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Antimicrobial Resistance, Phylogenetic Background, and Virulence Genes of Fecal *Escherichia coli* Isolates from Co-localized Chimpanzees and Humans in Uganda in Relation to Degree of Interspecies Contact

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Abstract

To what extent co-localized humans and wild animals share commensal *Escherichia coli* strains and the associated antimicrobial resistance determinants and virulence-associated genes is poorly defined. To study this, over two years (1995 and 1996) we collected fecal *Escherichia coli* isolates from two communities of chimpanzees that inhabit the Kibale National Park in Uganda and compared them to contemporaneous fecal *E. coli* isolates collected from villagers living at the boundary of the preserve and park workers (139 total isolates). We found that antimicrobial resistance was most prevalent among park workers (25 subjects), intermediately prevalent among Kanyawara community chimpanzees (which have human contact: 33 subjects), and least prevalent among villagers (6 subjects) and Ngogo community chimpanzees (which lack direct human contact: 20 subjects). Molecular analysis of the 139 isolates to assess the frequency and distribution of 54 molecular characteristics (phylogenetic group markers and virulence-associated genes) showed that strains from both groups of chimpanzees (Kanyawara, n = 64; Ngogo, n = 28) were more similar to strains from villagers (n = 16) than to strains from park workers (n = 31). Genes associated with extraintestinal disease in humans such as fimbrial and afimbrial adhesins, hemolysin, toxins, and iron uptake systems were, paradoxically, most prevalent among chimpanzee isolates. By multi-dimensional scaling, the closest between-species population similarity for molecular characteristics was between strains from Kanyawara chimpanzees and those from villagers. These data support the potential sharing of transmissible resistance markers between chimpanzees and humans in close contact, whereas similarities in commensal strains' genetic background is more related to shared habitat and host species.

ABBREVIATIONS

ANOVA: analysis of variance; BVAMC: Boston Veterans Affairs Medical Center; *E. coli*: *Escherichia coli;* ExPEC: Extraintestinal Pathogenic *E. coli;* LB: Luria-Bertan; MVAHCS: Minneapolis Veterans Affairs Health Care System; NCCLS: National Committee for Clinical Laboratory Standards; PFGE: Pulsed-field gel electrophoresis

INTRODUCTION

Humans have increasingly incurred into previously remote regions, resulting in both habitat encroachment and higher levels of human-animal interaction [1]. This increases the risk for physical contact and exchange of pathogenic organisms and/or genetic elements between species.

The extent of interspecies transfer of microbiota and microbial genetic elements could logically be predicted to vary in proportion to the level of interspecies contact. Human-to-animal transfer is of concern because it conceivably could increase the risk for human diseases in animals or establish animal reservoirs of pathologic organisms and/or disease-relevant microbial

elements that could later undergo zoonotic transmission back to humans [2,3]. However, clear documentation of a contact-proportionate risk for microbiologic transfer from a human source to animals is lacking, except in special cases.

With non-captive animals the interspecies transfer of bacteria and/or genetic elements between humans and animals is minimally documented. Two studies in wild baboons were the first to demonstrate that the level of contact with human habitats was associated with an increased risk of recovery of antimicrobial resistant fecal organisms [4,5].

In one of these studies, Rolland et al., studied three groups of baboons within the Amboseli National Park of Kenya [4]. Two of the groups had minimal human contact, whereas the third (Lodge group) raided refuse pits and latrines used by the lodge. Whereas cultures of feces taken from members of the Lodge group showed much higher percentages of antibiotic-resistant Gram-negative bacteria, there was also a larger fraction of non-lactose fermenting fecal organisms, i.e., bacteria other than *Escherichia coli* or *Klebsiella*. Resistance to the antibiotics studied – tetracycline, kanamycin, ampicillin, and cephalexin – is typically plasmid mediated, which these authors confirmed for the study isolates by conjugation experiments. Thus, whether the resistant population could be attributed to acquisition of antibiotic resistant plasmids by otherwise susceptible organisms such as *E. coli*, versus a change in fecal coliforms to an inherently resistant bacterial population, is unknown.

Likewise, Routman et al., studied five baboon communities in Tanzania [5]. Two communities raided garbage dumps of a local village, whereas three had minimal to no human contact. By contrast, with the above-mentioned Kenyan study, this study found no difference in the fraction of antimicrobial-resistant fecal organisms between animals with and without human contact. A relevant design difference between these studies is that in this Tanzanian study the human contact was with local villagers, who were agrarian and would have had limited outside contact and, presumably, less antimicrobial exposure, whereas in the Kenyan study it was at the site of a tourist lodge, with humans of higher socioeconomic status and, presumably, more antimicrobial exposure.

Field research into the primates of Kibale National Park, Uganda, has been ongoing since the 1970's. The Kibale Chimpanzee Project was established in the early 1980s as a field research program to assess the ecology, physiology, and behavioral science of endangered primates [6]. Within the park, primate research is conducted in three regions, Kanyawara, Ngogo, and Sebitoli [6], each with its own chimpanzee group. In particular, the Kanyawara group habitat overlaps with villages within the park and are human tolerant, whereas the Ngogo group has minimal human contact [6,7].

Four studies from the Kibale Chimpanzee Project are relevant. One found that respiratory illness was common in the Kanyawara chimpanzee group, but temporal patterns over a number of years did not match those in the neighboring village [8]. By contrast, two other studies documented outbreaks in the Kanyawara and Ngogo chimpanzees of human rhinovirus C, human metapneumovirus, and human respirovirus 3 (formerly parainfluenza virus 3), suggestive of spread of human pathogenic viruses to both of these chimpanzee populations, despite their differing degrees of human contact [9,10].

The only Kibale Chimpanzee Project study that has assessed possible interspecies transfer of bacteria was that of Goldberg, et al. [11]. In that study, chimpanzees harbored *Escherichia coli* strains genetically more similar (according to PCR-based genomic profiling) to those of humans employed in chimpanzee-directed research and tourism than to those of humans from a local village. The chimpanzee strains also exhibited antibiotic resistance levels intermediate between those of the two human groups. Notably, however, this study lacked a chimpanzee group without human contact, and did not define the phylogenetic group or virulence genes of the *E. coli* strains.

Here we investigated, within the Kibale National Park, the potential transfer from humans to chimpanzees of antibiotic resistance and/or other genetic elements associated with human disease. Specifically, we compared the prevalence of resistance phenotypes and selected genetic markers between fecal *E. coli* isolates from Kanyawara and Ngogo chimpanzees, local villagers, and park workers, to assess whether the outcome variables segregate by host species, lifestyle, or exposure to other hosts. We also assessed associations between different bacterial characteristics, independent of host group.

MATERIALS AND METHODS

Study populations

Stool samples from chimpanzees and human participants were collected in and near Kibale National Park, Uganda during June and July 1995 and in June 1996, using established methods that allowed sample assignment to specific donors [11]. Six human residents of a neighboring village and 25 park workers were sampled in 1995 and 1996, respectively. Chimpanzees from the Kanyawara and Ngogo communities were selected for study as representing contact and non-contact animals, respectively, as explained below.

The Kanyawara chimpanzees are human-habituated wild chimpanzees with years of daily exposure to field researchers and locally hired research assistants (hereafter, park workers). Park workers follow and observe the chimpanzees for several hours every day, thus serving as the greatest source of human exposure to the chimpanzees. The contact is indirect but high (defecation by chimps and humans, and movement of both species through those areas daily).

Chimpanzees also range across the park boundary and into adjacent agricultural fields to raid crops (plantains, corn, etc.). Local farmers and their families often camp in those fields to protect their crops from raiding by various wildlife species. Crop raiding outside the park along its borders (by chimpanzees),

and illegal hunting (by villagers crossing into the park) allow additional chimpanzee-human habitat overlap and interspecies exposures.

By contrast, the Ngogo chimpanzees live in a much more remote area of Kibale National Park, farther away from villages and crops. At the time of sample collection for the present study, the Ngogo chimpanzees were not human-habituated and had minimal overlapping habitat or exposure to humans, including park workers and villagers. (This has since changed; the Ngogo chimpanzees are now habituated and under study).

Isolate collection, antimicrobial resistance testing, pulsed-field gel electrophoresis

Stool was triple streaked onto MacConkey agar and incubated at ambient temp. From each plate six lactose-fermenting colonies (as available) were selected arbitrarily and inoculated into stabs containing trypticase-soy agar, which were then sealed and shipped in batches to the BVAMC for analysis.

At the BVAMC, material from each stab was streaked onto MacConkey agar. After overnight incubation a single lactose-fermenting colony per plate was picked and grown overnight in Luria-Bertani (LB) broth, from which aliquots were made and stored at -70°C.

For antimicrobial susceptibility testing, frozen isolates were freshly grown overnight in LB broth. Broth cultures were diluted to a 0.5 McFarland standard, then spread onto the surface of a 6" agar plate using a cotton-tipped swab. Antibiotic discs were placed onto the surface of each plate. After incubation overnight at 37°C, inhibition zones around each disc were measured. Zone diameter interpretations (susceptible, intermediate, and resistant) were per National Committee for Clinical Laboratory Standards (NCCLS) criteria [12].

Pulsed field gel electrophoresis (PFGE) was performed as described previously [13]. Each single-colony isolate from each fecal sample was incubated overnight in LB broth, and genomic DNA was isolated. Following restriction digestion with XbaI, DNA fragments were resolved in agarose gels and stained with ethidium bromide. PFGE profiles, as assessed by visual inspection, were compared within each subject. Isolates from a given host that exhibited indistinguishable PFGE profiles were regarded as representing the same strain, and those with different PFGE profiles as different strains.

For each study subject, one isolate per strain was selected and shipped in an agar stab to the Minneapolis VA Health Care System (MVAHCS) for further molecular analysis. Isolates collected in different years from the same subject were considered as independent collections.

PCR-based molecular analysis

At the MVAHCS, established conventional multiplex PCR-based assays, with primers and conditions as described elsewhere, were used to resolve seven major phylogenetic groups (A, B1, B2, C, D, E, F) [14], and to detect 48 genetic markers associated with extraintestinal virulence [15,16]. Boiled lysates of single-colony overnight broth cultures were used as target DNA. All PCR testing was done in duplicate, using relevant positive and negative controls. Using established molecular definitions, isolates were classified presumptively as extraintestinal pathogenic *E. coli* (ExPEC) if they contained ≥ 2 of *papAH* or *papC* (P fimbriae structural subunit and assembly; counted as one), *sfa/focDE* (S and F1C fimbriae), *afa/draBC* (Dr-binding adhesins), *kpsM* II (group 2 capsules), and *iutA* (aerobactin siderophore system) [17]. The number of discrete virulence gene operons detected was the virulence gene score.

Statistical analysis

Analyses involving antimicrobial resistance prevalence by study group were done at the by-subject level, for which each subject was scored for presence/absence of any detected resistance to a given agent, to account for possible non-independence between multiple isolates from a given subject due to within-host transfer of mobile resistance elements. Comparisons involving phylogenetic group and virulence genes, most of which are minimally or not horizontally mobile, and those involving antimicrobial resistance in comparison with other bacterial characteristics, were done at the by-isolate level.

For comparisons between study groups that involved dichotomous variables, an initial overall four-group comparison (which was tested using the Fisher-Freeman-Halton Exact Test) was followed by pairwise between-group comparisons using a Z-test. The number of statistically significant (i.e., $P \leq 0.05$) between-group differences was tabulated and compared with group identity. Because according to the Kolmogorov-Smirnov and Shapiro-Wilk tests virulence gene scores were not normally distributed, comparison involving them were tested using the Kruskal-Wallis test for an overall four-group comparison and the Mann-Whitney test for pairwise between-group comparisons.

For combined variables, we conducted multidimensional scaling in R, using antimicrobial resistance at the by-subject level to place individuals subjects on a two-dimensional plot (Figure 1), then used Analysis of Variance (ANOVA) to test for differences between groups on each dimension (Table 4). We likewise conducted multidimensional scaling using isolate-level virulence factors to place individual strains on a two-dimensional plot (Figure 5), then used ANOVA to test for differences between groups on each dimension (Table 5).

RESULTS

A total of 33 Kanyawara chimpanzees, 20 Ngogo chimpanzees, 25 park workers, and 6 villagers were sampled one or more times each, for 84 total subjects. For each animal (n = 53), and human (n = 31), participant, PFGE analysis of six presumptive *E. coli* colonies per fecal sample demonstrated a dominant (i.e., most abundant) strain, which in most cases accounted for all six colonies from that sample. Clonal deduplication yielded a total of 139 unique fecal *E. coli* strains, as defined based on source

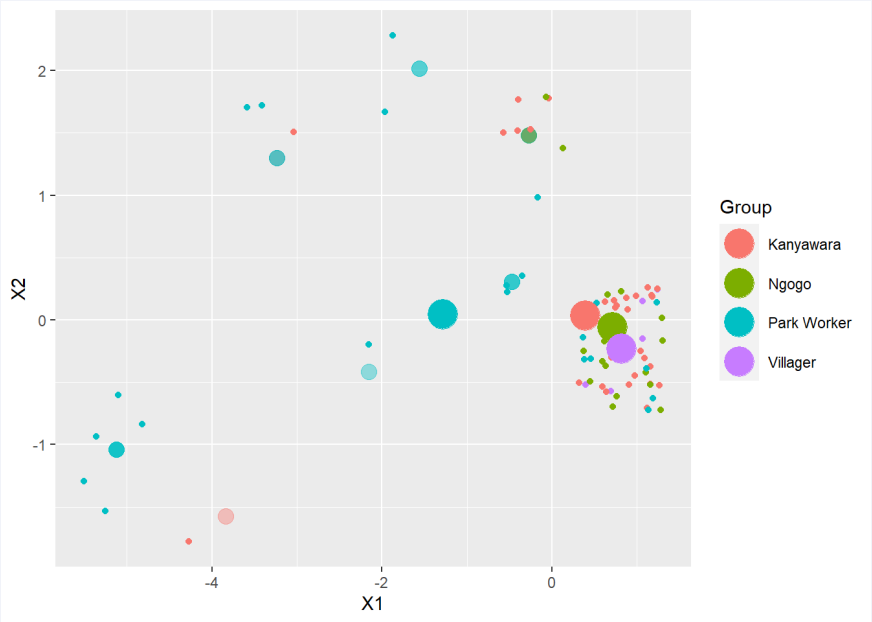

Figure 1 Multidimensional scaling of antibiotic resistance data. The analysis was based on the by-subject (n = 84) presence of fecal *E. coli* resistant (individually) to ampicillin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, or tetracycline. Axes X1 and X2, the most-informative axes, are orthogonal. Each subject appears once, either individually (small circles) or combined with others that share the same grid position (larger circles). Circle size and darkness reflects the number of strains at a given position, except for the four large dark circles, which are the group centroids. Kanyawara chimpanzees had human contact; Ngogo chimpanzees did not.

host and PFGE profile. The strains were distributed by host group as follows: Kanyawara chimpanzees, 64 strains; Ngogo chimpanzees, 28 strains; park workers, 31 strains; and villagers, 16 strains. Each host yielded from 1 to 7 unique strains (median, 1).

Antimicrobial resistance

The by-host prevalence of antimicrobial-resistant fecal *E. coli* varied significantly by host group for all four studied agents, including (overall resistance prevalence) ampicillin (23%), ampicillin-sulbactam (7%), trimethoprim-sulfamethoxazole (17%), and tetracycline (12%) (Table 1). Park workers consistently had the highest resistance prevalence values, Kanyawara chimpanzees had intermediate values, and villagers and Ngogo chimpanzees had the lowest values. In pairwise between-group comparisons (Tables 1 and 2), statistically significant differences were limited to comparisons of park workers vs. other groups.

Phylogenetic group distribution

In a by-strain analysis of the 139 total unique strains, phylogenetic group distribution varied significantly by host group (Table 3). Specifically, phylogenetic group B2 accounted for most strains from chimpanzees, whether Kanyawara (72%) or Ngogo (89%), and for 50% of strains from villagers, but was nearly absent from park workers (7%). Conversely, phylogenetic groups A and B1 accounted jointly for 77% of strains from park workers, but were scarce to absent among strains from chimpanzees or villagers (0-19% each per host group). The low-prevalence phylogenetic groups (C, D, E, and F) did not segregate significantly by host group. In pairwise between-group comparisons, significant differences in phylogenetic group distribution occurred between the park workers and the three other groups, and between villagers and Ngogo chimpanzees, but not between the two chimpanzee groups, or between villagers and Kanyawara chimpanzees.

Virulence genotypes

Forty (83%), of the 48 studied virulence-associated genes were detected, at overall prevalence levels ranging from 0.07% to 92%, and 34 (71%), were detected in ≥ 5% of strains (Table 3). Overall, 19% of strains qualified molecularly as ExPEC.

Of the 34 virulence-associated genes with ≥ 5% overall prevalence, 18 (53%) were distributed significantly by host group, in diverse patterns (Table 2). Two patterns predominated. One involved total, or near-total, confinement to chimpanzee strains, suggestive of a species effect. This occurred with *papAH* (P fimbriae structural subunit), *papC* (P fimbriae assembly), *papG* (P fimbriae adhesin), *hlyA* (alpha hemolysin), *ibeA* (invasion of brain endothelium), and *clbB/N* (colibactin). A similar but less extreme concentration among chimpanzee strains over human strains, consistent with a partial species effect, occurred with *fyuA* (yersiniabactin receptor) and *ompT* (outer membrane protease). The second main pattern involved a predominance

Table 1: Colonization with antimicrobial-resistant *Escherichia coli* among 84 co-localized chimpanzees and humans in Uganda.

	Prevalence of antimicrobial resistance by host group, no. of subjects (column%)							Pairwise between-group comparison P value[a]					
Anti-microbial agent[b]	Total (n = 84; 139 PFGE[c] types)	Kanyawara chimps[d] (K) (n = 33; 64 PFGE[c] types)	Ngogo chimps[d] (N) (n = 20; 28 PFGE[c] types)	Park workers (PW) (n = 25; 31 PFGE[c] types)	Villagers (V) (n = 6; 16 PFGE[c] types)	Four-group compa-rison P value[e]		K[f] vs. N[f]	K[f] vs. PW[f]	K[e] vs. V[e]	N[f] vs. PW[f]	N[f] vs. V[f]	PW[f] vs. V[f]
AMP[a]	19 (23)	7 (21)	2 (10)	10 (42)	0 (0)	0.04		NS	< 0.05	NS	< 0.05	NS	NS
SAM[a]	6 (7)	1 (3)	0 (0)	5 (21)	0 (0)	0.03		NS	< 0.05	NS	< 0.05	NS	NS
SXT[a]	14 (17)	1 (3)	0 (0)	13 (54)	0 (0)	< 0.001		NS	< 0.05	NS	< 0.05	NS	< 0.05
TET[a]	10 (12)	2 (6)	0 (0)	8 (33)	0 (0)	0.002		NS	< 0.05	NS	< 0.05	NS	NS

[a]Pairwise between-group comparison P values (according to the z-test), which are unadjusted for multiple comparisons, are shown dichotomously as P < 0.05 or NS (not significant, i.e., P ≥ 0.05).
[b]AMP, ampicillin; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.
[c]PFGE, pulsed-field gel electrophoresis
[d]Chimps, chimpanzees.
[e]Four-group comparison P values (by the Fisher-Freeman-Halton Exact Test).
[f]K, Kanyawara chimpanzees (human contact); N, Ngogo chimpanzees (no human contact); PW, park workers; V, villagers.

Table 2: Number of statistically significant pairwise differences between host groups for prevalence of individual bacterial characteristics.

Compared host groups	Antibiotic resistance	Molecular characteristics	Total
Ngogo chimps[b] vs. Kanyawara chimps[b]	0	4	4
Park workers vs. Kanyawara chimps[b]	4	18	22
Villagers vs. Kanyawara chimps[b]	0	8	8
Park workers vs. Ngogo chimps[b]	4	15	19
Villagers vs. Ngogo chimps[b]	0	9	9
Villagers vs. park workers	1	10	11

[a]Based on the statistical comparisons shown in Tables 1 and 2. Limited to variables that yielded P < 0.10 in an initial four-group comparison. The unit of analysis for antibiotic resistance was the subject, and for molecular characteristics was the strain.
[b]Chimps, chimpanzees. (Kanyawara chimps had human contact; Ngogo chimps did not.)

Table 3: Molecular characteristics of 139 fecal *Escherichia coli* isolates from co-localized chimpanzees and humans in Uganda.

Molecular characteristic		Prevalence of characteristic by host group, no. (column %)						Pairwise between-group comparison P value[a]					
Category	Specific trait[b,c,d]	Total (n = 139)	Kanya-wara chimps[e] (K) (n = 64)	Ngogo chimps[e] (N) (n = 28)	Park workers (PW) (n = 31)	Villagers (V) (n = 16)	Four-group comparison P value[f]	K[g] vs. N[g]	K[g] vs. PW[g]	K[g] vs. V[g]	N[g] vs. PW[g]	N[g] vs. V[g]	PW[g] vs. V[g]
Phygroup[h]	Group A	21 (15)	7 $(11)_1$	0 (0)	11 (36)	3 (17)	0.001	NS	P < 0.05	NS	P < 0.05	P < 0.05	NS
	Group B1	15 (11)	1 (2)	0 (0)	13 (42)	1 (6)	< 0.001	NS	P < 0.05	NS	P < 0.05	NS	P < 0.05
	Group B2	82 (58)	45 (71)	26 (90)	2 (7)	9 (50)	< 0.001	NS	P < 0.05	NS	P < 0.05	P < 0.05	P < 0.05
Adhesins	*papAH*	15 (11)	14 (22)	1 (4)	0 (0)	0 (0)	0.001	P < 0.05	P < 0.05	P < 0.05	NS	NS	NS
	papC	19 (13)	14 (22)	4 (14)	1 (3)	0 (0)	0.03	NS	P < 0.05	P < 0.05	NS	NS	NS
	papG	18 (13)	14 (22)	3 (11)	1 (3)	0 (0)	0.02	NS	P < 0.05	P < 0.05	NS	NS	NS
	afa/draBC	8 (6)	0 (0)	0 (0)	8 (26)	0 $(0)_1$	< 0.001	NS	P < 0.05	NS	P < 0.05	NS	P < 0.05
	bmaE	6 (4)	1 (2)	2 (7)	0 (0)	3 (19)	0.01	NS	NS	P < 0.05	NS	NS	P < 0.05
Toxins	*hlyA*	14 (10)	11 (17)	3 (11)	0 (0)	0 (0)	0.03	NS	P < 0.05	NS	NS	NS	NS
	sat	8 (6)	0 (0)	0 (0)	8 (26)	0 (0)	< 0.001	NS	P < 0.05	NS	P < 0.05	NS	P < 0.05
	vat	65 (46)	38 (59)	20 (71)	2 (7)	5 (31)	< 0.001	NS	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Iron[h]	*iroN*	32 (23)	17 (27)	10 (36)	0 (0)	5 (31)	0.005	NS	P < 0.05	NS	P < 0.05	NS	P < 0.05
	fyuA	74 (52)	38 (59)	20 (71)	10 (32)	6 (38)	0.008	NS	P < 0.05	NS	P < 0.05	P < 0.05	NS
	iutA	11 (8)	1 (2)	0 (0)	10 (32)	0 (0)	< 0.001	NS	P < 0.05	NS	P < 0.05	NS	P < 0.05
	chuA	101 (72)	56 (88)	28 (100)	6 (19)	11 (69)	< 0.001	P < 0.05	P < 0.05	NS	P < 0.05	P < 0.05	P < 0.05
Misc.[h]	*usp*	42 (30)	21 (33)	15 (52)	2 (7)	4 (22)	0.001	NS	P < 0.05	NS	P < 0.05	P < 0.05	NS
	ibeA	50 (35)	28 (44)	18 (64)	3 (10)	1 (6)	< 0.001	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	NS
	ompT	91 (65)	53 (83)	24 (86)	8 (26)	6 (38)	< 0.001	NS	P < 0.05	P < 0.05	P < 0.05	P < 0.05	NS
	iss	2 (1.4)	0 (0)	0 (0)	0 (0)	2 (13)	0.001	NS	NS	P < 0.05	NS	NS	NS
	malX	56 (40)	33 (52)	17 (61)	1 (3)	5 (31)	< 0.001	NS	P < 0.05	NS	P < 0.05	P < 0.05	P < 0.05
	clbB/N	6 (4)	1 (2)	5 (18)	0 (0)	0 (0)	0.001	P < 0.05	NS	NS	P < 0.05	NS	NS

[a]Pairwise between-group comparison P values (according to the z-test), which are unadjusted for multiple comparisons, are shown dichotomously as P < 0.05 or NS (not significant, i.e., P ≥ 0.05).
[b]Characteristics shown are those that yielded P < 0.05 in an overall four-group comparison. Definitions: *afa/draBC* (Dr-binding adhesins); *bmaE* (M fimbriae); *chuA* (heme uptake); *clbB/N* (colibactin synthesis); *fyuA* (yersiniabactin receptor); *hlyA* (alpha hemolysin); *ibeA* (invasion of brain endothelium); *iroN* (salmochelin receptor); *iss* (increased serum survival); *iutA* (aerobactin receptor); *malX* (pathogenicity island marker); *ompT* (outer membrane protein T); *papAH*, *papC*, *papG* (P fimbriae major pilin, assembly, and adhesin); *sat* (secreted autotransporter toxin); *usp* (uropathogenic-specific protein); *vat* (vacuolating toxin).
[c]Detected but not yielding P < 0.05, total n (% of 141): group C, 1 (0.7%); group D, 10 (7%); group E, 6 (4%); group F, 6 (4%); *astA* (enteroaggregative *E. coli* toxin), 24 (17%); *cdtB* (cytolethal distending toxin), 7 (5%); *clpG* (non-P adhesin), 1 (0.7%); *cnf1* (cytotoxic necrotizing factor), 3 (2%); ExPEC (extraintestinal pathogenic *E. coli*), 26 (19%); *fimH* (type 1 fimbriae), 130 (92%); *fliC* H7 (variant flagellin), 11 (7%); *focG* (F1C fimbriae), 11 (8%); *hlyF* (variant hemolysin), 1 (0.7%); *hra* (heat-resistant agglutinin), *iha* (adhesin-siderophore), 6 (43%); *ireA* (siderophore); *kpsM* II (group 2 capsules), 39 (28%); K1 (variant group 2 capsule), 19 (14%); K2 (variant group 2 capsule), 18 (13%); *papEF* (P fimbriae assembly), 18 (13%); *papG* allele II (variant P adhesin), 3 (2%); *papG* allele III (variant P adhesin), 12 (9%); *pic* (protein associated with intestinal colonization), 10 (7%); *rfc* (O4 lipopolysaccharide), 2 (1%); *sfa/focDE* (S and F1C fimbria); 38 (27%); *traT* (serum resistance-associated), 49 (35%); *tsh* (temperature-sensitive hemagglutinin), 6 (4%).
[d]Not detected: *cvaC* (colicin V), F17 (non-P adhesin), *gafD* (G fimbriae), *kpsMT* III (group 3 capsules), K5 (variant group 2 capsule), K15 (variant group 2 capsule), *papG* allele I (variant P adhesin), *sfaS* (S fimbriae).
[e]Chimps, chimpanzees.
[f]Four-group comparison P values (by the Fisher-Freeman-Halton Exact Test).
[g]K, Kanyawara chimpanzees (human contact); N, Ngogo chimpanzees (no human contact); PW, park workers; V, villagers.
[h]Phygroup, phylogenetic group. Iron, iron uptake. Misc., miscellaneous.

among chimpanzee strains and villager strains, with low or zero prevalence among park worker strain, suggestive of a within-park exposure effect. This occurred with *vat* (vacuolating toxin), *iroN* (siderophore), *chuA* (heme uptake), *usp* (uropathogenic-specific protein), and *malX* (pathogenicity island marker).

Two less common patterns also occurred. One was near or total confinement to park worker strains, consistent with an outside-park exposure; this applied to *afa/draBC* (Dr-binding adhesins), *sat* (secreted autotransporter toxin), and *iutA* (aerobactin receptor). The other was predominance specifically among villager strains, e.g., *bmaE* (M fimbriae), *ireA* (siderophore), and *iss* (increased serum survival), consistent with village-specific exposures.

Pairwise between-group comparisons for the frequency of individual virulence-associated genes identified numerous statistically significant differences. (Table 3). The number of such differences was greatest for comparisons between park workers versus chimpanzees (whether Kanyawara or Ngogo), intermediate for comparisons between villagers versus park workers or chimpanzees, and lowest for comparisons between Kanyawara versus Ngogo chimpanzees (Table 2).

Virulence gene scores (median, 6.0; range, 0 to 14.75) also varied significantly by study group (overall, P = 0.006), being higher (median [interquartile range]) among Kanyawara chimpanzees (6.0 [5.9]) and Ngogo chimpanzees (7.0 [5.3]) than among park workers (4.0 [3.0]) or villagers (5.0 [3.75]). The only statistically significant pairwise between-group differences were between park workers and Kanyawara or Ngogo chimpanzees. The proportion of strains qualifying as ExPEC, by contrast, did not vary significantly by study group (park workers, 29%; Ngogo chimpanzees, 25%; Kanyawara chimpanzees, 16%; villagers, 0%: P = 0.07).

Multidimensional scaling: antimicrobial resistance

In a two-dimensional plot of the X1 and X2 axis values from a multidimensional scaling analysis of the by-subject antimicrobial resistance data the subjects were distributed broadly, although concentrated near the origin (Figure 1). The centroids for three of the groups, i.e., the Kanyawara and Ngogo chimpanzees and villagers, were closely spaced on both axes, well separated on the X1 axis from the centroid for park workers. The centroid closest to the park workers' centroid was that for the Kanyawara chimpanzees. Statistical analysis of the X1 and X2 axis values showed no significant differences between the Kanyawara chimpanzees, Ngogo chimpanzees, and villagers, but on the X1 axis, significant differences between each of these groups and park workers (Table 4).

Multidimensional scaling: molecular characteristics

In a two-dimensional plot of the X1 and X2 axes from a multidimensional scaling analysis of the by-isolate molecular data the individual isolates were distributed in an inverted U-shaped pattern (Figure 2). The four study groups overlapped extensively, but had well separated centroids that were distributed along a diagonal in the upper left quadrant of the plot. The centroid for the villager strains was intermediate between the centroids for the park worker strains and the chimpanzee strains, closer to the centroid for Kanyawara chimpanzee strains than to that for Ngogo chimpanzee strains.

Statistical analysis of the X1 and X2 axis values identified no significant differences between Kanyawara and Ngogo chimpanzee strains, and only one difference – of marginal statistical significance (P = 0.04) – between chimpanzee strains (Ngogo group, X2 axis) and villager strains (Table 5). By contrast, park worker strains differed from both groups of chimpanzee strains on both axes (P < 0.001, all four comparisons), and from villager strains on axis X2 (P = 0.002).

Phylogenetic distribution of antimicrobial resistance and virulence genotypes

In a by-isolate phylogenetic analysis that was limited to isolates with available resistance data (131 [94%] of 139) and, for statistical reasons, the three most abundant phylogenetic groups (A, B1, and B2), antimicrobial resistance was significantly phylogenetically distributed for ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and tetracycline, and exhibited a similar trend for ampicillin (Table X). In each instance, resistance

Figure 2 Multidimensional scaling of molecular data. The analysis, which was by strain (n = 139), was based on phylogenetic group and virulence-associated gene content. Axes X1 and X2, the most-informative axes, are orthogonal. Each strain appears once, either individually (small circles) or combined with others that share the same grid position (larger circles). Circle size and darkness reflects the number of strains at a given position, except for the four large dark circles, which are the group centroids. Kanyawara chimpanzees had human contact; Ngogo chimpanzees did not.

Table 4: Statistical analysis of multidimensional scaling results for antimicrobial resistance data.[a]

	Axis X1		Axis X2	
Comparison groups	**Difference in means**	**P value[b]**	**Difference in means**	**P value[b]**
Ngogo chimps[c] vs. Kanyawara chimps[c]	0.32	0.87	-.09	0.97
Park workers vs. Kanyawara chimps[c]	-1.67	**< 0.001**	.01	0.99
Villagers vs. Kanyawara chimps[c]	.43	.93	-0.26	0.87
Park workers vs. Ngogo chimps[c]	-1.99	**< 0.001**	.10	0.96
Villagers vs. Ngogo chimps[c]	.11	0.99	-.17	0.97
Villagers vs. park workers	2.10	**0.02**	–.28	0.87

[a]Data were the by-subject detection of *E. coli* resistant to ampicillin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, or tetracycline. Each agent was counted separately.
[b]P values, by the F-test from ANOVA, are shown in boldface when P < 0.05.
[c]Chimps, chimpanzees. (Kanyawara chimps had human contact; Ngogo chimps did not.)

Table 5: Statistical analysis of multidimensional scaling results for molecular data[a].

	Axis X1		Axis X2	
Comparison groups	**Difference in means**	**P value[b]**	**Difference in means**	**P value[b]**
Ngogo chimps[c] vs. Kanyawara chimps[c]	0.39	0.94	1.05	0.11
Park workers vs. Kanyawara chimps[c]	-2.86	**< 0.001**	-2.97	**< 0.001**
Villagers vs. Kanyawara chimps[c]	– 1.93	.11	-0.67	0.64
Park workers vs. Ngogo chimps[c]	-3.25	**< 0.001**	-4.02	**< 0.001**
Villagers vs. Ngogo chimps[c]	-2.33	0.08	-1.72	**0.04**
Villagers vs. park workers	.92	0.76	2.29	**0.002**

[a]Molecular data included all phylogenetic groups and virulence-associated genes, regardless of the corresponding P values for between-group comparison.
[b]P values, by the F-test from ANOVA, are shown in boldface when P < 0.05.
[c]Chimps, chimpanzees. (Kanyawara chimps had human contact; Ngogo chimps did not.)

Table 6: Phylogenetic distribution of resistance phenotypes.

	No. of isolates resistant (column %)					P value[a], individual group vs. all other isolates		
Agent[b]	Total (n = 131)	Group A (n = 19)	Group B1 (n = 14)	Group B2 (n = 78)	Three-group comparison P value[a]	A vs. all	B1 vs. all	B2 vs. all
AMP	21 (16)	4 (21)	5 (36)	10 (13)	0.09	0.51	**0.049**	0.24
SAM	6 (5)	2 (11)	2 (14)	1 (1.3)	**0.04**	0.21	0.12	**0.04**
SXT	19 (15)	7 (37)	5 (36)	1 (1.3)	**< 0.001**	**0.001**	**0.01**	**< 0.001**
TET	19 (15)	5 (26)	3 (21)	1 (1.3)	**< 0.001**	**0.006**	0.075	**0.001**

[a]P values, by Fisher's exact test (two-tailed), are shown in boldface when P < 0.05.
[b]AMP, ampicillin; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

was most prevalent within groups A and B1, which had similar values, but scant within group B2.

Virulence gene scores also varied significantly by phylogenetic group (overall, P < 0.001). Among all 139 strains, virulence gene scores (median [interquartile range]) were typically much higher for group B2 (8.0 [3.5]) than for the other phylogenetic groups, including groups A (4.0 [3.6]), B1 (3.0 [3.0]), C (2.0 [n = 1]), D (3.0 [2.5]), E (3.0 [0.25]), and F (2.0 [6.25]). Statistically significant differences in pairwise between-group comparisons were limited to comparisons involving group B2 (not shown). By contrast, the proportion of strains qualifying as ExPEC (n = 26, 19%) did not vary significantly with phylogenetic group (A, 19%; B1, 27%; B2, 20%; C, 0%; D, 11%; E, 0%; F, 17%: overall P = 0.85).

DISCUSSION

In this study we compared fecal *E. coli* from four groups of co-localized humans and chimpanzees in rural Uganda that differed for the extent of between-group contact and other exposures. We sought for evidence of between-group commonality of strains, resistance elements, and virulence-associated genes in relation to host species, degree of inter-group contact, and shared environmental factors. Our findings support three main conclusions. First, antimicrobial resistance in the chimpanzees correlated directly with their level of human contact. Second, although phylogenetic group and virulence-associated genes tracked mainly with host species, they also followed patterns of between-group contact and shared environments. Third, these associations varied greatly between different traits, consistent with the traits' diverse functions and genetic backgrounds.

Evidence suggesting contact-related transmission was greatest for antimicrobial resistance, which followed a descending prevalence gradient from park workers (highest), through Kanyawara chimpanzees (intermediate), to villagers and Ngogo chimpanzees (lowest). A plausible explanation is that park workers, who presumably were most heavily exposed to antimicrobials and to external sources of resistant organisms, served as a reservoir of resistant *E. coli* and mobile resistance elements. It is notable that although the Kanyawara community is known to raid crops [18] and would thus have contact to villagers, these data would additionally suggest exposure to fecal flora from park workers as an explanation for antimicrobial transfer. Ngogo chimps, which had no such exposures, correspondingly nearly lacked resistance. Whereas a prior study reported similar findings [11], the present study extends the findings of Goldberg et al., by the inclusion of the Ngogo community, with minimal human contact, as well as determination of antibiotic resistance based on separate individuals or chimps, which eliminates potential overrepresentation of isolates from a single individual.

By contrast, a predominant species effect – as suggested by comparably high prevalence levels in both chimpanzee groups, and comparably low prevalence levels (or absence) in both human groups – was most apparent for certain group B2-associated virulence genes (*papAH/C/G*, *hlyA*, *ibeA*, *clbB/N*, *fyuA*, and *ompT*). Finally, a predominant environmental exposure effect was suggested by the disproportionately high prevalence of *iroN* among Kanyawara chimpanzees, Ngogo chimpanzees, and villagers (possibly related to their joint in-park residence); of group B1 strains, *afa/draBC*, and *sat* specifically among park workers (possibly reflecting outside-park exposures); and of *bmaE* and *iss* among villagers (possibly reflecting within-village exposures).

Multidimensional scaling allowed a consolidated analysis of all studied antimicrobial markers and, separately, all studied molecular markers. These results suggested that, for antimicrobial resistance prevalence, exposure-related effects dominated, given the outlier position of park workers relative to other groups in the X1-X2 axis plot. Contact effects – implying transmission – were intermediately important, whereas species effects were minimally important. By contrast, all three types of effect appeared to contribute substantially to the distribution of molecular traits, with species effects predominating, especially for chimpanzees. These relationships are consistent with the greater horizontal mobility (including between bacterial species) of resistance determinants, as compared with other accessory traits – including virulence-associated genes – and core genome elements, which by contrast are more important for host adaptation.

To our knowledge, this is the first study of the phylogenetic group distribution and virulence-associated gene content of fecal *E. coli* from nonhuman primates without human contact, or from chimpanzees irrespective of degree of human contact. Previous studies of fecal *E. coli* from nonhuman primates relied on zoo animals [19-21], or wild animals with human contact [22], included very few subjects [19,20,23], and/or did not address phylogenetic groups and virulence genes [11].

In that regard, three prior studies warrant mention. Foster-Nyarko et al., studied 101 fecal *E. coli* isolates from 43 human-associated monkeys and baboons in The Gambia [22]. As among our chimpanzee isolates, phylogenetic group B2 predominated (42%), but was followed closely by group B1 (35%), which was nearly absent among our chimpanzee isolates. Comparisons with archival Gambian human isolate genomes showed fewer antimicrobial resistance genes among the monkey and baboon isolates than among the human isolates and several instances of human-animal genomic similarity, consistent with recent host jumps. Murphy at al. [24], studied the genomes of 119 commensal *E. coli* isolates from wild animals in Mexico [23], including four monkey isolates. Comparisons with archival human-source genomes showed scant commonality at the strain level, but appreciable commonality for resistance and virulence genes, consistent with horizontal transfer. Lescat et al., studied fecal *E. coli* from humans, domestic animals, and wild animals (no primates) in rural Guyana [25]. As in our study, phylogenetic group B2 prevalence followed a descending gradient from wild animals (high), through domestic animals (intermediate), to humans (low), whereas group A prevalence and extent of antimicrobial resistance followed the reverse gradient (human isolates high, wild animal isolates low).

Our findings uniquely document, for 92 wild chimpanzee-source isolates, a heavily group B2-dominated fecal *E. coli* population, which contrasts with the groups A and B1-dominated, or more broadly distributed, fecal *E. coli* populations of the park workers and villagers. The predominance of group B2 among the chimpanzee isolates supports the concept that group B2-associated characteristics may have been selected evolutionarily because they promote commensalism, rather than virulence [26]. Notwithstanding the notable differences between the present chimpanzee-source and human-source *E. coli* populations, it is conceivable that their commonalities, albeit limited, facilitated interspecies transfer of resistance elements or resistant strains, and subsequent retention in the new host.

The highly divergent phylogenetic distribution of park worker vs. villager isolates, which echoes the findings of Goldberg et al. [11], supports the concept that the human fecal *E. coli* population is shaped heavily by environmental and other exposures, even within a given locale [27,28]. This precludes broadly valid statements about a 'typical' profile [29,30].

The observed phylogenetic distribution of resistance scores (group B2 lowest) and virulence gene scores (group B2 highest) is consistent with patterns observed in other settings [31,32], but here may additionally reflect confounding by study group and the associated species and exposure effects. Untangling such confounding would require a larger sample size and, possibly, a different study design.

The study has several limitations. First, the sample size and depth of sampling were limited, especially for villagers. Despite this, however, multiple statistically significant differences emerged. Second, transmission was inferred presumptively from observed prevalence trends and known inter-group contact patterns, not assessed directly. Third, exposures were inferred from the groups' known ecological characteristics, not measured directly. Fourth, the strain typing addressed a limited range of targets and used traditional methods rather than whole-genome sequencing, which could have allowed a more fine-grained phylogenetic analysis. Fifth, conditions at the resource-limited, tropical study site during sample collection and initial processing may have introduced unrecognized artifacts; these, however, would not be expected to bias the study's key comparisons.

The study also has notable strengths. Its setting and design provided a unique opportunity to assess host species, contact, and presumed exposures as determinants of colonization with antibiotic-resistant and virulent *E. coli*. It uniquely examined commensal *E. coli* from wild chimpanzees with and without human contact. The sample collection method allowed individual host attribution to each sample. Molecular deduplication of the multiple isolates per sample allowed resolution of unique strains, as needed for valid statistical analyses, while preserving clonal diversity. Finally, the multidimensional statistical analyses complemented conventional univariable analyses, allowing a consolidated overview of the extensive datasets without multiple-comparison artifacts.

CONCLUSIONS

Among fecal *E. coli* from co-localized humans and wild chimpanzees in rural Uganda we found evidence suggesting contact-related, inter-species transfer of antimicrobial resistance from park workers to chimpanzees. We also identified likely species and exposure-related influences on the phylogenetic background and accessory gene content of fecal *E. coli*. These findings provide novel insights into the phylogenetic composition and virulence-gene content of chimpanzee-associated *E. coli* and suggest that the fecal *E. coli* population in humans and chimpanzees is multiply determined.

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ETHICS STATEMENT

The study was reviewed and approved by the Boston Veteran Affairs Medical Center (BVAMC) Institutional Review Board.

REFERENCES

1. Muehlenbein MP. Human-wildlife contact and emerging infectious diseases. In Human-environment interactions. Human-environment interactions. 2013; 1: 79-94.

2. Liu C, Aziz M, Park D, Wu Z, Stegger M, Li M, et al. Using source-associated mobile genetic elements to identify zoonotic extraintestinal e. Coli infections. One Health. 2023; 16: 100518.

3. Kidsley AK, White RT, Beatson SA, Saputra S, Schembri MA, Gordon D, et al. Companion animals are spillover hosts of the multidrug-resistant human extraintestinal escherichia coli pandemic clones st131 and st1193. Front Microbiol. 2020; 11: 1968.

4. Rolland RM, Hausfater G, Marshall B, Levy SB. Antibiotic-resistant bacteria in wild primates: Increased prevalence in baboons feeding on human refuse. Appl Environ Microbiol. 1985; 49: 791-794.

5. Routman E, Miller RD, Philips-Conroy J, Hartl DL. Antibiotic resistance and population structure in escherichia coli from free-ranging african yellow baboons. Appl Environ Microbiol. 1985; 50: 49-754.

6. Thompson ME, Muller MN, Machanda ZP, Otali E, Wrangham RW. The kibale chimpanzee project: Over thirty years of research, conservation, and change. Biol Conserv. 2020; 252: 108857.

7. Muehlenbein MP. Parasitological analyses of the male chimpanzees (pan troglodytes schweinfurthii) at ngogo, kibale national park, uganda. Am J Primatol. 2005; 65: 167-179.

8. Thompson ME, Machanda ZP, Scully EJ, Enigk DK, Otali E, Muller MN, et al. Risk factors for respiratory illness in a community of wild chimpanzees (pan troglodytes schweinfurthii). Roy Soc open sci. 2018; 5: 180840.

9. Negrey J, RB R, Scully E, Phillips-Garcia S, Owens L, Langergraber K, et al. Simultaneous outbreaks of respiratory disease in wild chimpanzees caused by distinct viruses of human origin. Emerg Microbes Infect. 2019; 8: 139-149.

10. Scully E, Basnet S, Wrangham R, Muller M, Otali E, Hyeroba D, et al. Lethal respiratory epidemic in wild chimpanzees associated with human rhinovirus c. Emerg Inf Dis. 2018; 24: 267-274.

11. Goldberg TL, Gillespie TR, Rwego IB, Wheeler E, Estoffa EL, Chapman CA. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western uganda. Biol Conserv. 2007; 135: 511-517.

12. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing; eighth informational supplement. Nccls document m100-s8. National Committee for Clinical Laboratory Standards: Wayne, PA, 1998.

13. Maslow JN, Whittam TS, Gilks CF, Wilson RA, Mulligan ME, Adams KS, et al. Clonal relationships among bloodstream isolates of escherichia coli. Infect Immun. 1995; 63: 2409-2417.

14. Clermont O, Christenson JK, Denamur E, Gordon DM. The clermont escherichia coli phylo-typing method revisited: Improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 2012; 5: 58-65.

15. Johnson JR, Stell AL. Extended virulence genotypes of escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000: 181: 261-272.

16. Johnson JR, Johnston B, Thuras P, Launer B, Sokurenko EV, Miller L. Escherichia coli sequence type 131 h30 is the main driver of emerging extended-spectrum-β-lactamase-producing e. Coli at a tertiary care center. mSphere 2016; 1: e00314-00316.

17. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic escherichia coli from retail chicken products. Antimicrob. Agents Chemother. 2003; 47: 2161-2168.

18. Krief S, Cibot M, Bortolamiol S, Seguya A, Krief JM, Masi S. Wild chimpanzees on the edge: Nocturnal activities in croplands. PLoS One. 2014; 9: 1-11.

19. Ochman H, Selander RK. Standard reference strains of escherichia coli from natural populations. J Bacteriol. 1984; 157: 690-693.

20. Herzer PJ, Inouye S, Inouye M, Whittam TS. Phylogenetic distribution of branched rns-linked multicopy single-stranded DNA among natural isolates of escherichia coli. J Bacteriol. 1990; 172: 6175-6181.

21. Johnson JR, O'Bryan TT, Kuskowski MA, Maslow JN. Ongoing horizontal and vertical transmission of virulence genes and papa alleles among escherichia coli blood isolates from patients with diverse-source bacteremia. Infect Immun. 2001; 69: 5363-5374.

22. Foster-Nyarko E, Alikhan NF, Ravi A, Thilliez G, Thomson NM, Baker D, et al. Genomic diversity of escherichia coli isolates from non-human primates in the gambia. Microb Genomics. 2020; 6: 1-15.

23. Souza V, Rocha M, Valera A, Eguiarte LE. Genetic structure of natural populations of escherichia coli in wild hosts on different continents. Appl Environ Microbiol. 1999; 65: 3373-3385.

24. Murphy R, Palm M, Mustonen V, Warringer J, Farewell A, Parts L, et al. Genomic epidemiology and evolution of escherichia coli in wild animals in mexico. mSphere. 2021; 6: e00738-00720.

25. Lescat M, Clermont O, Woerther PL, Djossou F, Dupont C, Perroz G, et al. Commensal escherichia coli strains in guiana reveal a high genetic diversity with host-dependant population structure. Environ Microbiol Rep. 2013; 5: 49-57.

26. Le Gall T, Clermont O, Gouriou S, Picard B, Nassif X, Denamur E, et al. Extraintestinal virulence is a coincidental by-product of commensalism in b2 phylogenetic group escherichia coli strains. Mol Biol Evol. 2007; 24: 2373-2384.

27. Escobar-Paramo P, Le Menac'h A, Le Gall T, Amorin C, Gouriou S, Picard B, et al. Identification of forces shaping the commensal escherichia coli genetic structure by comparing animal and human isolates. Environ Microbiol. 2006; 8: 1975-1984.

28. Skurkink D, Bonnet D, Bernède-Bauduin C, Michel R, Guette C, Becker JM, et al. Characteristics of human intestinal escherichia coli with changing environments. Environ Microbiol. 2008; 10: 2132-2137.

29. Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventre A, Elion J, et al. Commensal escherichia coli isolates are phylogenetically distributed among geographically distinct human populations. Microbiol. 2001; 147: 1671-1676.

30. Bailey JK, Pinyon JL, Anantham S, Hall RM. Distribution of human commensal escherichia coli phylogenetic groups. J Clin Microbiol. 2010; 48: 3455-3456.

31. Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in escherichia coli. J Infect Dis. 2001; 183: 78-88.

32. Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, et al. Escherichia coli sequence type 131 (st131) as an emergent multidrug-resistant pathogen among u.S. Veterans. Clin Infect Dis. 2013; 57: 1256-1265.