JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3721–3725 Vol. 47, No. 11
0095-1137/09/$12.00 doi:10.1128/JCM.01581-09

Sharing of *Escherichia coli* Sequence Type ST131 and Other Multidrug-Resistant and Urovirulent *E. coli* Strains among Dogs and Cats within a Household▽†

James R. Johnson,[1]* Sybille Miller,[2]‡ Brian Johnston,[1] Connie Clabots,[1] and Chitrita DebRoy[3]

VA Medical Center and University of Minnesota, Minneapolis, Minnesota[1]; Veterinary Internal Medicine Practice of Northern Virginia, Manassas, Virginia[2]; and Escherichia coli Reference Center, Pennsylvania State University, University Park, Pennsylvania[3]

Received 16 August 2009/Accepted 20 August 2009

A multidrug-resistant (MDR) *Escherichia coli* strain from a human-associated pulsotype within sequence type ST131 (O25:H4) colonized three of five dogs and cats within a household. Of the four other colonizing strains identified, two were MDR and two colonized multiple hosts. The ST131 strain uniquely exhibited high resistance and virulence scores.

Within-household sharing of *Escherichia coli* strains among humans and pets has been documented in multiple studies (8, 12, 13, 18, 24). This phenomenon, which likely reflects host-to-host transmission, may facilitate the dissemination of virulent and antimicrobial-resistant *E. coli* within the community.

An extensively antimicrobial-resistant *E. coli* clonal group, sequence type ST131 (O25:H4), has been recognized as an important human pathogen only within the last several years, suggesting recent clonal dissemination and expansion (4, 7, 25). Currently best known for its association with extended-spectrum cephalosporin resistance, ST131 has contributed importantly to the global emergence of the CTX-M-15 extended-spectrum beta-lactamase (and, perhaps, vice versa) (4, 7, 25). *E. coli* ST131 also commonly occurs as a fluoroquinolone-resistant (FQ-R) but cephalosporin-susceptible pathogen (1, 15, 20).

Although ST131 has been isolated from a companion animal (a dog) (27), this report did not address other hosts from the same household or the isolate's relationship to human ST131 isolates. The recent finding by the *Escherichia coli* Reference Center (the Pennsylvania State University) of serotype O25:H4 for sequential FQ-R *E. coli* urine isolates from a dog with recurrent/persisting bacteriuria (see below) suggested *E. coli* ST131. The source household included multiple pets, providing an opportunity to investigate within-household sharing of the index FQ-R urine strain. We assessed whether (i) the dog's urine strain represented ST131, (ii) this strain was shared with other pets in the household, (iii) the strain resembled known human ST131 isolates, and (iv) additional sharing of antimicrobial-resistant and/or virulent *E. coli* clones could be found among the household pets.

Subjects and samples. The index canine subject was an Australian shepherd with persisting *E. coli* bacteriuria. Sequential urine samples were collected at a local veterinary clinic by cystocentisis over the 8 months following an initial diagnostic evaluation (in January 2008) for what proved to be immune-mediated thrombocytopenia. Cultures yielded various concentrations (usually >100,000 CFU/ml) of FQ-R *E. coli*. The dog exhibited no clinical signs of symptomatic urinary tract infection. Bacteriuria persisted despite repeated courses of antimicrobial therapy. The isolates exhibited fairly uniform susceptibility profiles, including resistance to ampicillin, fluoroquinolones, and gentamicin and susceptibility to amikacin, amoxicillin (amoxicilline)-clavulanate, extended-spectrum cephalosporins, chloramphenicol, nitrofurantoin, and tobramycin. The only variation involved resistance to tetracycline, ticarcillin, and trimethoprim-sulfamethoxazole, which appeared in month 5. To confirm that serial isolates represented the same strain, and to identify characteristics possibly explaining this strain's persistence, isolates from months 7 and 8 (July and August 2008) were analyzed as described below.

The other household pets were a dog (an Akita) and five cats (three Maine Coons and two short-haired domestic cats). The Akita had a history of chronic extremity infection, treated empirically with various antimicrobial agents over the preceding 2 years without cultures, and asymptomatic bacteriuria, documented in January 2008 and involving *E. coli* (with a susceptibility profile different from that of the index dog's isolates) and *Pseudomonas aeruginosa*. None of the cats had prior infections or antimicrobial therapy.

Fecal samples. Approximately 1 month after collection of the urine samples that yielded the index dog's serotyped urine isolates, a fecal culture was collected from the index dog and four canine and feline housemates by swabbing freshly passed feces. Swabs were processed in a stepwise fashion, largely as previously described (12), to recover total and antimicrobial-resistant *E. coli* by using tryptic soy broth and gram-negative selective agars, supplemented selectively with antimicrobials. Supplementation (when used) was with (i) ciprofloxacin alone (4 mg/liter), (ii) ceftazidime alone (2 or 20 mg/liter), or (iii) trimethoprim (1.6 mg/liter)-sulfamethoxazole (100 mg/liter)

* Corresponding author. Mailing address: Infectious Diseases (111F), Veterans Affairs Medical Center, 1 Veterans Drive, Minneapolis, MN 55417. Phone: (612) 467-4188. Fax: (612) 727-5995. E-mail: johns007@umn.edu.

† Supplemental material for this article may be found at http://jcm.asm.org/.

‡ Present address: Leesburg Veterinary Internal Medicine, 165 Fort Evans Rd. NE, Leesburg, VA.

▽ Published ahead of print on 26 August 2009.

TABLE 1. Host associations and bacterial characteristics of five *Escherichia coli* strains (and variants) from two dogs and three cats within a household

Category	Data for indicated strain or variant[a]					
	968	1049	1051	1052a	1052b	1050
Medium(a)[b]	Cip	Plain	Plain	Plain, Gen-Sxt	Plain	Cip
Host identification no.[c]	1, 3, 4	2, 3, 4	5	1, 5	1	2
Host species	Dog, cat	Dog, cat	Cat	Dog, cat	Dog	Dog
Phylogenetic group	B2 (ST131)	B2	B2	D	D	A
O-antigen type (*rfb* allele)	O25b	O17	O6	O7	O7	Unknown[d]
Virulence genes[e]						
papA allele[f]	−	F12	F10	−	−	−
papG allele[g]	−	III	−	−	−	−
sfa-focDE	−	+	+	−	−	−
sfaS	−	+	+	−	−	−
focG	−	+	−	−	−	−
iha	+	−	−	−	−	−
fimH	+	+	+	+	+	−
hra	−	+	+	−	−	−
hlyF	−	−	−	−	+	−
cnf1	−	+	−	−	−	−
sat	+	−	+	−	−	−
vat	−	−	+	−	−	−
astA	−	+	−	−	−	−
iroN	−	+	+	−	+	−
fyuA	+	+	+	−	−	−
iutA	+	−	−	−	+	+
kpsM II	+	−	+	+	+	−
kfiC	+	−	−	−	−	−
K15	−	−	−	+	+	−
cvaC	−	−	−	−	+	−
usp	+	−	+	−	−	−
traT	+	−	+	+	+	−
ompT	+	+	+	+	+	−
iss	−	−	−	−	+	−
H7*fliC*	−	+	−	−	−	−
malX	+	+	+	−	−	−
clbB	−	−	+	−	−	−
Virulence score[h]	10	13	14	4	9	1

[a] All representatives of each strain or variant gave identical results. Plus and minus signs indicate the presence and absence of a trait, respectively.

[b] The medium used was MacConkey or tergitol-7 agar, plain or supplemented with ciprofloxacin (4 mg/liter) (Cip) or gentamicin (10 mg/liter) plus trimethoprim (1.6 mg/liter)-sulfamethoxazole (100 mg/liter) (Gen-Sxt).

[c] Hosts: 1, Australian shepherd (index subject; dog); 2, Akita (dog); 3 and 4, Maine Coon cats; 5, short-haired domestic cat. The index dog (host 1) provided urine and fecal samples. Hosts 2 to 5 provided fecal samples only.

[d] Clone 1050 was negative by the O-antigen PCR assay (for 14 sepsis-associated *rfb* variants).

[e] The virulence genes indicated are those detected in at least one isolate. Abbreviations: *papA*, P fimbria structural subunit (with alleles F10 and F12); *papG*, P fimbria adhesin (with allele III); *sfa-focDE*, S and F1C fimbriae; *focG*, F1C fimbriae; *sfaS*, S fimbriae; *iha*, adhesin-siderophore; *fimH*, type 1 fimbriae; *hra*, heat-resistant agglutinin; *hlyA*, alpha hemolysin; *hlyF*, variant hemolysin; *cnf1*, cytotoxic necrotizing factor; *sat*, secreted autotransporter toxin; *vat*, vacuolating toxin; *astA*, enteroaggregative *E. coli*-associated toxin; *iroN*, siderophore receptor; *fyuA*, yersiniabactin receptor; *iutA*, aerobactin receptor; *kpsM* II, group 2 capsule; *kfiC*, K5 capsule; K15, group 2 capsule; *cvaC*, microcin (colicin) V; *usp*, uropathogenic-specific protein; *traT*, serum resistance associated; *ompT*, outer membrane protease; *iss*, increased serum survival; H7*fliC*, flagellar variant; *malX*, pathogenicity island marker; *clbB*, colibactin.

[f] Isolates with a detectable *papA* allele (F10 and F12) were also positive for *papC* (P fimbria assembly). All other isolates were *papC* negative.

[g] Isolates with a detectable *papG* allele (*papG* III) were also positive for *papEF* (P fimbria tip pilins). All other isolates were *papEF* negative.

[h] The virulence score is number of markers detected (adjusted for multiple detection of *pap*, *sfa*, *foc*, and *kps* operons).

plus gentamicin (10 mg/liter). Five putative *E. coli* colonies from each plate, as available, were identified presumptively on the basis of lactose, indole, and citrate phenotype.

Molecular and phenotypic analysis. The two index urine isolates and five *E. coli* colonies from each antimicrobial-supplemented and nonsupplemented fecal culture plate underwent genomic profiling using random amplified polymorphic DNA (RAPD) analysis, followed by pulsed-field gel electrophoresis (PFGE) analysis of one representative per unique RAPD type per specimen (12, 28). PFGE profiles that, according to Bionumerics (Applied Maths, Austin, TX), exhibited ≥94% Dice similarity (9), which corresponds to a ≤3-band difference (J. R. Johnson, unpublished results), which in turn implies a close genetic relationship (30), were regarded as representing the same PFGE type or strain. Comparisons were made against a private PFGE library maintained within the laboratory of the investigator (J. R. Johnson), which at the time of the study included 255 ST131 isolates from multiple U.S. and international locales, representing 125 different PFGE types.

One representative per strain per sample underwent species confirmation by API-20E (bioMérieux)- and PCR-based analysis for determination of *E. coli* phylogenetic group (A, B1, B2, or D) (2, 26) and ST131 status (17) and detection of 62 ex-

TABLE 2. Antimicrobial resistance profiles of five *Escherichia coli* strains (and variants) from two dogs and three cats within a household[a]

Agent(s) or parameter	Result for indicated strain or variant					
	968	1049	1051	1052a	1052b	1050
Ampicillin	R	S[b]	S	R	R	R
Amoxicillin-clavulanate	R	S[b]	S	R	R	R
Piperacillin	R[b]	S	S	R	S	R
Cephalothin	S[b]	S	S	R	R	S
Cefoxitin	S	S	S	R	R	S
Ceftriaxone	S	S	S	R	R	S
Ceftazidime	S	S	S	R	R	S
Chloramphenicol	S	S	S	R	R	R
Tetracycline	R	S	S	R	R	R
Nalidixic acid	R	S	S	S	S	R
Ciprofloxacin	R	S	S	S	S	R
Gentamicin	R	S	S	R	S	R
Streptomycin	R	S	S	R	R	R
Sulfisoxazole	R	S	S	R	R	R
Trimethoprim	R	S	S	R	S	R
Trimethoprim-sulfamethoxazole	R	S	S	R	S	R
Resistance score	11	0	0	14	10	12

[a] The drugs indicated are the 16 for which resistance was detected. Strains were susceptible (S) to the corresponding drugs except where indicated as resistant (R). All isolates were susceptible to amikacin, aztreonam, cefepime, imipenem, nitrofurantoin, and piperacillin-tazobactan. The resistance score is the number of resistance results detected (regardless of drug class) among the 22 tested drugs.

[b] Intermediate susceptibility was observed for a minority of representatives of strain 968 with cephalothin and piperacillin and for a minority of representatives of strain 1049 with ampicillin and amoxicillin-clavulanate.

traintestinal pathogenic *E. coli*-associated virulence genes (Table 1) (16), the O25b *rfb* (lipopolysaccharide) variant (4), and $bla_{\text{CTX-M-15}}$ (21). The virulence score, which corresponds epidemiologically and experimentally to virulence (14, 29), was the number of virulence genes detected, adjusted for multiple detection of the *pap*, *sfa-foc*, and *kps* operons.

Susceptibility to 22 antimicrobial agents (Table 2) was determined by disk diffusion (5, 6). For minor discrepancies among the multiple representatives of a given strain for susceptibility to a given drug (i.e., intermediate susceptibility versus susceptibility or resistance), the majority result (which in all instances was susceptibility or resistance) was used. The resistance score was the number of drugs to which a strain exhibited resistance. Representatives of a given strain that differed consistently for multiple virulence traits and resistance markers were regarded as variants of that strain.

Serotyping. For the index urine isolates, O antigens were detected by agglutination using 180 O-antigen-specific antisera (produced by the Pennsylvania State University serotyping laboratory), whereas H types were defined by PCR-restriction fragment length polymorphism analysis of *fliC* (flagellin) (22). For one representative per strain (or variant) per fecal sample, 14 sepsis-associated *rfb* variants (O1, O2, O4, O6, O11, O12, O15, O16, O17, O18, O25a, O25b, O75, and O157) were sought by PCR (3, 4).

Results: urine isolates. The index dog's two urine isolates (O25:H4) derived from phylogenetic group B2 exhibited ST131-specific single nucleotide polymorphisms in *gyrA* and *mdh*, contained the ST131-associated O25b *rfb* variant, were indistinguishable from ST131 reference strains according to RAPD analysis, and lacked $bla_{\text{CTX-M-15}}$. Their shared PFGE profile (Fig. 1) represented the second-most-prevalent PFGE type (type 968) within a private database of human ST131 isolates; the other representatives of type 968 were from Lexington, KY, Minneapolis, MN, New York, NY, Omaha, NE, and Mohali, India. The canine urine isolates' virulence gene profiles were typical of previously reported ST131 isolates (25) and included *iha*, *fimH*, *sat*, *fyuA*, *iutA*, *kpsM* II, *kfiC*, *usp*, *traT*, *ompT*, and *malX* (Table 1).

Fecal clones. Fecal samples from the participating pets (two dogs and three cats) yielded five distinct strains (one with two variants), which exhibited diverse phylogenetic backgrounds, virulence genotypes, and resistance profiles (Fig. 1 and Tables 1 and 2). Virulence genotypes were more extensive among the three group B2 strains (virulence scores, 10 to 14) than between the two non-B2 strains (virulence scores, 1 to 9), whereas resistance profiles were more extensive between the non-B2 strains (resistance scores, 9 to 13 versus 0 to 11). One of the non-B2 strains exhibited resistance to extended-spec-

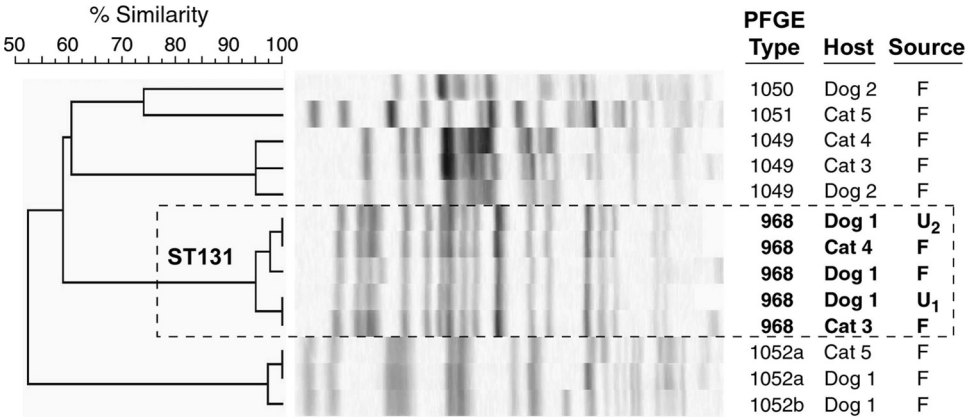

FIG. 1. XbaI PFGE profiles of *Escherichia coli* isolates from two dogs and three cats within a household. The dendrogram was inferred on the basis of Dice similarity coefficients. PFGE types were defined at the 94% similarity level. Sources: F, fecal sample; U, urine sample. Sequential urine isolates (separated by approximately 1 month) are designated U_1 and U_2. A dashed rectangle encloses isolates of PFGE type 968 (i.e., the index dog's urine isolates, plus fecal isolates from dog 1 and cats 3 and 4), which correspond to ST131.

trum cephalosporins and cefoxitin. The ST131 strain uniquely exhibited high virulence and resistance scores.

Host associations of strains. The five strains were identified in one to three (median, two) hosts each. Each host shared one or two strains with one to three (median, three) other hosts (Table 1; see also Fig. S2 in the supplemental material). The two most widely shared strains (three hosts each) were from group B2; one of these, the index ST131 strain, occurred in the index dog and both Maine Coon cats. Of the 10 potential strain-sharing host pairs, 6 exhibited strain sharing, which involved one strain for 5 pairs and two strains for 1 pair. Interestingly, strain sharing was documented for five of six across-species (i.e., dog-cat) pairs but only one of four same-species (i.e., dog-dog or cat-cat) pairs.

Comment. This molecular epidemiological analysis of *E. coli* isolates from two dogs and three cats within a household confirmed that the index dog's recurrent bacteriuria strain represented not just *E. coli* ST131 but a prevalent human-associated pulsotype within ST131. This strain, which was FQ-R but extended-spectrum cephalosporin susceptible and $bla_{\text{CTX-M-15}}$ negative, was widely shared within the household, occurring in two cats in addition to the index dog. Two other *E. coli* strains (one extensively antimicrobial resistant) also were shared among multiple hosts, across species lines.

Although best known for its association with extended-spectrum cephalosporin resistance (and CTX-M-15), ST131 is probably much more common as an FQ-R but cephalosporin-susceptible pathogen (1, 20, 23), as observed here. To our knowledge, this is the first report of isolation of cephalosporin-susceptible *E. coli* ST131 from animal hosts. The present findings, together with the recently reported CTX-M-15-positive ST131 canine isolate (27), support the assumption that companion animals may represent a reservoir of ST131. Likewise, the present ST131 strain's human-associated PFGE type suggests that the strain may be adapted for human colonization and pathogenicity. If animal-human transmission of ST131 occurs in either direction, it may be contributing to the ongoing global dissemination and emergence of ST131.

The extensive strain sharing that we documented (involving all five hosts, three of five strains, and 6 of 10 host-host pairs) further supports the assumption that sharing of fecal *E. coli* isolates among cohabiting hosts is the norm (11–13, 18, 24). The two most widely shared strains were from group B2 and had extensive virulence genotypes, confirming that group B2 and some of its characteristic virulence genes are associated with prolonged and multiple-host colonization (10, 13, 24).

Competing hypotheses for the observed strain sharing include within-household transmission versus parallel acquisition from an external source, e.g., the food supply (19). Host-to-host transmission is suggested by the prolonged colonization of the index dog with the ST131 strain prior to its detection in other household members. However, the evidence is inconclusive. A longitudinal study, ideally with environmental sampling, would be needed to clarify these points.

The limitations of the study include the relatively small study population, the analysis of only five colonies per plate, the absence of sampling of the environment or human household members, the cross-sectional study design, and the limited information regarding host antimicrobial use. The strengths include the participation of multiple dogs and cats from the same household, the extensive molecular analysis, and the comparison with a large private ST131 PFGE reference library.

Summary. In this study of five household pets, we found that the index dog's recurrent urinary tract inflection strain represented a prevalent human-associated variant of *E. coli* ST131 (O25:H4) and colonized two cats in addition to the index dog. Two other virulent-appearing and/or multidrug-resistant *E. coli* strains were also shared across species lines. These findings provide novel evidence of cephalosporin-susceptible, FQ-R ST131 in companion animals and suggest host-to-host transmission of ST131 among household pets.

This material is based upon work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (J.R.J.).

J.R.J. has received grant support and/or honoraria from Bayer, Wyeth-Ayerst, Ortho-McNeil, Merck, Procter and Gamble, and Rochester Medical Group. The other authors have no conflicts of interest regarding this work.

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