1993. Focal dermatitis and cellulitis in broiler chickens: bacteriological and pathological findings. Avian Dis. **37:**839–844.
24. **Moll, A., P. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bacterial activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum in *Escherichia coli*. Infect. Immun. **28:**359–367.
25. **Morris, M. P.** 1994. Broiler cellulitis update. Broiler Ind. **1994**(March)**:**36–39.
26. **Nei, M.** 1987. Molecular evolutionary genetics. Columbia University Press, New York.
27. **Neilands, J. B.** 1992. Mechanism and regulation of synthesis of aerobactin in *Escherichia coli* K12 (pColV-K30). Can J. Microbiol. **38:**728–733.
28. **Ngeleka, M., J. Harel, M. Jacques, and J. M. Fairbrother.** 1992. Characterization of a non acidic polysaccharide capsular antigen of septicemic *Escherichia coli* O115:K″V1651″:F165 and evaluation of its role in pathogenicity. Infect. Immun. **60:**5048–5056.
29. **Orskov, F., and I. Orskov.** 1992. *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. **38:**699–704.
30. **Peighambari, S. M., R. J. Julian, J. P. Vaillancourt, and C. L. Gyles.** 1995. *Escherichia coli* cellulitis: experimental infections in broiler chickens. Avian Dis. **39:**125–134.
31. **Peighambari, S. M., J. P. Vaillancourt, R. A. Wilson, and C. L. Gyles.** 1995. Characteristics of *Escherichia coli* isolates from avian cellulitis. Avian Dis. **39:**116–124.

32. **Purcell, B. K., J. Pruckler, and S. Clegg.** 1987. Nucleotide sequences of genes encoding type 1 fimbrial subunits of *Klebsiella pneumoniae* and *Salmonella typhimurium*. J. Bacteriol. **169:**5831–5834.
33. **Qadri, F., A. Haque, S. M. Faruque, K. A. Bettelheim, R. Robins-Browne, and M. J. Albert.** 1994. Hemagglutinating properties of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. **32:**510–514.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51:**873–884.
36. **Smith, H. W.** 1974. A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicin V. J. Gen. Microbiol. **83:**95–111.
37. **Strömberg, N., P.-G. Nyholm, I. Pascher, and S. Normark.** 1991. Saccharide orientation at the cell surface affects glycolipid receptor function. Proc. Natl. Acad. Sci. USA **88:**9340–9344.
38. **Taylor, P. W.** 1985. Measurement of the bactericidal activity of serum, p. 445–456. *In* M. Sussman (ed.), The virulence of *Escherichia coli*: reviews and methods. Academic Press, Inc. (London), Ltd., London.
39. **Valvano, M. A.** 1992. Pathogenicity and molecular genetics of O-specific side-chain lipopolysaccharides of *Escherichia coli*. Can. J. Microbiol. **38:**711–719.
40. **Waters, L. V., and J. H. Crosa.** 1991. Colicin V virulence plasmids. Microbiol. Rev. **55:**437–450.
41. **White, D. G., M. Dho-Moulin, R. A. Wilson, and T. S. Whittam.** 1993. Clonal relationships and variation in virulence among *Escherichia coli* strains of avian origin. Microb. Pathog. **14:**399–409.
42. **White, D. G., R. A. Wilson, D. A. Emery, K. V. Nagaraja, and T. S. Whittam.** 1993. Clonal diversity among strains of *Escherichia coli* incriminated in turkey colisepticemia. Vet. Microbiol. **34:**19–34.
43. **White, D. G., R. A. Wilson, A. S. Gabriel, M. Saco, and T. S. Whittam.** 1990. Genetic relationship among strains of avian *Escherichia coli* associated with swollen-head syndrome. Infect. Immun. **58:**3613–3620.
44. **Whittam, T. S., and R. A. Wilson.** 1988. Genetic relationships among pathogenic strains of avian *Escherichia coli*. Infect. Immun. **56:**2458–2466.

Editor: B. I. Eisenstein