Molecular Comparison of Extraintestinal \textit{Escherichia coli} Isolates of the Same Electrophoretic Lineages from Humans and Domestic Animals

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Molecular typing methods were used to characterize 38 \textit{Escherichia coli} strains that originally were isolated from extraintestinal infections and represented 5 multilocus enzyme electrophoretic types (ETs) recovered from both humans and animals. Within each ET, the human and animal isolates did not consistently segregate by host group, according to individual virulence factors (VFs), composite VF-serotype profiles, or pulsed-field gel electrophoresis profiles. Several close matches with respect to VF-serotype profiles were identified between human and canine isolates from different locales. One canine and 2 human isolates of sero-group O6 closely resembled archetypal human pyelonephritis isolate 536 (O6:K15:H31), according to \textit{pap}A sequence and VF-serotype profile. These findings support the hypothesis that certain pathogenic lineages of \textit{E. coli} cause disease in both humans and animals and that humans may acquire pathogenic \textit{E. coli} from domestic pets.

The controversial hypothesis that humans may acquire extraintestinal pathogenic \textit{Escherichia coli} (ExPEC) from their household pets has been suggested by the many similarities among \textit{E. coli} isolates recovered from urinary tract infections in dogs, cats, and humans [1]. These similarities include the frequent presence of variant III of the digalactoside-specific P-fimbrial adhesin molecule PapG and other extraintestinal virulence factors (VFs) and derivation from some of the same evolutionary lineages, as defined by multilocus enzyme electrophoresis and serotyping [1, 2]. However, despite the observed similarities, human and animal ExPEC could represent host-specialized clones that differ with respect to specific VFs or distinct combinations of (shared) VFs. Such host-specialized clones could be undetected within multilocus enzyme electrophoresis–defined lineages (electrophoretic types [ETs]) based on genetic polymorphisms at conserved housekeeping genes.

To test these hypothesis, we determined extended VF genotypes and pulsed-field gel electrophoresis (PFGE) profiles for all the animal and human extraintestinal infection isolates from the 5 ETs that in a previous study had included isolates from both domestic animals and humans [2]. We compared the animal and human isolates within each ET with respect to O:H serotype, extended virulence genotype, and PFGE profile, to determine whether any trait or combination of traits consistently differentiated animal from human isolates.

Methods

\textit{Strains.} The study population (figure 1) included 38 independent \textit{E. coli} isolates from dogs (n = 15), cats (n = 4), and humans (n = 19) with urinary tract infections, with or without extravesical involvement, from ETs 1, 12, 14, 21, and 35, as defined elsewhere by Whittam et al. [2], by multilocus enzyme electrophoresis with use of 16 metabolic enzymes. Epidemiological data available for the isolates included state of origin (Florida, Michigan, Pennsylvania, or Tennessee), year of submission to the \textit{E. coli} Reference Center (ECRC; University Park, PA), ECRC accession number within that calendar year, and specimen type (if other than urine). Fourteen of the original 52 isolates, for which epidemiological data and PFGE results suggested possible multiple isolations of a particular strain from a single host, were excluded from analysis. Human pyelonephritis isolate 536 (O6:K15:H31) [3] was provided by Gabrielle Blum-Oehler (Universität Würzburg, Germany).

\textit{Serotyping.} O:H serotypes were determined by the ECRC. For the H antigen, strains that were nonmotile were designated as NM, and those that were motile but nontypeable were designated as NT. OX14 is the conditional designation used by the ECRC for a novel O antigen (personal communication, M.A. Davis).

\textit{VF genotyping.} Isolates were tested for 30 putative virulence
Table 1. Characteristics of 38 *Escherichia coli* isolates from humans and animals with extraintestinal infections. All hosts had urinary tract infections; certain animal isolates were recovered from sources other than urine (conj., conjunctiva; prost., prostate; and intest., intestine). Isolates indistinguishable from strain 840619 were recovered also from the spleen and the uterus of same host as 840619, and an isolate indistinguishable from strain 830815 was recovered also from the lung of same host as 830815 (not shown). For H antigens: NM, nonmotile; and NT, nontypeable. F type (allele of *papA*, F-pilus structural subunit gene) was determined by comparative sequence analysis of predicted PapA peptides (isolates 840379, 830043, 830428, and 840222 [shown as "48" or "536"]) or by multiplex polymerase chain reaction (PCR; other *papA*-positive isolates). For virulence factor (VF) genes, 1 and 2 indicate presence and absence of gene, respectively. Presence or absence of F-pilus adhesin gene *papG* (by flanking primer PCR) corresponded precisely with presence or absence of detectable *papG* allele (by internal primer PCR). Only those genes for which ≥1 strain was positive are shown. No strains were positive for *afa*/dra (Dr family adhesins), *nfaE* (non-F pilus adhesins), or *cvaC* (colicin V). Other VFs: *sfa*/foc (common to S- and F1C-pilus operons), *sfaS* (S-pilus adhesin), *focG* (F1C-pilus adhesin), *bma* (M-adhesins), *gaf* (G-adhesins), *iba* (putative adhesin-siderophore), *fenH* (type 1-fimbrial adhesin), *hlyA* (hemolysin), *cfa* (cytotoxic necrotizing factor), *yfaA* (yersiniabactin), *iutA* (aerobactin), *kpsMT*-II and -III (group 2 and 3 capsule synthesis), *kpsMT* K1, K2, and "K5" (variants of *kpsMT*-II; "K5" primers react with all non-K1 and non-K2 group II *kpsMT* variants), *rfa* (O4 lipopolysaccharide synthesis), *ibeA* (invasion of brain endothelium), *sfeT* (serum-resistance associated), and PAI (pathogenicity-associated island marker *malX* from strain CFT073). ET, electrophoretic type; FL, Florida; MI, Michigan; PA, Pennsylvania; PFGE, pulsed-field gel electrophoresis; TN, Tennessee.
factor genes of extraintestinal pathogenic E. coli, including papAH, papC, papEF, papG, and the 3 alleles of papG, using a multiplex polymerase chain reaction (PCR) assay, as described elsewhere [4, 5] (figure 1). Five genes (iha, hlyA, cnf1, iroN, and kpsMT-II) also were detected by dot-blot hybridization, as described elsewhere [4, 5]. Twelve alleles of the P-fimbrial major pilin gene papA were detected by multiplex PCR, as described elsewhere [6].

For strains that were papAH-positive by PCR but negative in the papA allele assay, papAH amplicons were directly sequenced, as described elsewhere [6]. Predicted PapA peptides were compared with reference peptide sequences for the 12 defined PapA variants and for PapA from human pyelonephritis isolate 536 [3] (as recently sequenced by the authors: GenBank accession no. AF237477; unpublished data) by use of CLUSTAL-W [7], as described elsewhere [6].

Cluster analysis of virulence genotype and serotype data. Distance matrices based on virulence factor profiles (including F types) and O:H serotypes, as calculated for all pairwise combinations of the isolates within a given ET, were used to infer similarity dendrograms for each ET, according to the unpaired group method with averaging [1]. Each isolate was assessed visually as to whether its nearest neighbor in the dendrogram represented the same host group (i.e., human-human or animal-animal), the alternate host group (i.e., human-animal or animal-human), or was a mixed cluster comprising animal and human isolates.

PFGE. Macrorestriction analysis of XbaI-digested total DNA by PFGE was done as described elsewhere [1]. Isolates from a given ET were analyzed in parallel in a single gel, with repeat analyses done as needed to clarify ambiguities. Genomic patterns that differed by >3 bands, compared with previously defined patterns within the same ET, were assigned new unique genotype numbers, whereas new patterns that differed by ≤3 bands from a previously defined pattern were designated as subtypes of the index type [1].

Statistical analysis. Comparisons of proportions were tested by use of Fisher’s exact test. Comparisons of the prevalence of different characteristics in the same population were tested by use of McNemar’s test [8].

Nucleotide sequence accession numbers. papA sequences from the present study were deposited in GenBank, under accession numbers AF255002 (strain 830043), AF255003 (strain 840222), AF255004 (strain 830428), and AF287159 (strain 840379).

Results

VF profiles, including papG and papA alleles. Twenty-eight of the 30 VF genes (all except afal, dra, and nfaE) were detected in ≥1 strain each and ranged in prevalence from 3% (gafD) to 97% (fimH; figure 1). The most prevalent papG allele, by far, was allele III (74%: vs. other alleles; < .01, McNemar’s test). According to the PCR assay for F type, 25 of the 29 pap-positive isolates contained ≥1 of the 12 defined papA alleles. The remaining 4 papA-positive isolates, strains 840379 [ET 1], and 830043, 830428, and 840222 [ET 21], were negative by PCR for an F type and thus underwent direct sequencing of papA. The predicted PapA peptides for 3 of these strains (2 human and 1 canine) were virtually identical (99.5%) in amino acid sequence to the PapA variant from human pyelonephritis iso-

late 536, whereas the next closest reference PapA peptide was the F7-2 variant (78.4% identity). These 3 isolates exhibited a consensus VF profile that differed from that of strain 536 only by the presence of focG instead of sfaS (figure 1; authors’ unpublished data). The predicted PapA peptide from the fourth strain, a cat isolate from ET 1, was most similar to the F48 PapA variant (85.3% amino acid identity), with the next closest reference PapA peptide being the F11 variant (83.7% identity).

Comparison of individual characteristics between animal and human isolates. With few exceptions, within each ET, the human and animal isolates overlapped with respect to O:H serotypes and all individual VFs (figure 1). The only traits that differentiated animal from human isolates within a given ET were papG allele 1 and the F14 papA allele within ET 12 and the X14 O antigen and K1 kpsMT variant within ET 35 (figure 1).

Cluster analysis of VF profiles and O:H serotypes. To determine whether human and animal E. coli isolates segregate according to composite VF-serotype profiles, similarity relationships among the isolates within each ET were assessed by cluster analysis of the data (figure 1). Thirty-six discrete patterns were resolved among the 38 isolates (figure 2); 34 of these were specific to a single isolate, whereas 2 patterns were shared by 2 isolates, as indicated by the 2 ties in the ET 21 dendrogram (figure 2). One of the shared profiles involved 2 human isolates from Michigan, and the other involved a human isolate from Michigan and a dog isolate from Florida (figure 2). When the criterion for matching was relaxed to the 98% similarity level, 4 additional pairs of matching isolates were identified, 2 comprising members of the same host group and 2 comprising members of different host groups (figure 2).

Similarity relationships also were assessed by use of a “nearest neighbor” analysis. The nearest neighbor in the dendrogram represented the same host group for 15 of the 38 isolates, the alternate host group for 16 isolates, and a mixed cluster containing representatives from both host groups for 7 isolates (figure 2). Thus, isolates were somewhat more likely to have as their nearest neighbor in the VF-serotype dendrogram a mixed cluster or an isolate from the alternate host group (n = 23) than an isolate from the same host group (n = 15).

PFGE analysis. PFGE resolved 35 discrete genomic patterns (types). Of these, 32 types had no subtypes and were represented by a single isolate each. The other 3 types (5, 17, and 27) each exhibited 2 closely related subtypes (a and b), which also were represented by a single isolate each; thus, no 2 isolates had indistinguishable PFGE patterns. In the 3 instances in which 2 isolates exhibited subtypes of the same PFGE type, both isolates represented the same host group (human, for PFGE types 17 and 27; animal, for PFGE type 5) but were also from the same locale (Tennessee, type 17; Michigan, type 27; Florida, type 5; figures 1 and 3). Certain other strains (including dog-human combinations) exhibited related PFGE patterns that were insufficiently similar to qualify as subtypes of a single PFGE type (figure 3).
Figure 2. Cluster analysis (unpaired group method with averaging) of extended virulence factor-serotype profiles of animal and human Escherichia coli isolates from 5 electrophoretic types (ETs). Scale markings differ among ETs, but all dendrograms are drawn to same scale. Host species: C, cat; D, dog; H, human. Locales: FL, Florida; MI, Michigan; PA, Pennsylvania; TN, Tennessee.

Discussion

In the present study, we found that, within the 5 ETs that were analyzed, the human and animal isolates did not segregate according to host group, with respect to individual VFs, combined VF-serotype profiles, or PFGE types. Instead, we observed considerable intermingling of human and animal isolates, including several precise or extremely close VF profile matches between certain human and dog isolates from different locales. These findings, which suggest that some lineages of ExPEC are pathogenic for humans and animals, are consistent with the hypothesis of direct or indirect animal-to-human transmission of extraintestinal pathogens [1].

We found nearly complete overlap between animal and human ExPEC within each ET, with respect to individual VFs. In addition, close correspondence, according to extended VF-serotype profiles, was as frequent among isolates from different host groups as among isolates from the same host group. For a given isolate, the strain with the next most similar VF-serotype profile was as likely to represent the alternate host group (or a cluster containing isolates from both host groups) as the same host group.

In several instances, the VF profiles of certain animal isolates revealed similarities between the animal isolates and archetypal human ExPEC strains. The 2 animal isolates from ET 12 exhibited the F13 and F14 papA and papG alleles I and III. These characteristics, plus the remainder of these strains’ VF profiles, closely resembled those of archetypal human ExPEC isolate CP9 (O4:K54:H5;F13,F14), a representative of the “J96-like” clonal group of E. coli O4:H5, whose members have caused diverse infections in humans and animals in the United States and Europe [9, 10] (unpublished data). Similarly, presence of the distinctive “F536” PapA variant in 3 O6:H31 isolates (2 human and 1 canine) from ET 21 led to the discovery that, according to extended VF profiles and serotype, these isolates are highly similar to archetypal pyelonephritis isolate 536 (O6:K15:H31) [3]. ET 21 from the present study thus appears to correspond with “clonal group 2” of Cherif et al. [11], which contained O6:K15:H31 ExPEC isolates from both animals and humans.

PFGE analysis demonstrated that the most closely related strains occurred within the same host group but were also from the same locale. Sharing of closely related PFGE profiles among hosts from a particular locale is consistent with the possibility of local predominance of specific clones [12], despite the wide-
spread distribution of broader clonal groups. Because of the tremendous diversity of PFGE patterns within *Escherichia coli* and geographic segregation of clones, examination of larger numbers of isolates from different host groups from a single locale would be needed to optimally assess strain sharing among host groups.

Although the present study provides clear evidence of commonality between human and animal ExPEC, it does not prove cross-species transmission or exclude the possibility that humans and animals acquire the same *E. coli* types from common external sources without directly exchanging strains. However, given the documented transmission of other pathogenic microorganisms from dogs and cats to humans [13], it seems highly probable that humans encounter canine- or feline-source *E. coli* with some frequency, whether on the pets themselves or in the environment. Studies of transmission of *E. coli* between domestic animals and humans, and of the health consequences of such transmission, are needed.

The possibility that certain *E. coli* strains are pathogenic for both animals and humans has several implications. First, if animal-to-human transmission contributes appreciably to disease burden in humans, then the interruption of such transmission may provide a new mechanism for disease prevention [1]. Second, if similar virulence mechanisms are operative in human and animal infections due to ExPEC, then vaccines developed for one host group may have cross-protective applicability for the other host group [14]. Third, analysis of virulence mechanisms within one host group may provide insights into pathogenesis in the other host group. Fourth, if domestic animals do transmit *E. coli* to humans, veterinary antibiotic use may be an important driving force for antibiotic-resistant bacteria in humans and thus may warrant the same level of scrutiny as is currently given to antibiotic use in human medicine and in food production [15].

It should be noted that lineages other than the 5 analyzed here also might include both human and animal isolates. If so, the range of *E. coli* VF*s applicable across host species may be even broader than documented in the present study.

**Acknowledgments**

We thank Dave Prentiss and Ann Emery (Minneapolis Veterans Affairs Medical Center) for preparing the figures and for helping with
manuscript preparation, respectively, and Gabriele Blum-Oehler (Universität Würzburg, Germany) for providing human pyelonephritis isolate 536 (O6:K15:H31).

References