JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 218–226 Vol. 41, No. 1
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.218–226.2003

Extended Virulence Genotype of Pathogenic *Escherichia coli* Isolates Carrying the *afa-8* Operon: Evidence of Similarities between Isolates from Humans and Animals with Extraintestinal Infections

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Received 6 May 2002/Returned for modification 18 June 2002/Accepted 24 October 2002

The afimbrial AfaE-VIII adhesin is common among *Escherichia coli* isolates from calves with intestinal and/or extraintestinal infections and from humans with sepsis or pyelonephritis. The virulence genotypes of 77 *Escherichia coli afa-8* isolates from farm animals and humans were compared to determine whether any trait of commonality exists between isolates of the different host species. Over half of the extraintestinal *afa-8* isolates were associated with *pap* and *f17Ac* adhesin genes and contained virulence genes (*pap*, *hly*, and *cnf1*) which are characteristic of human extraintestinal pathogenic *E. coli* (ExPEC). PapG, which occurs as three known variants (variants I to III), is encoded by the corresponding three alleles of *papG*. Among the *pap*-positive strains, new *papG* variants (*papGrs*) that differed from the isolates with genes for the three adhesin classes predominated over isolates with *papG* allele III, which in turn were more prevalent than those with allele II. The data showed the substantial prevalence of the enteroaggregative *E. coli* heat-stable enterotoxin gene (*east1*) among *afa-8* isolates. Most of the *afa-8* isolates harbored the high-pathogenicity island (HPI) present in pathogenic *Yersinia*; however, two-thirds of the HPI-positive strains shared a truncated HPI integrase gene. The presence of ExPEC-associated virulence factors (VFs) in extraintestinal isolates that carry genes typical of enteric strains and that express O antigens associated with intestinal *E. coli* is consistent with transfer of VFs and O-antigen determinants between ExPEC and enteric strains. The similarities between animal and human ExPEC strains support the hypothesis of overlapping populations, with members of certain clones or clonal groups including animal and human strains. The presence of multiple-antibiotic-resistant bovine *afa-8* strains among such clones may represent a potential public health risk.

The fimbrial and afimbrial adhesins of the Afa family mediate the adherence of uropathogenic and diarrhea-associated *Escherichia coli* to various host tissues. Among the Afa-related surface antigens, the Afa(Dr^+) adhesins (AfaE-I, AfaE-III, Dr, and F1845) bind to epithelial cells via recognition of the decay-accelerating factor (DAF; also referred to as CD55) carrying the Dr blood group antigen (15, 33, 36, 37, 44). We recently described a new *afa* operon (*afa-8*) encoding afimbrial adhesin AfaE-VIII which does not recognize DAF molecules (Afa(Dr^-) adhesin). The *afa-8* operon can be either chromosome or plasmid borne, suggesting that it may be carried by a mobile element, facilitating its dissemination (16, 34). It therefore appears that in some strains the *afa-8* operon is carried by a 61-kb pathogenicity island (PAI) inserted into *pheV* and/or *pheR* tRNA-encoding genes. Partial characterization of the *afa-8*-containing PAI indicated that this PAI carries the *afa-8* operon as the only known virulence determinant (35).

The *afa-8* operon is common among pathogenic *E. coli* strains isolated from animals and humans (16, 34, 52). It was frequently found in animal and human isolates producing CNF toxins, but it has also been detected in CNF-negative strains isolated from calves and piglets (16, 41). Bloodstream infections in which the bacteria are derived from the intestinal flora by bacterial translocation are common in patients with cancer. Preliminary results showed that the *afa-8* operon is found in CNF1-producing strains associated with this type of bacteremia (22, 38, 42). Therefore, *afa-8* is probably involved in the development of extraintestinal infections associated with primary colonization of the intestine. However, *afa-8* has never been detected in diarrhea-associated human isolates (38). In addition to CNF1, certain *afa-8*-positive strains carry virulence factor (VF) genes including *pap*, *sfa*, *f17A*, and *clpG* (P, S, F17, and CS31A adhesins, respectively) and *hlyA* (hemolysin), which are frequently detected in extraintestinal pathogenic *E. coli* (ExPEC) (1, 17, 36, 41, 42).

ExPEC strains are the major cause of neonatal meningitis, gram-negative organism-associated bacteremia, pyelonephritis, cystitis, and prostatitis. Most ExPEC strains are derived from phylogenetic group B2 (and, to a lesser extent, from group D) and belong to restricted O serogroups (serogroups O1, O2, O4, O6, O18, O75, and O83) (25, 26, 47). The virulence potential of ExPEC is determined largely by the presence of specialized VFs, which can be located on large plasmids or on the chromosomes of pathogenic strains. Recognized VFs of ExPEC, which are infrequent among commensal strains, include adhesins (P, S, and F1C fimbriae and Afa-related adhesins), toxins (hemolysin and cytotoxic-necrotizing factor 1),

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siderophores (aerobactin and yersiniabactin), capsules (K1, K5, and K12), invasins (*Ibe10* and *afaD* genes), and factors contributing to serum resistance (5, 22, 28, 55). Most VF genes are located on PAIs, which are large segments of chromosomal DNA inserted within or near tRNA genes. These PAIs may provide a mechanism of horizontal transfer of genes between lineages within *E. coli* and even between species (6, 7, 19, 20). A high prevalence of VFs typical of human ExPEC among animal isolates allows one to speculate about cross-infection between different host species (40, 42). This idea was supported by phylogenetic studies, which have demonstrated clonal overlap between pathogenic isolates from humans and animals (poultry, calves, piglets, and dogs) belonging to serogroups O4, O6, and O78 that contain CNF1, hemolysin, and other putative VFs (9, 10, 25, 29, 30).

In the present study, to determine whether any trait of commonality exists between animal and human *afa-8* isolates, 32 human and 45 animal pathogenic isolates were characterized with respect to the prevalence of a broad range of VFs. We also compared the susceptibilities of the isolates to antimicrobial agents in relation to the epidemiological source.

MATERIALS AND METHODS

Bacterial isolates. The population studied included 77 *E. coli* isolates from humans ($n = 32$) with bacteremia or urinary tract infections (UTIs; with or without extraurinary tract involvement) and from calves ($n = 42$) and piglets ($n = 3$) with intestinal or extraintestinal colibacillosis. The human, bovine, and porcine isolates were partially characterized previously (13, 22, 42). All the strains carried sequences of the *afa-8* operon, as evidenced by detection of the *afaD8* and *afaE8* genes by PCR or colony hybridization assays as described in a previous report (38).

Human isolates included *afa-8* strains of different origins. Fourteen *E. coli* strains were isolated between 1992 and 1993 from the blood of cancer patients who were hospitalized with bacteremia at the Institut Gustave Roussy (a French cancer reference center). This collection was obtained from A. Andremont (Groupe Hospitalier Bichat-Claude Bernard, Paris, France). Fifteen strains were isolated from the urine of patients with pyelonephritis who were admitted to five different hospitals over the period from 1985 to 1989. This collection was obtained from M. Archambaud (Centre Hospitalier Universitaire Rangueil, Toulouse, France). Three *afa-8* strains isolated in 1999 from patients hospitalized with UTIs were kindly provided by F. Bourlioux (EPS Perray-Vaucluse, Epinay sur Orge, France).

Forty-two *E. coli* strains obtained from intestinal or extraintestinal sites in calves with signs and lesions typical of septicemia ($n = 16$) or enteritis ($n = 26$) in European countries (Belgium, France, Spain) were also studied. All these strains were obtained from J. G. Mainil (Faculté de Médecine Vétérinaire, Université de Liège, Liège, Belgium) and from M. Contrepois (Laboratoire de Microbiologie, Centre de Clermont-Ferrand/Theix, Institut National de la Recherche Agronomique, St Genès Champanelle, France). Three strains isolated from extraintestinal sites of piglets with septicemia were provided by J. Fairbrother (Faculty of Veterinary Medicine, University of Montreal, Montreal, Quebec, Canada).

Uropathogenic *E. coli* strain J96 was used as a reference strain for *papG* alleles I and III, and IA2 was used as a reference strain for *papG* allele II and for other VF genes (*papAH*, *papC*, *papEF*, *sfa*/*foc*, *hlyA*, *cnf1*). CFT073 was used as a reference strain for PAI I_{CFT073} markers *malX*, *cysB*, and *modB*. Bovine septicemia-associated strain 31A was used as a reference strain for the *f17Ac* (also called 20K or *gafD*), *papGrs* (a new *papG* variant), *clpG* (major subunit of the CS31A adhesin), *east1* (enteroaggregative *E. coli* heat-stable enterotoxin 1), and *iutA* (aerobactin) genes.

Bacterial cultures, DNA extraction, and serotyping. *E. coli* strains were grown in Luria-Bertani broth at 37°C overnight. Genomic DNA was extracted by rapid lysis, as described previously (3), and stored at −20°C for further analysis.

Fifty-two strains were O serotyped by the International *Escherichia* and *Klebsiella* Center, Statens Serum Institut, Copenhagen, Denmark.

Genotyping of VFs. Isolates were tested by multiplex PCR assays (28) for putative VF genes of extraintestinal pathogenic *E. coli*, including *pap*-encoding sequences (*papAH*, *papC*, *papEF*, and the three *papG* alleles), *sfa*/*foc* (central region of the *sfa*/*foc* operon), *hlyA* (hemolysin), *cnf1* (cytotoxic necrotizing factor type 1), *cnf2* (cytotoxic necrotizing factor type 2), *iutA* (aerobactin), *kpsMT* II (group II capsule synthesis), *iha* (nonhemagglutinating adhesin-associated PAI I_{CFT073}), and *iroN* (catechol siderophore receptor-associated PAI_{CP9}). Hemolysin was detected both by PCR and by detection of hemolysis on sheep blood agar.

As reported previously (4), bovine septicemia-associated strain 31A was positive for the different *pap* elements but negative for all three recognized *papG* adhesin classes. However, hybridization experiments revealed that strain 31A carried a complete *pap* operon, suggesting the presence of a new *papG* variant (referred to as *papGrs*). Detection of such a *papG*-related sequence was done by a PCR assay with a new pair of primers: *papG4* (3′-CCTGTCAGGCTGTAATGATGCT-5′) and *papG-C* (3′-CAAGACACAGAAAGAGTCTGAGCC-5′). PCR was carried out as described previously (4) at an annealing temperature of 49°C. When used in combination, primers *papG4* and *papG-C* revealed a product of 708 bp for *pap*-positive strains that were negative for the three recognized adhesin classes. Slight differences in the sizes of the PCR products suggest that different variants of *papGrs* may exist.

To detect the F17-related fimbrial adhesins and to identify the four alleles of the major structural subunit (F17A), strains were tested for the *f17Aa*, *f17Ab*, *f17Ac*, and *f17Ad* genes as described by Bertin et al. (2). Genes *clpG* and *east1* were detected by PCR as described previously (3). Production of F17 and CS31A adhesins was detected by both PCR and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of protein extracts. Concordant results were obtained by the two methods (data not shown). Investigation of the location of the *afa-8* operon by hybridization between the *afaD8* and *afaE8* amplification products, which were used as probes, and plasmid DNA from the isolates was done as described previously (35).

The genes *malX*, *cysB*, and *modD* were used as markers for PAI I_{CFT073} (18, 31). The primers used for PCR and the sizes of the amplified fragments were as follows: for *malX*, primers *malX-L* (5′-GCGATCGGCCAACCTGTTCT-3′) and *malX-R* (5′-CGGTTCGGCTGTGATTGGTG-3′), 429 bp; for *cysB*, primers *cysB-L* (5′-GGATAACCAATAGCAGAACAA-3′) and *cysB-R* (5′-AGTTATTGACATCGCATGGT-3′), 682 bp; and for *modD*, primers *modD-L* (5′-AGCTGAAGTACGTCTGGTTG-3′) and *modD-R* (5′-TTCTGTCGCTTGTAAGATGT-3′), 510 bp. PCR was carried out at an annealing temperature of 52°C. The *espB* gene from the LEE locus carried by enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) and the *stx* (Shiga-like toxin) virulence gene of Shiga toxin-producing *E. coli* (STEC) were detected by a PCR assay as described previously (43).

Detection of *Yersinia* HPI and determination of integration site of HPI in *afa-8* strains. PCR assays were performed as described previously (49) to detect marker genes (*irp1*, *irp2*, and *fyuA*) for the high-pathogenicity island (HPI) of *Yersinia*. To examine the linkage of HPI to the *asnT* tRNA gene, the primer pair *Ec-chrom* and *HPI-5′ end* generated by Schubert et al. (49) was used. As most of the HPI-positive strains did not generate any PCR fragments with this primer pair, a new primer, *P4del* (5′-GAACACATGCAGCATCAGCAG-3′), derived from the 5′ end of the HPI integrase gene, was defined. By using the primer pair *Ec-chrom* and *P4del*, PCR amplicons were obtained with all of the HPI-positive strains. To examine the HPI integration site and the HPI integrase gene, the PCR fragments obtained with primer pair *Ec-chrom* and *P4del* were sequenced for representative HPI-positive strains. Automated DNA sequencing was performed on double-stranded DNA templates with the Prism Ready Reaction dye dideoxy terminator cycle-sequencing kit (Applied Biosystems Inc.) according to the instructions of the manufacturer. Electrophoresis of the sequencing products was performed with an automated sequencer (model 373A; Applied Biosystems Inc.).

Identification of DNA polymorphisms in the *mutS-rpoS* region. In comparison to *E. coli* MG1655, previous studies (14, 21, 39) revealed that EPEC, EHEC, and *E. coli* group B2 strains harbor specific DNA insertions within the *mutS-rpoS* intergenic region. A PCR assay was developed to determine the nature of the *mutS-rpoS* intergenic region of the *afa-8* isolates. The production of two amplicons of 798 bp (primers *O454* and *yclC*) and 608 bp (primers *slyA* and *rpoS*) indicated the presence of the inserted sequence of EPEC 1, EPEC 2, and EHEC 2 groups within the *mutS-rpoS* intergenic region. The production of two amplicons of 1,072 bp (primers *O218* and *yclC*) and 608 bp (primers *slyA* and *rpoS*) indicated the presence of the inserted sequence of O157:H7 and EHEC 1 strains within the *mutS-rpoS* intergenic region. The production of two amplicons of 747 bp (primers *O454* and *O347*) and 473 bp (primers *O183* and *rpoS*) indicated the presence of the inserted sequence of the phylogenetic group B2 strains within the *mutS-rpoS* intergenic region. The production of a 650-bp amplicon (primers *O454* and *rpoS*) indicated the presence of the *mutS-rpoS* intergenic region in *E. coli* K-12. The cycling conditions were as follows: denaturation for 60 s at 95°C,

annealing for 90 s at 52°C, and extension for 90 s at 72°C (30 cycles). The primers used for the PCR analysis were as follows: *O454* (3′-GATTGACCTGCCTCTG TTAC-5′), *yclC* (3′-GAATATGTCCGTGCTGGAA-5′), *slyA* (3′-GATAGAAG CAGCTGTCAGCA-5′), *rpoS* (3′-GATATGAAGCAGAGCATC-5′), *O218* (3′-CGTTGGTGATATTCATGGTG-5′), *O347* (3′-CGCTGAAGGTTCAACATA AC-5′), and *O183* (3′-TTGCAGATAGAT-CAGCGTAAC-5′).

Serum bactericidal assay. Survival of the bacteria in 90% fresh nonimmune human serum was examined by the bactericidal assay described by Taylor and Kroll (53). Serum-resistant strain 31A (an *E. coli* strain causing septicemia) and serum-sensitive strain HB101 were used as positive and negative controls, respectively.

Antibiotic susceptibility. The susceptibilities of the isolates to 19 antimicrobial agents were determined in a semisolid medium by use of a commercially available microdilution system (ATB G-strips; BioMerieux, Lyon, France), as recommended by the manufacturer. The following antibiotics and amounts (in micrograms per milliliter of semisolid medium) were used per assay: amoxicillin (16 μg/ml), ticarcillin (16 μg/ml), piperacillin (8 μg/ml), piperacillin-tazobactam (8 and 4 μg/ml, respectively), imipenem (4 μg/ml), cephalothin (8 μg/ml), cefoxitin (8 μg/ml), cefotaxime (4 and 32 μg/ml), ceftazidime (1 and 4 μg/ml), cefepime (4 μg/ml), cefpirome (4 μg/ml), tobramycin (4 μg), amikacin (8 μg/ml), gentamicin (4 μg/ml), netilmicin (4 μg/ml), sulfamethoxazole-trimethoprim (2 and 38 μg/ml, respectively), nalidixic acid (8 μg/ml), pefloxacin (1 and 4 μg/ml, respectively), and ciprofloxacin (1 and 2 μg/ml).

Statistical analysis. The data obtained for the different groups were compared by using the chi-square test and Fisher's exact test. Correlations between traits were assessed by using Pearson's correlation. Comparison of the prevalences of different traits within the same population were made by McNemar's test. The threshold for statistical significance was a P value <0.05. An aggregate VF score was calculated for each strain by summing the number of nonredundant VFs present in the same strain, and VF scores were compared as described by Johnson et al. (23).

Distance matrices based on VF genotype and phenotype data (including biotype and serum resistance) were calculated for all pairwise combinations and were used to infer a similarity dendrogram by the unweighted pair group method with averaging (UPGMA) (50) by use of the numerical taxonomy and multivariate analysis system (SPSS software for Windows, version 10.1; SPSS, Paris, France).

RESULTS

Prevalence of VFs. All of the *afa-8* strains contained at least one of the VF genes studied, which ranged in prevalence from 7% (*kpsMT* II) to 80% (*iutA*) (Table 1). Of the adhesin-encoding genes studied, *pap* and *f17Ac* were more prevalent than *clpG*, *iha*, and *sfa*/*foc* (for all comparisons, $P < 0.05$ by McNemar's test). Over half of the isolates contained *pap* elements (*papAHCEF*) and various *papG* alleles. The newly identified variants (*papGrs*) predominated over those with allele III, which were in turn more prevalent than those with allele II (for all comparisons, $P < 0.05$ by McNemar's test). *papG* allele I was not encountered among the *afa-8* isolates. Of the siderophore-encoding genes studied, *iutA* and *fyuA* were more prevalent than *iroN*. Of note was the substantial prevalence of the enteroaggregative *E. coli* heat-stable enterotoxin gene (*east1*) and the low prevalence of the PAI I_{CFT073} marker genes.

Serotyping. The O serogroups of 52 isolates were determined. Although over half of the *afa-8* strains carried ExPEC-associated virulence genes, serotyping showed the paucity of ExPEC-associated O antigens among these strains (only six isolates had the O4, O83, and O78 antigens). The most frequently observed serogroups were O11 (21 isolates), O101 (9 isolates), and O8 (6 isolates). Two isolates each were serogroup O118, O86, and O9; and four strains were nontypeable.

Antibiotic susceptibility. Most of the isolates (88%) were resistant to at least two antimicrobial agents; only five strains were sensitive to all of the antimicrobial agents tested (Table 2). Only one bovine isolate was resistant to imipenem and ciprofloxacin. A high prevalence of resistance (>50% of isolates) to piperacillin, ticarcillin, and trimethoprim-sulfamethoxazole was observed. Compared with all other epidemiological sources, antimicrobial resistance was highest among animal bacteremia isolates (antimicrobial resistance score, 9.0 versus 5.9).

Bactericidal effect of normal human serum. Among the 77 isolates, 52 (67%) were resistant to killing by 90% human serum (Table 1). Compared to the isolates associated with intestinal infections, extraintestinal isolates exhibited a significantly higher prevalence of resistance to serum ($P < 0.05$). Several VFs were associated with increased serum resistance, including *pap* genes ($P = 0.02$), *hlyA* ($P = 0.01$), and HPI ($P = 0.03$). In contrast, serum resistance was negatively associated with *cnf2* ($P < 0.01$) and *iha* ($P = 0.005$).

HPI of *Yersinia*. Among the *afa-8* isolates, HPI was the second most frequent VF overall (Table 1). Compared to the diarrhea-associated isolates, the extraintestinal isolates had a higher prevalence of HPI (77 versus 51%; $P = 0.02$). The products obtained by PCR with the *Ec-chrom* and *P4del* primer pair with 19 (36%) of the 52 HPI-positive strains had the predicted lengths (1,360 bp). Sequencing of this 1,360-bp amplicon showed that the HPIs of these strains were directly linked to the *asnT* tRNA gene and had an intact HPI integrase gene. Interestingly, the PCR products obtained with the remaining 33 (64%) HPI-positive strains were about 350 bp smaller than those obtained with the other strains (1,010 bp instead of 1,360 bp), indicating a deletion in the HPI integrase gene. Sequencing of the 1,010-bp amplicon from four different strains (data not shown) revealed a 347-bp deletion, resulting in a truncated HPI integrase gene.

Plasmid-borne *afa-8* operon. The location of the *afa-8* operon was investigated by hybridization between *afaD8* and *afaE8* amplification products, which were used as probes, and plasmid DNA from the 77 isolates. Hybridization with plasmid DNA was detected for 16 of the 77 (20%) isolates. Several VFs displayed specific associations with either the chromosome- or the plasmid-borne *afa-8* operon. Although *clpG*, *papG* allele III, *hly*, and *east1* were significantly associated with the chromosomal location of the *afa-8* operon (for all comparisons, $P < 0.03$), for the bovine isolates, *cnf2* and *papG* allele II were significantly associated with a plasmid location of the *afa-8* operon (for all comparisons, $P < 0.01$) (Table 1).

Polymorphism in the *mutS-rpoS* region. Among the 77 *afa-8* strains, 22 (29%) differed from *E. coli* K-12 in the *mutS-rpoS* intergenic region (Table 1). A first group (10 isolates) comprising essentially animal isolates (including 6 of the 7 STEC isolates) harbored the *mutS-rpoS* intergenic region of EPEC group 1, EPEC group 2, and EHEC group 2 strains. A second group (seven isolates) comprising members of different host groups (five human extraintestinal isolates and two bovine diarrhea-associated isolates) harbored the *mutS-rpoS* intergenic region of *E. coli* O157:H7 and EHEC group 1 strains. A third group (five isolates), which included exclusively the O4 and O83 human isolates, harbored the *mutS-rpoS* intergenic region of the phylogenetic group B2 strains.

Cluster analysis of VF profiles and O antigens. To determine whether the isolates segregate according to their VF

TABLE 1. Distribution of VFs in the 77 *E. coli afa-8* strains by epidemiological source

VF gene[a] (no. of strains)	Prevalence (%) of VF					
	Total *afa-8* strains (n = 77)	Human pyelonephritis + UTI (n = 18)	Human bacteremia (n = 14)	Animal bacteremia (n = 19)	Animal enteritis (non-STEC) (n = 19)	Animal enteritis (STEC) (n = 7)
afa-8 operon (chromosome)	75	88	100	82	57[f]	50
afa-8 operon (plasmid)	25	12	0[d,f]	18[d]	43[f]	50
papAHCEF (39)	51	61	57[f]	78[e]	26[e,f]	0
papG$_{\text{allele II}}$ (5)	6	0	0	16	10	0
papG$_{\text{allele III}}$ (15)	19	28	22	24	10	0
papGrs (19)	25	33	35[f]	38[e]	5[e,f]	0
sfa/*foc* (8)	10	22	14	0	0	0
iha (14)	18	17	37[d]	8[d]	8	42
F17 (39)	51	55	36	61	56[b]	0[b]
f17Aa (6)	8	0	0	5	25	0
f17Ab (6)	8	0	0	5	20	0
f17Ac/*gafD* (27)	35	55	35	50[e]	10[e]	0
clpG (31)	41	44	36[f]	68[e]	21[e,f]	0
hlyA (15)	20	28	21	31	10	0
cnf1 (15)	20	28	21	31[e]	10[b,e]	0[b]
cnf2 (13)	17	0	0[f]	14[e]	55[b,e,f]	0[b]
east1 (24)	32	28	43	42	21	28
kpsMT IIp (8)	7	38[c]	0[c]	0	0	0
iutA (60)	80	78	86	77	85	57
HPI (*fyuA, irp1, irp2*) (52)	69	72	78[f]	84[e]	47[e,f]	57
HPI *intN* (19)	25	39	21	50	15	0
HPI *intD* (33)	43	33	57	50	35	57
iroN (24)	31	55	50[f]	33[e]	5[e,f]	0
modD (14)	18	44	28[d]	5[d]	10	0
PAI$_{\text{CFT073}}$ (6)	8	28[c]	7[c]	0	0	0
Polymorphism *mutS-rpoS* (22)	29	44	14	5[e]	15[e]	100
EHEC group 1 strains (7)	9	22	7	0	5	14
EPEC group 1 and 2 strains (10)	13	6	0	5	0[b]	86[b]
Group B2 strains (5)	7	22	7	0	0	0
Serum resistance (52)	67	72	86[f]	88[e]	35[e,f]	57

[a] VF genotypes were detected by PCR; for F17 and CS31A adhesins, serum resistance, and antimicrobial resistance, however, phenotypic observations were used.
[b] For comparisons between animal enteritis (STEC) and animal enteritis (non-STEC), $P < 0.01$ for *cnf1, cnf2,* F17, and *mutS-rpoS* intergenic region of EPEC group 1 and EPEC group 2.
[c] For comparisons between human pyelonephritis and human bacteremia isolates, $P < 0.01$ for *kpsMT* II and PAI I$_{\text{CFT073}}$.
[d] For comparisons between human bacteremia and animal bacteremia isolates, $P < 0.01$ for *iha, modD,* and the plasmid-borne *afa-8* operon.
[e] For comparisons between animal bacteremia and animal enteritis isolates (non-STEC), $P < 0.01$ for *pap* elements, *papGrs*, *f17A*, *clpG*, *cnf2*, HPI, and serum resistance.
[f] For comparisons between human bacteremia and animal enteritis isolates (non-STEC), $P < 0.01$ for *papGrs*, *f17Aa*, *cnf2*, HPI, *iroN*, the plasmid-borne *afa-8* operon, and serum resistance.

serotype profiles, similarity relationships among the isolates were assessed by UPGMA. The dendrogram (data not shown) resolved the population into five main clusters. All strains containing traits of ExPEC were segregated into clusters I, III, and V. The remaining isolates segregated into clusters II and IV according to their diarrhea-associated VF profiles (Table 3).

Cluster I was the largest and accounted for 52% of the *afa-8* isolates. Within this cluster, five subclusters with different O antigens and VF profiles were resolved. Of particular interest

TABLE 2. Antibiotic resistance of the *E. coli afa-8* strains by epidemiological source

Host	Source	ARS[a]	% Strains resistant to the following antimicrobial agent[b]:																
			AMX	TIC	TZP	PIP	CEF	CXT	FOX	CAZ	FEP	CPO	TOB	AMK	GEN	NET	SXT	NAL	PEF
Human	Pyelonephritis + UTI (n = 18)	5.6	83	66	5	72	83	16	0	16	0	0	11	22	0	0	36	11	6
Human	Bacteremia (n = 14)	7.6	86	86	46	86	94	33	13	26	0	0	20	6	6	6	50	40	6
Animal	Bacteremia (n = 19)	9.1	94	52	10	47	100	74	47	63	47	47	32	42	26	31	47	31	31
Animal	Enteritis, non-STEC (n = 19)	5.4	76	52	16	56	80	32	8	28	8	8	24	12	20	16	56	20	12
Animal	Enteritis, STEC (n = 7)	5.3	86	71	14	42	57	14	0	0	0	0	0	0	0	0	42	14	0

[a] ARS, antibiotic resistance score, which corresponds to the mean number of agents to which the strains were found to be resistant.
[b] AMX, amoxicillin; TIC, ticarcillin; TZP, piperacillin-tazobactam; PIP, piperacillin; CEF, cephalothin; CTX, cefotaxime; FOX, cefoxitin; CAZ, ceftazidime; FEP, cefepime; CPO, cefpirom; TOB, tobramycin; AMK, amikacin; GEN, gentamicin; NET, netilmicin; STX, sulfamethoxazole-trimethoprim; NAL, nalidixic acid; PEF, pefloxacin.

were the seven bovine and the three human isolates that constituted subcluster ID. With one exception, all isolates expressed the O11 antigen and displayed the profile of uropathogenic *E. coli* strains, including the *pap* operon with *papG* allele III, *fyuA*, *iutA*, *cnf1*, *hlyA*, and serum resistance. Interestingly, all isolates in this subcluster shared, in addition to ExPEC-associated traits, the two diarrhea-associated traits *clpG* and *east1*. Cluster II comprised 17 bovine isolates. None of these strains contained ExPEC-associated traits, but most of the isolates bearing the *f17Ab* and *cnf2* genes and the plasmid-borne *afa-8* operon were concentrated in this cluster. Cluster III, comprising exclusively human extraintestinal isolates, had the highest aggregate VF score (12.4 versus 6.0; $P = 0.01$). Isolates of this cluster exhibited ExPEC-associated VF serotype profiles and shared the *mutS-rpoS* intergenic region of group B2 strains. The markers PAI I_{CFT073} (*malX* and *cysB*) and *sfa*/*foc* were found exclusively within this cluster. In addition to ExPEC-associated traits, all isolates in this cluster expressed bovine intestinal adhesin F17c. In cluster IV, seven diarrhea-associated bovine isolates displayed the VF profile of STEC. All strains in this cluster shared the *mutS-rpoS* intergenic region of EPEC or EHEC strains. In cluster V, seven human isolates associated with extraintestinal infections exhibited VF profiles that differed from modal VF profiles for both intestinal and extraintestinal strains in that they had a combination of the VFs of the two groups (*kpsMT* II, *east1*, *modD*, and serum resistance). All isolates that constituted this cluster shared the *mutS-rpoS* intergenic region of EPEC or EHEC strains.

Distribution of VFs by epidemiological source. The distributions of the VFs among the 77 *afa-8* strains were analyzed in relation to the epidemiological sources of the isolates (Table 1), with strains stratified into five groups (human pyelonephritis [n = 18], human bacteremia [n = 14], animal bacteremia [n = 19], non-STEC animal enteritis [n = 19], and STEC [n = 7]). No statistically significant difference ($P < 0.01$) between human and animal extraintestinal isolates with respect to ExPEC-associated traits (including the uniform presence of *pap*, *F17Ac*, *hly*, *cnf1*, *fyuA*, and serum resistance) was observed. However, a significant difference with respect to the prevalence of individual VFs was observed, including *sfa*/*foc*, *kpsMT* II, *iroN*, PAI I_{CFT073}, and the *mutS-rpoS* intergenic region of the group B2 strains (which were more prevalent in human isolates than in animal isolates), *east1* (which was more prevalent in bacteremia-associated isolates than in isolates of any of the other groups), and *pap*, *clpG*, *iroN*, *fyuA*, and serum resistance (which were more prevalent in bacteremia-associated isolates than in enteritis-associated isolates). When the prevalence of individual traits was examined, the 26 diarrhea-associated bovine isolates differed from the extraintestinal isolates with respect to the lack of ExPEC-associated traits. Most of the *cnf2* genes and the plasmid-borne *afa-8* operon were found among diarrhea-associated bovine isolates.

DISCUSSION

Previous studies have reported the association of the *afa-8* operon with CNF1- or CNF2-producing *E. coli* strains isolated from intestinal or extraintestinal infections in humans and animals (16, 38, 41). The present study provides novel epidemiological information on the distributions of the various VFs of AfaE-VIII-producing *E coli* strains.

Although the enteroaggregative *E. coli* heat-stable enterotoxin 1 gene (*east1*) was common in *E. coli* diarrheagenic isolates from humans and animals and EAST1 has primarily been regarded as an enteric VF (48, 54), this study revealed the substantial prevalence of the *east1* gene among extraintestinal *afa-8* isolates. Little is known about the pathogenic significance of EAST1 in diarrhea, but previous reports (3, 54) indicated that it may be found in association with different diarrheagenic VFs, including adhesins (CS31A, CFA/II, K88) and toxins (heat-stable toxin STa, heat-labile toxin). Consistent with these previous findings, a significant association between *east1* and *clpG* (the major subunit of CS31A) was observed among extraintestinal *afa-8* isolates. The recent finding that fecal strains with ExPEC-associated traits were isolated from dogs with diarrhea (51) raises the question whether these isolates are capable of causing intestinal as well as extraintestinal infections. In our opinion, the different findings from this study yield further evidence for the involvement of diarrheagenic isolates in the development of extraintestinal infections: (i) cluster 1D isolates from calves with diarrhea were indistinguishable in terms of their O types and VF profiles from isolates from calves with extraintestinal infections; (ii) among certain extraintestinal isolates, enteritis-associated traits (*f17Ac*, *clpG*, *east1*) were found to be associated with ExPEC-associated traits (*papG* allele III, *hlyA*, *cnf1*); and (iii) many extraintestinal *afa-8* isolates with ExPEC-associated traits exhibited the O8, O9, O11, and O101 antigens, which are commonly found in fecal or diarrheagenic isolates (11, 13, 55).

The HPI of *Yersinia*, which carries the yersiniabactin siderophore system, was common in *afa-8* isolates. Consistent

TABLE 3. Characteristics of the 77 *E. coli* isolates from humans and farm animals with intestinal or extraintestinal infections

Cluster and strain[a]	Host	Source[b]	O antigen	ARS[c]	SR[d]	*afa-8*[e]	Putative VF[f]																	
							kpsII	*pap*	*pagG*	*clpG*	*f17A*	*sfa*	*iha*	*hly*	*cnf*	*east1*	*stx*	*iutA*	*iroN*	HPI	*int*	*modD*	PAI	*mutS/ rpoS*
IA																								
29	Human	P	O+	1	−	C	−	−	−	+	c	−	−	−	−	−	−	+	+	+	N	−	−	−
32	Human	P	O78	6	+	C	+	−	−	−	c	−	−	−	−	−	−	+	+	+	N	−	−	−
48	Porcine	E	O118	16	+	C	−	−	−	−	c	−	−	−	−	−	−	+	+	+	N	−	−	−
49	Porcine	E	O118	19	+	C	−	−	−	−	c	−	−	−	−	−	−	+	+	+	N	−	−	−
13	Human	S	O11	9	+	C	−	−	−	−	a	−	−	−	−	−	−	+	+	+	D	−	−	−
8	Human	S	O+	11	+	C	−	−	−	+	c	−	+	−	−	+	−	+	+	+	D	+	−	−
11	Human	S	ND[g]	1	+	C	−	−	−	−	c	−	−	−	−	−	−	+	−	+	D	−	−	−
IB																								
41	Bovine	E	O8	12	+	P	−	+	II	+	c	−	−	−	−	−	−	+	+	+	D	−	−	−
40	Bovine	E	O8	11	+	P	−	+	II	+	c	−	−	−	−	−	−	+	+	+	D	−	−	−
39	Bovine	E	O8	13	+	P	−	+	II	+	c	−	−	−	−	+	−	+	+	+	N	−	−	−
47	Bovine	I	O11	1	−	C	−	+	II	+	b	−	−	−	2	−	−	+	−	+	D	−	−	−
58	Bovine	I	O8	4	+	P	−	+	II	−	−	−	−	−	2	−	−	+	−	−	−	+	−	EHEC
43	Bovine	E	O11	10	+	C	−	+	rs	−	a	−	−	−	−	−	−	−	−	+	D	−	−	−
68	Bovine	E	O11	5	+	C	−	+	rs	+	c	−	−	−	−	−	−	+	−	+	−	−	−	−
45	Bovine	E	O8	3	+	C	−	+	rs	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−
50	Bovine	E	O11	8	+	C	−	+	rs	+	c	−	−	−	−	+	−	+	−	+	D	−	−	−
42	Bovine	E	O8	6	−	C	−	+	rs	+	c	−	−	−	−	−	−	−	−	+	N	−	−	−
27	Human	P	ND	7	+	C	−	+	rs	−	c	−	−	−	−	−	−	+	+	+	D	−	−	−
24	Human	P	O+	7	+	C	−	+	rs	+	c	−	−	−	−	−	−	+	−	+	D	−	−	−
26	Human	P	O9	6	+	C	−	+	rs	+	c	−	−	−	−	−	−	+	+	+	D	−	−	−
44	Bovine	E	O11	6	+	C	−	+	rs	+	c	−	−	−	−	−	−	+	−	+	D	−	−	−
14	Human	S	O11	7	+	C	−	+	rs	+	c	−	−	−	−	−	−	+	+	+	D	−	−	−
7	Human	S	ND	6	+	C	−	+	rs	−	−	−	−	−	−	−	−	−	−	+	D	−	−	−
12	Human	S	O11	11	+	C	−	+	rs	−	−	−	−	−	−	−	−	+	−	+	D	−	−	−
IC																								
23	Human	P	O101	5	−	C	−	+	rs	−	−	−	+	−	−	−	−	+	+	+	N	−	−	−
51	Porcine	E	O101	7	+	C	−	+	rs	−	−	+	−	+	1	−	−	+	+	−	−	−	−	−
8	Human	S	O101	8	+	C	−	+	rs	−	−	−	−	−	−	−	−	+	+	+	N	−	−	−
10	Human	S	O101	6	+	C	−	+	rs	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
ID																								
35	Bovine	E	O11	4	+	C	−	+	III	+	−	−	+	+	1	+	−	+	−	+	D	−	−	−
33	Bovine	E	O11	3	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
52	Bovine	I	O11	8	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
53	Bovine	I	O11	12	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
36	Bovine	E	O11	17	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
3	Human	S	O11	12	+	C	−	+	III	+	−	−	+	+	1	+	−	+	−	+	D	−	−	−
4	Human	S	O11	12	+	C	−	+	III	+	−	−	+	+	1	+	−	+	−	+	D	−	−	−
34	Bovine	E	O11	8	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
19	Human	E	O11	8	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
46	Bovine	E	O88	17	+	C	−	+	III	+	−	−	−	+	1	+	−	+	−	+	D	−	−	−
IE																								
25	Human	P	O11	11	+	C	−	−	−	+	c	−	−	−	−	+	−	+	−	+	D	−	−	−
55	Bovine	I	O86	7	−	C	−	−	−	+	−	−	+	−	−	+	−	+	−	+	D	−	−	−
5	Human	S	O11	7	−	C	−	−	−	+	−	−	+	−	−	+	−	+	−	−	−	−	−	−
II																								
69	Bovine	I	O101	10	+	C	−	−	−	−	b	−	−	−	−	−	−	−	−	−	−	−	−	−
21	Human	P	ND	0	−	P	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−
70	Bovine	I	ND	6	−	C	−	−	−	−	b	−	−	−	−	−	−	−	−	−	−	−	−	−
61	Bovine	I	ND	4	+	P	−	−	−	−	b	−	−	−	2	−	−	+	−	−	−	−	−	−
64	Bovine	I	ND	0	−	P	−	−	−	−	−	−	−	−	2	−	−	+	−	−	−	−	−	−
57	Bovine	I	ND	0	−	P	−	−	−	−	−	−	+	−	2	−	−	+	−	+	D	−	−	−
66	Bovine	I	ND	0	−	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
62	Bovine	I	ND	7	−	P	−	−	−	−	−	−	−	−	2	+	−	+	−	+	D	−	−	−
63	Bovine	I	ND	10	−	P	−	−	−	−	b	−	−	−	2	−	−	+	−	−	−	−	−	−
60	Bovine	I	O101	4	+	P	−	−	−	−	b	−	−	−	2	−	−	+	−	+	N	−	−	−
38	Bovine	E	O11	1	−	C	+	−	−	−	c	−	−	−	2	+	−	+	−	−	−	−	−	−
65	Bovine	I	ND	13	−	C	−	−	−	−	c	−	−	−	2	−	−	+	−	−	−	−	−	−

Continued on following page

TABLE 3—*Continued*

Cluster and strain[a]	Host	Source[b]	O antigen	ARS[c]	SR[d]	*afa-8*[e]	Putative VF[f]																
							kpsII	*pap*	*pagG*	*clpG*	*f17A*	*sfa*	*iha*	*hly*	*cnf*	*east1*	*stx*	*iutA*	*iroN*	HPI	*int*	*modD*	PAI	*mutS/rpoS*
56	Bovine	I	O11	3	−	C	−	−	−	−	c	−	−	−	2	−	−	+	−	−	−	+	−	EPEC
37	Bovine	E	ND	13	+	P	−	−	−	−	b	−	−	−	2	−	−	−	−	+	N	−	−	EPEC
67	Bovine	I	O101	8	−	C	−	+	rs	−	a	−	−	−	−	−	−	+	−	−	−	−	−	−
III																								
15	Human	P	O83	2	+	C	+	+	III	−	c	+	−	+	1	−	−	+	+	+	N	+	+	B2
16	Human	P	O83	2	+	C	+	+	III	−	c	+	−	+	1	−	−	+	+	+	N	+	+	B2
30	Human	P	O83	8	+	C	+	+	III	−	c	+	−	+	1	−	−	+	+	+	N	+	+	B2
1	Human	S	O4	1	+	C	−	+	III	−	c	+	−	+	1	−	−	+	+	+	N	+	+	B2
31	Human	P	O4	7	−	C	−	+	III	−	c	+	−	+	1	−	−	+	+	+	N	+	+	B2
68	Bovine	I	O101	3	−	C	−	−	−	−	a	+	−	−	−	−	−	+	+	+	N	+	−	−
6	Human	S	O+	8	−	C	−	−	−	−	−	+	+	−	−	+	−	+	+	+	N	+	−	−
IV																								
75	Bovine	Stx	ND	7	−	ND	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−	+	−	EHEC
77	Bovine	Stx	ND	8	+	ND	−	−	−	−	−	−	−	−	−	−	+	+	−	+	O	−	−	EPEC
71	Bovine	Stx	ND	5	+	ND	−	−	−	−	−	−	+	−	−	−	+	+	−	+	D	−	−	EPEC
74	Bovine	Stx	O101	3	−	P	−	−	−	−	+	−	+	−	−	−	+	+	−	−	+	−	−	EPEC
73	Bovine	Stx	ND	6	+	C	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	EPEC
76	Bovine	Stx	ND	2	+	G	−	−	−	−	−	−	−	−	−	−	+	−	−	+	D	−	−	EPEC
72	Bovine	Stx	ND	6	−	P	−	−	−	−	−	−	+	−	−	−	+	+	−	+	D	−	−	EPEC
V																								
28	Human	P	O9	6	+	P	+	+	rs	−	−	−	−	−	−	+	−	+	+	+	D	+	−	EHEC
20	Human	P	ND	7	−	C	+	−	−	+	−	−	+	−	−	+	−	+	−	−	−	+	−	EHEC
22	Human	P	ND	4	+	C	−	+	rs	−	−	−	−	−	−	+	−	−	−	−	−	+	−	EPEC
18	Human	P	ND	0	+	C	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	−	EHEC
17	Human	P	ND	5	+	C	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	+	EHEC
59	Bovine	I	101	3	−	C	−	−	−	−	a	−	−	−	−	−	−	−	−	+	N	−	−	EPEC
2	Human	S	ND	8	+	C	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	EHEC

[a] Clusters and subclusters were resolved by similarity relationship analysis (UPGMA).
[b] Source, epidemiological sources: P, patients with pyelonephritis; S, cancer patients with sepsis; E, animals with extraintestinal infections; I, animals with intestinal infections; Stx, intestinal infections with STEC strains.
[c] ARS, antibiotic resistance score, which corresponds to the number of agents to which the strains were found to be resistant (28).
[d] SR, serum resistance; +, survival in fresh human serum; −, killing by fresh human serum.
[e] *afa-8, afa-8* operon location; P, plasmid; C, chromosome; ND, not determined.
[f] For VF genes, + and − indicate the presence or absence of the given gene, respectively. Putative VF genes are further described as follows: *kpsMT II,* group II capsule synthesis; *pap, papAH, papC, papEF;* for *papG,* III, allele III; II, allele II; rs, *papGrs; clpG,* major subunit of CS31A; for *f17A,* a, b, and c indicate alleles *f17Aa, f17Ab,* and *f17Ac,* respectively; *sfa,* central region of the *sfa/foc* operon; *iha,* nonhemagglutinating adhesin-associated PAI I_{CFT073}; *hly,* hemolysin; for *cnf,* 1, cytotoxic necrotizing factor type 1; 2, cytotoxic necrotizing factor type 2; *east1,* enteroaggregative *E. coli* heat-stable enterotoxin 1; *stx,* STEC markers *sepB* and *stx; iutA,* aerobactin; *iroN,* catechole siderophore receptor-associated PAI_{CP9}; HPI, pathogenicity-associated island markers *irp1, irp2,* and *fyuA* from pathogenic *Yersinia* HPI; *int,* HPI integrase gene (D, truncated *int* gene; N, intact *int* gene); *modD,* molybdenum transport; PAI, pathogenicity-associated island markers *malX* and *cysB* from strain CFT073; *mutS/rpoS,* DNA polymorphism in the *mutS-rpoS* region (EHEC, typical of EHEC group 1; EPEC, typical of EPEC groups 1 and 2; B2, typical of strains from phylogenetic group B2). Genotypes were obtained by PCR; hemolysin, however, was detected by both PCR and hemolysis on sheep blood agar, and adhesins F17 and CS31A were detected by both PCR and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[g] ND, not determined.

with previous reports involving other human clinical isolates (12, 28, 49), HPI was significantly more prevalent among extraintestinal *afa-8* isolates than among diarrhea-associated *afa-8* isolates. Among the HPI-positive strains, those which carried a 347-bp deletion in their HPI integrase genes predominated over strains with intact HPI integrase genes. Karch et al. (32) previously identified an identical deletion in the integrase gene of the HPI carried by Shiga toxin-producing *E. coli* serotype O26 strains. The presence of this deletion in the HPI integrase gene carried by all strains in subcluster ID suggests the fixation of HPI in the genomes of these strains.

Recent studies revealed a pathotype-associated polymorphism in the *mutS-rpoS* region of the *E. coli* chromosome (14, 21, 39). PCR analysis showed that 29% of the *afa-8* isolates differed from *E. coli* K-12 in the *mutS-rpoS* intergenic region. The five O4 and O83 human isolates included in cluster III harbored the DNA insertion characteristic of group B2 strains, suggesting that these strains belong to phylogenetic group B2. Among the strains in cluster IV, the concordant distribution of STEC-associated traits and the *mutS-rpoS* intergenic region of EPEC or EHEC strains suggests that these *afa-8* isolates are true STEC strains. Of particular interest was the concentration of the DNA segment specific to EPEC or EHEC strains within human pyelonephritis-associated isolates that lack modal ExPEC-associated traits (cluster V). The DNA sequence conserved in the *mutS-rpoS* intergenic region of EPEC and EHEC isolates encodes the *Salmonella enterica* transcriptional regulator (SlyA). A function of SlyA in pathogenesis is suggested by the results for *Salmonella*, in which SlyA has been shown to play a role in bacterial survival in the intracellular environment

of host macrophages and in the invasion of M cells (8). This finding raises the question whether SlyA has a function in the pathogenesis of certain *E. coli* extraintestinal isolates that lack ExPEC-associated traits.

Two-thirds of the extraintestinal *afa-8* isolates contained *pap* elements and various *papG* alleles. However, half of the *pap*-positive strains carried a *papG* variant which differd from the three recognized adhesin classes. Recently, Bertin et al. (4) demonstrated that bovine septicemia-associated strain 31A carried a complete *pap* operon with a structural subunit (*papA*) which is highly homologous to F11 and an unknown *papG* allele (*papGrs*). The present study indicates that among *afa-8* isolates, *papGrs* predominates over *papG* alleles II and III. Strains containing *papG* allele III differed significantly from those containing *papGrs* and *papG* allele II with respect to the association with *hlyA* and *cnf1*. The observed association of *papG* allele II with intestinal adhesins CS31A and F17 is a novel finding of this study. Consistent with recent findings for avian pathogenic *E. coli* strains (52), this study revealed a high prevalence of intestinal adhesin F17c among human and bovine extraintestinal *afa-8* isolates. In addition to *afa-8,* a significant proportion (22%) of the extraintestinal isolates displayed the associated set of adhesin genes, *pap*, *clpG*, and *f17Ac.*

The hypothesis that humans may acquire ExPEC strains from their domestic animals has been suggested by the similarities observed among isolates recovered from dogs, cats, and humans with UTIs. Recently, Johnson et al. (25) provided evidence of a commonality between canine and human ExPEC strains and suggested that canine feces represent a potential reservoir of *E. coli* with infectious potential for humans. Our finding that bovine and human isolates in subcluster ID were indistinguishable with respect to their O-type and VF profiles also supports the hypothesis of overlapping populations. With respect to their VF profiles, the two human O4 isolates appeared to be highly similar to an isolate taken from a dog with an UTI (strain 840383) described by Johnson et al. (24). Given these similarities, it is possible that certain clones within the *afa-8* population are also pathogenic for humans and dogs.

The AfaE-VIII adhesin is very similar to the M adhesin, an afimbrial adhesin encoded by the *bma* gene cluster (46). The prevalence of the M adhesin in pathogenic *E. coli* strains from humans and dogs has been estimated to be on the order of 1% (24), whereas the prevalence of the *afa-8* operon in pathogenic *E. coli* isolates from humans and farm animals has been estimated to be significantly higher (5 to 25%) (16, 39, 52). Since the prevalence of *afa-8* and *bma* was assessed by PCR assays with different primer sets, differences in the prevalence of *afa-8* and *bma* need to be evaluated in terms of the primers used.

A large number of extraintestinal *afa-8* isolates were resistant to several of the antimicrobial agents frequently used in human medicine. *afa-8* isolates from farm animals were significantly more likely to be multidrug resistant than human isolates. Similar results have been observed with other pathogenic *E. coli* isolates and may reflect the selection of a resistant population due to the use of antibiotic additives in animal feed and the extensive use of antibiotics in veterinary medicine. Previous studies have reported that pathogenic isolates that produce VFs are more sensitive to antimicrobial agents than those without VFs (27, 28, 45). In contrast, the antimicrobial resistance of *afa-8* strains was not correlated with the prevalence of the VFs.

Taken together, our findings suggest that extraintestinal *afa-8* isolates represent distinct clonal groups that differ from other ExPEC isolates. The zoonotic potential of certain clones and the high prevalence of multiple antibiotic resistance among bovine *afa-8* isolates suggest that such strains present a risk to public health.

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