JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6064–6072 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6064–6072.2005

Bacterial Characteristics in Relation to Clinical Source of *Escherichia coli* Isolates from Women with Acute Cystitis or Pyelonephritis and Uninfected Women

James R. Johnson,[1,2]* Krista Owens,[1,2] Abby Gajewski,[1,2] and Michael A. Kuskowski[3,4]

Mucosal and Vaccine Research Center[1] and Geriatric Research, Education, and Clinical Center,[3] VA Medical Center, Minneapolis, Minnesota, and Departments of Medicine[2] and Psychiatry,[4] University of Minnesota, Minneapolis, Minnesota

Received 6 July 2005/Returned for modification 6 September 2005/Accepted 28 September 2005

Characteristics differentiating *Escherichia coli* strains that cause cystitis or pyelonephritis from fecal *E. coli* remain incompletely defined, particularly among adult women in the United States. Accordingly, phylogenetic group, O antigens, and virulence factors (VFs) were analyzed among 329 *E. coli* isolates from the mid-to-late 1990s from women in the United States with acute pyelonephritis ($n = 170$), cystitis ($n = 83$), or no infection (fecal; $n = 76$). Compared with fecal and cystitis isolates, pyelonephritis isolates exhibited a greater prevalence of phylogenetic group B2, most virulence-associated O antigens, and most VFs and had higher VF scores. In contrast, cystitis and fecal isolates differed minimally. By stepwise multivariable logistic regression, significant ($P \leq 0.015$) predictors of cystitis and/or pyelonephritis (versus fecal) included *afa*/*dra* (Dr-binding adhesins), *ibeA* (invasion of brain endothelium), *iha* (putative adhesin-siderophore), *malX* (pathogenicity island marker), the O75 antigen, *papEF* (P fimbriae), *papG* allele II (P adhesin variant), group B2, and *sfa*/*foc* (S and F1C fimbriae). However, virulence profiles overlapped considerably among source groups and varied greatly within each group. *E. coli* "clonal group A" (CGA) and the O2:K5/K7:H1 and O75:K+ clonal groups were significantly associated with cystitis and/or pyelonephritis. These findings identify potential vaccine targets, suggest that urovirulence is multiply determined, and confirm the urovirulence of specific *E. coli* clonal groups, including recently recognized CGA.

Acute cystitis is extremely common among reproductive-age women, whereas acute pyelonephritis, while much less common, is associated with high per-episode costs and morbidity (40). Together, cystitis and pyelonephritis are major contributors to the overall health burden and costs attributable to urinary tract infection (UTI) among women (40). Better characterization of the distinctive extraintestinal pathogenic *Escherichia coli* (ExPEC) strains that cause most episodes of cystitis and pyelonephritis in women could conceivably help guide the development of preventive measures against these important diseases (33, 41).

Most previous epidemiological comparisons involving *E. coli* urine isolates from patients with cystitis or pyelonephritis and fecal isolates from healthy controls were done with children and/or outside the United States and prior to the recent rise in antimicrobial resistance among uropathogens (1, 2, 5–7, 27, 28, 34). Since the human fecal *E. coli* flora can vary dramatically by locale (4, 44, 50) and since assays are now available for *E. coli* phylogenetic group (3) and for many more virulence factors (VFs) than were included in most previous studies (21, 24, 25), we chose to revisit this issue. Specifically, we analyzed *E. coli* urine isolates from women with acute uncomplicated cystitis or pyelonephritis and fecal isolates from uninfected women, as collected in the mid-to-late 1990s in the United States. We sought to identify syndrome-specific differences in the distribution of *E. coli* phylogenetic groups, O antigens, individual VFs, and composite virulence profiles.

* Corresponding author. Mailing address: Infectious Diseases (111F), Minneapolis VA Medical Center, 1 Veterans Drive, Minneapolis, MN 55417. Phone: (612) 467-4185. Fax: (612) 727-5995. E-mail: johns007@umn.edu.

MATERIALS AND METHODS

Subjects and isolates. The project was approved by the relevant institutional review boards. Fecal *E. coli* isolates were obtained in 1998 and 1999 from consenting women volunteers who did not have UTI-associated symptoms and were receiving (or were eligible to receive) health care at the University of Minnesota student health service (Minneapolis, MN). Fecal swabs were collected and processed within 48 h to isolate *E. coli* (12). One arbitrarily selected *E. coli* colony per sample was analyzed (42).

Cystitis isolates were recovered from urine samples from consenting women volunteers who presented to the University of Minnesota student health service in 1998 or 1999 with symptoms of uncomplicated acute cystitis and who had pyuria on microscopic analysis (30). Urine samples were refrigerated until cultured (100 μl) on MacConkey agar, within 72 h of collection. Lactose-fermenting, indole-positive colonies with an appropriate colonial morphology were defined as *E. coli*. From the 82 samples that yielded ≥10 CFU/ml *E. coli* (46), one arbitrarily selected *E. coli* colony per sample was analyzed (42).

Pyelonephritis isolates included the 170 available pretherapy *E. coli* urine isolates from women with acute uncomplicated pyelonephritis of mild-to-moderate severity from a recent multicenter treatment trial (1994 to 1996) (17, 48). The subjects were from 25 widely distributed U.S. locales, including five subjects from Minneapolis, MN (University of Minnesota Hospital or Student Health Service) (43).

Phylotyping, virulence genotyping, and O typing. Isolates were tested in duplicate for the four main *E. coli* phylogenetic groups (A, B1, B2, and D), 31 ExPEC-associated VFs, and 13 *papA* (P fimbriae subunit) alleles by using established PCR- and dot blot-based assays, together with appropriate positive and negative controls (3, 21, 24, 25). The VF score was the number of VFs detected, adjusted for multiple detection of the *pap* and *kps* (capsule) operons. Such molecular characteristics have previously been shown to predict experimental in vivo virulence (15, 36). O antigens were determined by the *E. coli* Reference Center (University Park, PA). The O1, O2, O4, O6, O7, O16, O18, O25, and O75 antigens were regarded as UTI-associated (O-UTI) (11).

TABLE 1. Distribution of phylogenetic groups, O antigens, and *papA* alleles (P fimbriae structural subunit) by source group among 329 *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women

Trait[a]	Prevalence of trait (no. [%]) among isolate group (*n*):				*P* value[b]		
	Total (329)	Fecal (76)	Cystitis (83)	Pyelo[c] (170)	Fecal vs cystitis isolates	Fecal vs pyelo isolates	Cystitis vs pyelo isolates
Group A	37 (11)	9 (12)	15 (18)	13 (8)			0.02
Group B1	23 (7)	9 (12)	12 (14)	2 (1)		0.001	<0.001
Group B2	197 (60)	40 (53)	37 (45)	120 (71)		0.009	<0.001
Group D	72 (22)	18 (24)	19 (23)	35 (21)			
O2	62 (19)	14 (18)	8 (10)	40 (24)			0.01
O16	10 (3)	1 (1)	0	9 (5)			0.03
O75	22 (7)	2 (3)	2 (2)	18 (11)		0.04	0.03
O-UTI	185 (56)	35 (46)	35 (42)	115 (67)		0.002	<0.001
F7-1 *papA* allele	17 (5)	2 (3)	0	15 (9)			0.003
F7-2 *papA* allele	16 (5)	0	2 (2)	14 (8)		0.006	
F10 *papA* allele	51 (16)	7 (9)	7 (8)	37 (21)		0.02	0.008
F14 *papA* allele	26 (8)	2 (3)	4 (9)	20 (12)		0.03	
F16 *papA* allele	31 (9)	2 (3)	10 (12)	19 (11)	0.03	0.03	

[a] Only those O antigens and *papA* alleles are shown that yielded a *P* value of <0.05 in both the initial screen for heterogeneity among groups and at least one pairwise comparison.
[b] *P* values (by Fisher's exact test) are shown where *P* is <0.05.
[c] Pyelo, pyelonephritis.

Statistical analysis. Comparisons of proportions were tested using Fisher's exact test for unpaired comparisons and McNemar's test for paired comparisons. (For multiple-group comparisons, an initial chi-square or Fisher's exact test for heterogeneity was done, and only if this yielded a *P* value of <0.05 were the individual pairwise comparisons tested.) Comparisons involving VF scores were tested using the Mann-Whitney U test. (For multiple-group comparisons, an initial test for heterogeneity across groups was done using a Kruskal-Wallis one-way analysis of variance, and only if this yielded a *P* value of <0.05 were individual pairwise comparisons examined.) Stepwise multivariable logistic regression analysis was used to assess multiple bacterial characteristics simultaneously as predictors of source group. Cluster analysis of VF profiles was done according to the unweighted pair group method with arithmetic averaging. The resulting tree was inspected (i) to determine whether each isolate's nearest neighbor in the tree represented the same source group, an alternate source group, or (if a cluster) multiple source groups and (ii) to identify clades exclusively representing a specific source group. The threshold for statistical significance was a *P* value of <0.05, with a *P* value of <0.015 used in the multivariable models.

RESULTS

Phylogenetic group. The three source groups (fecal, cystitis, and pyelonephritis) differed significantly for the prevalence of phylogenetic groups A, B1, and B2 but not D (Table 1). All differences were between the pyelonephritis group versus the fecal and cystitis groups rather than between the fecal and cystitis groups. Although phylogenetic group B2 predominated overwhelmingly among the pyelonephritis isolates, it was also the most prevalent group among the fecal and cystitis isolates (Table 1).

O antigens. The 9 UTI-associated O antigens were encountered, in descending order of frequency, as follows (with percentage of 329 isolates indicated in parentheses): O2 (19%); O6 (10%); O75 (7%); O1 and O18 (5% each); O4, O16, and O25 (3% each); O7 (2%). Overall, 185 (56%) isolates exhibited a UTI-associated O antigen. The O11/O17/O73/O77 antigens, characteristic of *E. coli* clonal group A (CGA) (17, 19, 30), occurred in 23 (7%) isolates. The O2, O16, and O75 antigens, and the O-UTI antigens collectively, were significantly associated with pyelonephritis, whereas the fecal and cystitis isolates did not differ significantly for any O antigen(s) (Table 1).

Virulence factors. Of the 31 VFs sought, all but *papG* allele I (P fimbriae adhesin variant) and *bmaE* (M fimbriae) were detected in ≥1 isolate each (Table 2). Of the detected VFs, 21 (72%) were significantly distributed by source group. All but 3 were significantly more prevalent among pyelonephritis isolates than among fecal and/or cystitis isolates. Exceptions included the K1 *kpsM* (capsule) variants (most prevalent among fecal isolates) and *ibeA* (invasion of brain endothelium; most prevalent among cystitis isolates) (Table 2). Only these two VFs differed significantly in prevalence between the fecal and cystitis isolates, and these differences were modest (Table 2).

***papA* alleles.** Eleven *papA* variants were detected, in order of descending frequency as follows (with the percentage of 329 isolates indicated in parentheses): F10 (15%); F11 (13%); F16 (9%); F14 (8%); F7-1, F7-2, and F48 (5% each); F8 (3%); F9 and F12 (2%); F13 (1.5%). Five alleles (F7-1, F7-2, F10, F14, and F16) were significantly associated with pyelonephritis (Table 1). The only apparent difference between the fecal and cystitis isolates involved the (CGA-associated) F16 allele (17, 19, 30), which was more prevalent among cystitis isolates (12% versus 3%; $P = 0.03$).

Virulence scores. Aggregate VF scores were significantly higher among pyelonephritis isolates (median, 9; range, 1 to 14) than among fecal isolates (median, 7; range, 0 to 13) or cystitis isolates (median, 7; range, 1 to 14) ($P < 0.001$ for both comparisons). Virulence scores did not differ significantly between fecal and cystitis isolates.

Multivariable analysis. Because of the known associations among VFs, O antigens, and phylogenetic groups (22, 24), stepwise multivariable logistic regression analysis was used to identify bacterial characteristics significantly associated with each source group. First, separate models were constructed to differentiate between the groups in pairwise comparisons (Table 3). In one set of models, the candidate predictor variables included all bacterial traits analyzed, i.e., the 4 phyloge-

TABLE 2. Distribution of virulence-associated traits by source group among 329 *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women

Trait[a]	Prevalence of trait (no. [%]) among isolate group (*n*):				*P* value[b]		
	Total (329)	Fecal (76)	Cystitis (83)	Pyelo[c] (170)	Fecal vs cystitis isolates	Fecal vs pyelo isolates	Cystitis vs pyelo isolates
papA/*C*/*EF*/*G*	178–180 (54)	28 (37)	34–35 (41–42)	116–117 (68–69)		<0.001	<0.001
papG allele II	141 (43)	19 (25)	20 (24)	102 (60)		<0.001	<0.001
papG alleles II + III	20 (6)	3 (4)	1 (1)	16 (9)			0.01
focG	41 (12)	4 (5)	4 (5)	33 (19)		0.003	0.002
afa/*draBC*	40 (12)	4 (5)	7 (8)	29 (17)		0.01	
iha	132 (40)	13 (17)	22 (27)	97 (57)		<0.001	<0.001
fimH	318 (97)	70 (92)	80 (96)	168 (99)		0.01	
hlyD	104 (32)	15 (20)	23 (28)	66 (39)		0.003	
cdtB	28 (9)	4 (5)	2 (2)	22 (13)			0.006
fyuA	256 (78)	51 (67)	57 (69)	148 (87)		<0.001	0.001
iutA	151 (46)	20 (26)	24 (29)	107 (63)		<0.001	<0.001
kpsM II	259 (79)	53 (70)	59 (71)	147 (86)		0.003	0.005
K1 *kpsM*	104 (32)	37 (49)	24 (29)	43 (25)	0.01	<0.001	
traT	218 (66)	45 (59)	49 (59)	124 (73)		0.04	0.03
ibeA	43 (13)	7 (9)	18 (22)	18 (11)	0.048		0.02
ompT	250 (76)	52 (68)	58 (70)	140 (82)		0.02	0.03
malX	212 (64)	46 (61)	38 (46)	128 (75)		0.02	<0.001
ExPEC	178 (54)	28 (37)	34 (41)	116 (69)		<0.001	<0.001

[a] Traits shown are those that yielded a *P* value of <0.05 in both the initial screen for heterogeneity among groups and at least one pairwise comparison, including *afa*/*dra* (Dr-binding adhesins), *cdtB* (cytolethal distending toxin), ExPEC (defined as presence of ≥2 of *papA* and/or *papC, afa*/*dra, sfa*/*foc, kpsM* II, and *iutA*), *fimH* (type 1 fimbriae), *focG* (F1C fimbriae), *fyuA* (yersiniabactin siderophore system), *hlyD* (hemolysin), *ibeA* (invasion of brain endothelium), *iha* (adhesin siderophore), *iutA* (aerobactin system), *kpsM* II (group 2 capsule), K1 (K1 *kpsM* II variant), *malX* (pathogenicity island marker), *ompT* (outer membrane protease T), *papA*/*C*/*EF*/*G* (P fimbriae structural subunit, assembly, tip pilins, and adhesin), *papG* alleles II and III (P adhesin variants), and *traT* (serum resistance associated). Traits detected in ≥1 isolate each, but not significantly distributed by source group, include (the percentage of the total [*n* = 329] number of isolates is indicated in parentheses) *cnf1* (cytotoxic-necrotizing factor 1; 22%), *cvaC* (colicin [microcin] V; 3%), *gafD* (G fimbriae; 0.6%), *iroN* (siderophore receptor; 31%), *iss* (increased serum survival; 4%), K2 *kpsM* variant (4%), *kpsM* III (group 3 capsule; 2%), *rfc* (O4 lipopolysaccharide synthesis; 2%), *sfa*/*foc* (S and F1C fimbriae; 24%), and *sfaS* (S fimbriae adhesin; 9%). *papG* allele I and *bmaE* were not detected in any isolate.

[b] *P* values (by Fisher's exact test) are shown where *P* is <0.05.

[c] Pyelo, pyelonephritis.

netic groups, the 9 UTI-associated O antigens, the O11/O17/O73/O77 antigens (collectively), and the individual VFs and *papA* alleles. In these models, at the significance level of a *P* value of ≤0.015, 2 variables significantly predicted cystitis (versus fecal), including *ibeA* (positive) and the K1 *kpsM* variant (negative). In contrast, six variables significantly predicted pyelonephritis (versus fecal), including *papEF* (P fimbriae subunits), *iha* (putative adhesin-siderophore), and *ibeA* (all positive), and the F8 *papA* allele, *papG* allele III (P adhesin variant), and the K1 *kpsM* variant (all negative). Likewise, 4 variables significantly predicted pyelonephritis (versus cystitis), including *papG* allele II and the O75 antigen (both positive), and the F16 *papA* allele and group B1 (both negative). In a similar set of models in which candidate predictor variables were limited to *papA* alleles and VFs (without phylogenetic groups and O antigens), the same predictors of cystitis or pyelonephritis (versus fecal) emerged. However, newly predicting pyelonephritis (versus cystitis) were *afa*/*draBC* (Dr-binding adhesins) and *malX* (pathogenicity island marker), with no negative predictors (Table 3).

Next, multivariable models were constructed to differentiate fecal isolates from all urine isolates (i.e., cystitis plus pyelonephritis isolates) and pyelonephritis isolates from all nonpyelonephritis isolates (i.e., fecal plus cystitis isolates), using the same two sets of candidate predictor variables as above (Table 4). Significant predictors of fecal isolates (versus urine isolates) with both sets of candidate predictor variables included *iha*, *papEF*, and *ibeA* (all negative), and K1 (positive). For pyelonephritis isolates (versus all other isolates), with the first (extended) set of candidate predictors, significant predictors in-

TABLE 3. Stepwise multivariable logistic regression analysis for bacterial predictors of individual source groups among 329 *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women

Outcome variable (referent)	Predictor variable[a]	*P* value	Odds ratio	95% CI[b]
Cystitis (fecal)	K1 *kpsM* variant	0.001	0.30	0.15–0.63
	ibeA	0.004	4.54	1.62–12.73
Pyelonephritis (fecal)	K1 *kpsM* variant	<0.001	0.06	0.018–0.20
	F8 *papA* allele	0.008	0.07	0.009–0.050
	papG allele III	0.001	0.095	0.02–0.38
	iha	<0.001	5.96	2.47–14.40
	ibeA	<0.001	23.66	5.21–107.39
	papEF	<0.001	29.72	7.74–114.04
Pyelonephritis (cystitis) I[a]	F16 *papA* allele	0.004	0.23	0.008–0.63
	Group B1	0.013	0.35	0.16–0.81
	papG allele II	<0.001	8.44	3.93–18.12
	O75	0.002	11.22	2.35–53.59
Pyelonephritis (cystitis) II[a]	*papG* allele II	<0.001	5.20	2.78–9.82
	afa/*draBC*	0.007	3.68	1.44–9.43
	malX	0.003	2.48	1.36–4.53

[a] Predictor variables shown are those from the last step in which all variables included in the model yielded a *P* value of ≤0.015. Two different sets of candidate predictor variables were used for each endpoint (I, full set; II, short set). The first (full) set included all bacterial characteristics analyzed, i.e., the four phylogenetic groups, the UTI-associated O antigens (individually and collectively), the O11/O17/O73/O77 antigens, the individual *papA* alleles, individual virulence factors, and ExPEC status. The second (short) set included only the *papA* alleles, individual virulence factors, and ExPEC status. Results for "Cystitis (fecal)" and "Pyelonephritis (fecal)" were the same with either set of candidate predictor variables. For "Pyelonephritis (cystitis)," the two sets of candidate predictor variables yielded different results, as shown.

[b] CI, confidence interval.

TABLE 4. Stepwise multivariable logistic regression analysis for bacterial predictors of fecal or pyelonephritis source among 329 *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women[a]

Outcome variable (referent)	Predictor variable[b]	*P* value	Odds ratio	95% CI
Fecal (cystitis or pyelo)	*iha*	0.004	0.34	0.17–0.71
	K1	<0.001	5.05	2.46–10.16
	papEF	<0.001	0.27	0.14–0.54
	ibeA	<0.001	0.16	0.06–0.44
Pyelo (fecal or cystitis) I[b]	Group B2	0.001	1.37	1.14–1.64
	O75	0.003	6.00	1.81–19.84
	papG allele II	<0.001	6.30	3.71–10.70
	K1	<0.001	0.31	0.17–0.56
Pyelo (fecal or cystitis) II[b]	*papG* allele II	<0.001	7.65	4.43–13.21
	sfa/*focDE*	0.004	2.50	1.34–4.65
	afa/*draBC*	<0.001	6.21	2.73–14.12
	K1	0.006	0.47	0.47–0.81

[a] Pyelo, pyelonephritis; CI, confidence interval; *afa*/*draBC*, Dr-binding adhesins; *ibeA*, invasion of brain endothelium; *iha*, putative adhesin siderophore; K1, K1 capsule variant; *papEF*, P fimbriae minor pilins; *papG* allele II, P fimbriae adhesin variant; *sfa*/*focDE*, S and F1C fimbriae.

[b] Predictor variables shown are those from the last step in which all variables included in the model yielded a *P* value of ≤0.015. Two different sets of candidate predictor variables were used for each endpoint (I, full set; II, short set). The first (full) set included all bacterial characteristics analyzed, i.e., the four phylogenetic groups, the UTI-associated O antigens (individually and collectively), the O11/O17/O73/O77 antigens, the individual *papA* alleles, individual virulence factors, and ExPEC status. The second (short) set included only the *papA* alleles, individual virulence factors, and ExPEC status. Results for "Fecal (cystitis or pyelo)" were the same with either set of candidate predictor variables. For "Pyelo (fecal or cystitis)," the two sets of candidate predictor variables yielded different results, as shown.

cluded group B2, the O75 antigen, and *papG* allele II (all positive), and K1 (negative), whereas with the second (restricted) set of candidate predictors, *afa*/*draBC* and *sfa*/*focDE* replaced O75 and group B2 as positive predictors (Table 4).

Virulence profiles. To determine whether distinctive combinations of VFs also might differentiate among the three clinical source groups, aggregate VF profiles were analyzed. The 329 isolates exhibited 190 unique VF profiles. Of these profiles, 148 (78%) occurred in a single isolate each (148 isolates total), 31 (16%) occurred in from 2 to 4 isolates each (70 isolates total), and 11 (6%) occurred in ≥5 isolates each (range, 5 to 22; median, 7; 111 isolates total) (Table 5). Of these 11 frequent profiles, profiles 1 to 7 corresponded with familiar ExPEC clonal groups, including CGA (profiles 1 and 2; differing only for *fyuA* [yersiniabactin]), the O1/O2:K1:H7 clonal group (profile 3), the O18:K1:H7 clonal group (profile 4), the O6:K15:H31 clonal group (profile 5), and the O75:K+ clonal group (profiles 6 and 7; differing only for *iutA* [aerobactin]). These 11 VF profiles were broadly distributed by source group; 5 included pyelonephritis isolates plus either fecal ($n = 3$) or cystitis isolates ($n = 2$), whereas 6 included all 3 source groups. Notably, none included fecal and cystitis isolates without pyelonephritis isolates, or a single source group only.

Nonetheless, 5 of the 11 frequent VF profiles did exhibit some degree of syndrome specificity (Table 5). Two (profiles 1 and 6) were significantly overrepresented among pyelonephritis isolates, and 2 others (profiles 2 and 11) were overrepresented among cystitis isolates, with profile 11 also being significantly underrepresented among pyelonephritis isolates. In contrast, profile 3 was significantly overrepresented among fecal isolates, and profile 9 exhibited a similar trend ($P = 0.052$). In comparisons limited to fecal and cystitis isolates, profile 2 ($P = 0.03$) and profiles 1 and 2 combined ($P = 0.01$) were significantly associated with cystitis, whereas profile 3 was significantly associated with fecal isolates ($P = 0.03$).

TABLE 5. Frequently encountered virulence profiles among *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women

Profile no.[a]	Cluster[a]	No. of isolates[b]	Phylogenetic group(s)[c]	Main O group(s)[d] (no.)	Distribution of isolates by source[e]			Constituent gene(s)
					Fecal (76)	Cyst (83)	Pyelo (170)	
1	Ia	11	D	O17/O77 (6)	0	1	10**	*papA*/*C*/*EF*/*G*, F16, *papG* II, *iha*, *fim*, *iutA*, *kpsM* II, *traT*, *ompT*
2	Ia	9	D	O17/O77 (6)	0	6**	3	Same as profile 1, plus *fyuA*
3	Ia	22	B2	O1/O2 (19)	9*	2	11	*papA*/*C*/*EF*/*G*, F11, *papG* II, *iha*, *fim*, *fyuA*, *kpsM* II, K1, *traT*, *ompT*, *malX*
4	Ia	6	B2	O18 (5)	0	2	4	*papA*/*C*/*EF*/*G*, F10, *papG* III, *sfa*/*foc*, *sfaS*, *fim*, *hly*, *cnf*, *fyuA*, *kpsM* II, K1, *traT*, *ibeA*, *ompT*, *malX*
5	Ib	5	B2	O6 (5)	1	0	4	*papA*/*C*/*EF*/*G*, F48, *papG* III, *sfa*/*foc*, *sfaS*, *fim*, *hly*, *cnf*, *iroN*, *fyuA*, *kpsM* II, *traT*, *ompT*, *malX*
6	Ib	12	B2	O75 (12)	1	0	11**	*afa*/*dra*, *fim*, *fyuA*, *iutA*, *kpsM* II, *traT*, *ompT*, *malX*
7	IIa	5	B2	O75 (4)	1	0	4	Same as profile 6, minus *iutA*
8	IIa	7	Mult	Mult	0	3	4	*fim*, *fyuA*, *kpsM* II, K1, *traT*, *ibeA*, *ompT*
9	IIb	7	Mult	Mult	4	1	2	*fim*, *ompT*
10	IIb	5	Mult	Mult	2	2	1	*fim*, *iroN*, *fyuA*, *kpsM* II, K1, *traT*, *ibeA*, *ompT*
11	IIb	22	A, B1	Mult	6	10*	6(*)	*fim*

[a] Pathotypes and clusters were numbered sequentially as encountered in the virulence profile-based tree.

[b] Number of isolates with indicated pathotype.

[c] For pathotypes 1 to 7, all isolates were from the phylogenetic group shown for that pathotype. Pathotypes 8 to 10 were represented by isolates from three or four phylogenetic groups each. Pathotype 11 was represented by 15 group A isolates and 7 group B1 isolates. Mult, multiple groups.

[d] Within pathotypes 1 and 2, 12 isolates expressed either the O17 or O77 antigen ($n = 6$) or both antigens ($n = 6$). Additionally, pathotype 2 contained one isolate each that expressed either the O11 or O73 antigen. Mult, multiple O antigens.

[e] Numbers shown in parentheses are total numbers in each source group (fecal, cystitis, pyelonephritis). *P* value symbols (*, $P < 0.05$; **, $P < 0.01$), by Fisher's exact test, are for prevalence of the indicated pathotype within the particular source group versus among all other isolates combined. Symbols shown in parentheses denote negative associations. Cyst, cystitis; Pyelo, pyelonephritis.

TABLE 6. Distribution within virulence profile-based tree of 325 *Escherichia coli* isolates from uninfected women and women with acute cystitis or pyelonephritis

Cluster or subcluster[a]	No. of isolates (% of total) (n = 329)[a]	No. (%) of isolates of cluster or subcluster		
		Fecal (76 [23%] of 329)	Cystitis (83 [25%] of 329)	Pyelonephritis (166 [50%] of 329)[a]
I	175 (53)	28 (16)[b]	35 (20)[c]	112 (64)[d]
Ia	114 (35)	17 (15)[d]	20 (18)	77 (68)[d]
Ib	61 (19)	11 (18)	15 (25)[c]	35 (57)
II	150 (46)	48 (32)[b]	48 (32)[c]	54 (36)[d]
IIa	80 (24)	19 (24)	21 (26)	40 (50)
IIb	70 (21)	29 (41)[d]	27 (39)[c]	14 (20)[d]

[a] In the virulence profile-based dendrogram, 4 additional pyelonephritis isolates were placed outside the 2 main clusters (and 4 main subclusters).
[b] $P = 0.002$, for prevalence of source group within indicated cluster or subcluster versus the rest of the population.
[c] $P \leq 0.03$, for prevalence of source group within indicated cluster or subcluster versus the rest of the population.
[d] $P \leq 0.001$, for prevalence of source group within indicated cluster or subcluster versus the rest of the population.

TABLE 7. Phylogenetic distribution of O antigens and *papA* alleles (P fimbriae structural subunit) by source group among 329 *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women

Trait[a]	Prevalence of trait (no. of isolates [%]) within group[b]:			
	A (n = 37)	B1 (n = 23)	B2 (n = 197)	D (n = 72)
O2	2 (5)*	1 (4)	53 (27)***	6 (8)(*)
O6	0*	1 (4)	31 (16)***	1 (1)(**)
O7	0	0	1 (0.5)(*)	5 (7)**
O11/O17/O73/O77	0	0	2 (1)(***)	21 (30)***
O18	1 (3)	0	15 (8)**	0 (*)
O75	0	1 (4)	21 (11)***	0 (**)
O-UTI	3 (8)(***)	3 (13)(***)	163 (83)***	16 (22)(***)
F10 *papA* allele	0 (**)	0 (*)	43 (22)***	8 (11)
F11 *papA* allele	0 (**)	0	43 (22)***	0 (***)
F14 *papA* allele	0	0	26 (13)***	0 (**)
F16 *papA* allele	1 (3)	0	4 (2)(***)	26 (36)***
F48 *papA* allele	0	0	14 (7)*	2 (3)

[a] Only those O antigens and *papA* alleles are shown that yielded a P value of <0.05 in both the initial screen for heterogeneity among groups and at least one pairwise comparison.
[b] P values (by Fisher's exact test) are indicated by asterisks where P is <0.05. Symbols: *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Parentheses indicate negative associations.

VF profile tree. Cluster analysis of aggregate VF profiles yielded a tree consisting of two major clusters (I and II), each comprising two subclusters (not shown). Isolates were significantly distributed within this tree according to clinical group (Table 6). Within cluster 1 (n = 175), pyelonephritis isolates predominated overwhelmingly, whereas within cluster II (n = 150), the three source groups were evenly distributed. Overall, pyelonephritis isolates exhibited a declining prevalence gradient, from predominance within subcluster Ia to a minority position within subcluster IIb. Fecal isolates exhibited an opposite trend, whereas cystitis isolates exhibited an intermediate pattern of distribution. These prevalence shifts were statistically significant within the two main clusters and within three of the four subclusters (Ia, Ib, and IIb) (Table 6).

In the VF profile tree, 3 smaller clades were identified that contained ≥5 isolates each, exclusively from a single source group. All 3 of these clades contained only pyelonephritis isolates. The numbers of isolates per clade (the P value for prevalence of pyelonephritis isolates in clade versus remainder of population is given in parentheses) were as follows: clades 1 and 2, 9 isolates each (P = 0.004); clade 3, 7 isolates (P = 0.015). In clades 2 and 3 (which were closely related), all 16 constituent isolates expressed the O2 antigen and had virulence profiles corresponding with the O2:K5/K7:H1 clonal group (24).

The fecal and cystitis isolates did not differ significantly according to relative prevalence by major cluster or subcluster (Table 6). Likewise, no clade with ≥5 members contained only fecal or only cystitis isolates, without representatives of another source group. However, when analyzed apart from the pyelonephritis isolates, fecal and cystitis isolates were significantly more likely to have as their nearest neighbor in the VF tree a representative of the same source group, as opposed to a member of the alternate source group (P = 0.01, McNemar's test).

As for segregation of urine isolates (i.e., cystitis plus pyelonephritis isolates) from fecal isolates by virulence profiles, 4 clades with ≥5 members contained only urine isolates, without fecal isolates. The numbers of isolates per clade (the P value for prevalence of urine isolates in the clade versus the remainder of population is given in parentheses) were as follows: clade A, 19 isolates (P = 0.01); clade B, 24 isolates (P = 0.002); clade C, 12 isolates (P = 0.08); clade D, 6 isolates ($P > 0.10$); and clade D, 7 isolates ($P > 0.10$). The associated VF profiles were quite extensive (not shown). By VF profile, O antigens, and phylogenetic group, clade B corresponded with CGA and clade C corresponded with the O2:K5/K7:H1 clonal group. In contrast, 3 other clades with ≥5 members each contained only fecal and cystitis isolates, without pyelonephritis isolates. P values for the prevalence of nonpyelonephritis isolates in these clades, versus the remainder of the population, ranged from 0.01 to 0.03. These clades were smaller and more diverse than the pyelonephritis-associated clades. Each comprised only 5 to 6 isolates (typically with sparse virulence profiles) but multiple O antigens and phylogenetic groups (not shown).

Bacterial characteristics in relation to colony count. The similarity of the cystitis and fecal isolates suggested the possibility that, in some instances, fecal contaminants were misclassified as cystitis due to the low colony count criterion used for cystitis. Accordingly, bacterial characteristics were analyzed in relation to colony count among the cystitis isolates, with the expectation that low-count isolates (if fecal contaminants) should exhibit a lower prevalence of UTI-associated O antigens, VFs, and phylogenetic group B2 than high-count isolates. However, with three different colony count thresholds used to dichotomously stratify the population, the few significant differences that were detected (0 to 4 per stratification) actually involved a higher prevalence of virulence-associated traits among low-count isolates (not shown).

Phylogenetic distribution of bacterial traits. The similarity of cystitis and fecal isolates also prompted an assessment of whether bacterial traits were phylogenetically distributed as expected. Indeed, 4 of the UTI-associated O antigens were significantly concentrated within group B2, and the O7 and

TABLE 8. Phylogenetic distribution of virulence-associated traits among 329 *Escherichia coli* isolates from women with cystitis or pyelonephritis and uninfected women

Trait[a]	Prevalence of trait (no. [%] of isolates) within phylogenetic group[b]:			
	A (n = 37)	B1 (n = 23)	B2 (n = 197)	D (n = 72)
papA/C/EF/G	2–3 (5–8)(***)	0 (***)	134–135 (68)***	42 (58)
papG allele II	2 (5)(***)	0 (***)	97 (49)***	42 (58)**
papG allele III	0 (***)	0 (*)	54 (27)***	3 (4)(***)
sfa/focDE	0 (***)	1 (4)(*)	76 (39)***	1 (1)(***)
sfaS	0	1 (1)	26 (13)***	1 (1)(**)
focG	0 (**)	1 (1)	40 (20)***	0 (***)
afa/draBC	6 (16)	0	31 (16)*	3 (4)(*)
iha	7 (19)(**)	0 (***)	82 (42)	43 (60)***
fimH	32 (86)(**)	20 (87)(*)	197 (100)***	69 (96)
hlyD	2 (5)(***)	1 (4)(**)	90 (46)***	11 (15)(***)
cnf1	0 (***)	2 (9)	71 (36)***	1 (1)(***)
cdtB	0	1 (4)	27 (13)***	0 (***)
iroN	1 (3)(***)	4 (17)	89 (45)***	7 (10)(***)
fyuA	14 (38)(***)	6 (26)(***)	195 (99)***	41 (57)(***)
iutA	8 (22)(**)	1 (4)(***)	98 (50)	44 (61)**
kpsM II	9 (24)(***)	5 (22)(***)	184 (93)***	61 (85)
K1 *kpsM*	3 (8)(***)	4 (17)	82 (42)***	15 (21)*
traT	12 (32)(***)	7 (30)(***)	148 (75)***	51 (71)
ibeA	0 (**)	3 (13)	35 (18)**	5 (7)
ompT	6 (16)(***)	9 (39)(***)	183 (93)***	52 (72)
malX	2 (5)(***)	1 (4)(***)	190 (96)***	19 (26)(***)
ExPEC	2 (5)(***)	0 (***)	135 (69)***	41 (57)

[a] Traits shown are those that yielded P values of <0.05 in both the initial screen for heterogeneity among groups and at least one pairwise comparison, including *afa/dra* (Dr-binding adhesins), *cdtB* (cytolethal distending toxin), *cnfI* (cytotoxic-necrotizing factor 1), *fimH* (type 1 fimbriae), *focG* (F1C fimbriae), *fyuA* (yersiniabactin siderophore system), *hly* (hemolysin), *iha* (adhesin-siderophore), *ireA* (siderophore receptor), *ibeA* (invasion of brain endothelium), *iroN* (siderophore receptor), *iutA* (aerobactin system), *kpsM* II (group 2 capsule), K1 (K1 *kpsM* II variant), K2 (K2 *kpsM* II variant), *malX* (pathogenicity island marker), *ompT* (outer membrane protease T), *papA/C/EF/G* (P fimbriae structural subunit, assembly, tip pilins, and adhesin), *papG* alleles II and III (P fimbriae adhesin variants), *rfc* (O4 lipopolysaccharide synthesis), *sfa/foc* (S and F1C fimbriae), *sfaS* (S fimbriae adhesin), and *traT* (serum resistance associated). Traits detected in ≥1 isolate each, but not significantly phylogenetically distributed, include (the percentage of the total of 329 isolates is indicated in parentheses) *cvaC* (colicin [microcin] V; 3%), *gafD* (G fimbriae; 0.6%), *iss* (increased serum survival; 4%), K2 *kpsM* II variant (4%), *kpsM* III (group 3 capsule; 2%), and *rfc* (O4 lipopolysaccharide synthesis; 2.4%); *papG* allele I and *bmaE* were not detected in any isolate.

[b] P values (by Fisher's exact test) are indicated by asterisks where P is <0.05. Symbols: *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Parentheses indicate negative associations.

(CGA-associated) O11/O17/O73/O77 antigens were concentrated within group D (Table 7). Likewise, 4 *papA* alleles were significantly associated with group B2, and the F16 allele was associated with group D (Table 7). Similarly, 23 VFs were significantly phylogenetically distributed, with group B2 exhibiting the highest prevalence for all VFs but *papG* allele II, *iha*, and *iutA*, which instead were significantly concentrated within group D (Table 8). Accordingly, aggregate VF scores (medians and ranges are given in parentheses) were highest within group B2 (9; 2 to 14), intermediate within group D (7; 0 to 12), and lowest within groups A (2; 1 to 11) and B1 (2; 0 to 10) ($P <$ 0.001 for each group versus all others).

DISCUSSION

We found that among *E. coli* isolates from U.S. women, acute pyelonephritis isolates differed greatly from contemporaneous cystitis and fecal isolates according to phylogenetic group, O antigens, and VFs, whereas cystitis and fecal isolates exhibited considerably fewer (and subtler) differences. Additionally, although certain virulence profiles and groups of related profiles were significantly distributed by source group, no one VF or profile was confined to a single source group. These findings identify potential targets for anti-UTI vaccines, suggest that UTI pathogenesis is multiply determined, and indicate that most ExPEC clonal groups and their distinctive VFs may be syndromically versatile.

Not surprisingly, pyelonephritis isolates exhibited a much greater prevalence of phylogenetic group B2, UTI-associated O antigens, and individual VFs, plus higher aggregate VF scores, than did cystitis and fecal isolates. They also accounted for several prominent clades within the VF profile tree, two of which corresponded with familiar ExPEC clonal groups, specifically *E. coli* O2:K5/K7:H1 and O75:K+ (24), thereby identifying these as "pyelonephritogenic clones" (35). Notably, the individual VFs that, in various multivariable models, were significant predictors of pyelonephritis included three traditionally recognized "uropathogenic" traits (*papG* allele II, *afa/dra*, and *sfa/foc*), plus a more recently identified meningitis-associated trait (*ibeA*) (10) and two relative newcomers, *iha* and *malX* (21, 24). Experimental data indicate that *papG* II, *afa/dra*, *sfa/foc*, and *iha* contribute to the pathogenesis of pyelonephritis (8, 14, 31, 38). Likewise, *malX*, heretofore regarded only as a pathogenicity island marker, was recently shown to contribute to experimental avian septicemia (29). Experimental assessment of *malX* and *ibeA* as uro-VFs is warranted, since these may be useful targets for interventions such as vaccines.

In contrast, the cystitis isolates differed surprisingly little from the fecal isolates. Only two individual VFs differentiated these groups, and the differences were in opposing directions,

resulting in similar aggregate VF scores. Between-group differences in virulence profiles were also subtle. However, statistically significant clustering of virulence profiles by source group (cystitis versus fecal) was found, both by prevalence assessments involving individual profiles or groups of related profiles and by "nearest neighbor" analysis. Likewise, in the multivariable analyses, the fecal and cystitis isolates exhibited nonoverlapping sets of predictor variables that differentiated either group from pyelonephritis isolates. Thus, the cystitis and fecal isolates differed statistically but in ways not obviously suggesting greater virulence for the cystitis isolates or identifying useful targets for interventions. One exception was the significant association with cystitis (versus fecal) of *E. coli* CGA. This provided novel epidemiological evidence of enhanced urovirulence for this recently emerged multidrug-resistant clonal group and suggested that interventions against it could be protective (17, 19, 30).

Several possible explanations for the considerable similarity of cystitis and fecal isolates were considered. Misclassification bias seemed unlikely, since low-count cystitis isolates appeared as virulent as or more virulent than high-count isolates. The phylogenetic distribution of VFs, VF scores, and O antigens within the total population was consistent with previous work, evidence against laboratory artifacts, or anomalies within the collection. Significant host mismatching between the fecal and cystitis subjects also seemed unlikely, since these isolates were collected approximately concurrently from women eligible to attend the same university student health service. Future analysis of host characteristics in relation to bacterial traits within the cystitis and fecal groups might be informative (18, 23), as might comparisons of unique fecal and urine clones from subjects with cystitis (22). It is probable that the fecal group included a number of potential cystitis isolates that were residing innocuously within the fecal reservoir (47), the presence of which would tend to bias comparisons toward the null. Finally, our presence-absence testing may have overlooked key determinants of cystitis, possibly including unrecognized VFs (49), minor sequence variants of known VFs (45), or differences in VF expression (9).

Interestingly, whereas by molecular criteria the present cystitis isolates appear to be generally as virulent as previously analyzed cystitis isolates, the present fecal isolates appear more virulent than other reported fecal isolates (4, 16, 20, 22, 28, 32, 39, 44, 50). Since fecal *E. coli* populations can vary by locale and host group (4, 44, 50), it may be that the present control subjects have a comparatively high-virulence colonic flora. If so, and if colonization with virulent *E. coli* is a rate-limiting step in the development of UTI (47), it may be that the incidence of UTI is higher in the present control population than in similar populations with a less-virulent fecal flora. Future comparative studies of UTI incidence in relation to the molecular virulence characteristics of the local fecal *E. coli* population would be of interest.

Our findings suggest that no single VF or virulence profile and few groups of related profiles are entirely specific to cystitis, pyelonephritis, or UTI in general. Although statistically significant differences were detected, most were relative, not categorical. Conversely, VF profiles were quite diverse within each source group, and multiple VFs contributed significantly in combination to statistical differentiation among source groups. These observations argue against a strict linkage of specific VFs or VF profiles (and the corresponding clonal groups) with a particular clinical source and against the concept that a single VF underlies extraintestinal virulence. Accordingly, optimal anti-VF interventions may need to address multiple targets (33); this may provide the added benefit of protection against multiple clinical syndromes (13, 26).

Limitations of the study include the modest numbers of fecal and cystitis isolates, paucity of clinical/epidemiological data, lack of attention to gene expression and minor sequence variants, and use of multiple comparisons, resulting in an increased risk for type I error. Since this was an exploratory, hypothesis-generating study involving multiple comparisons, the putatively significant associations require validation in future studies before being considered definitive. (Notably, however, many of the observed associations were extremely strong and, hence, are quite unlikely to represent chance findings.) The necessary use of stepwise regression modeling techniques to isolate important predictor variables may have led to a further capitalization on chance variation at the expense of true underlying effects, although to guard against this in these analyses we used a more stringent criterion for statistical significance. In principle, it would be preferable to study pyelonephritis isolates from the same locale as the comparison cystitis and fecal isolates; however, this was logistically unfeasible. Moreover, we are unaware of evidence that the *E. coli* clonal groups that cause acute uncomplicated pyelonephritis are differentially distributed across the United States in a way that would bias these comparisons.

The strengths of the study include the large number of clinically well defined, prospectively collected pyelonephritis isolates, inclusion of (prospectively collected) fecal isolates from the same locale and similar time frame as the (prospectively collected) cystitis isolates, the extensive array of traits assessed, which have been shown to predict experimental virulence (15, 37), and the use of multiple complementary analytical approaches.

In summary, we found that *E. coli* isolates from women with acute pyelonephritis differed considerably from contemporaneous cystitis and fecal isolates, which in contrast differed minimally. Although certain virulence profiles were significantly distributed by source group, no single VF or profile was confined to a single source group. *E. coli* CGA was prominent in both cystitis and pyelonephritis, whereas the O2:K5/K7:H1 and O75:K+ clonal groups were particularly prominent in pyelonephritis. These findings identify potential vaccine targets, suggest that urovirulence is multiply determined, and indicate that many ExPEC clonal groups and VFs may be pathogenically versatile.

ACKNOWLEDGMENTS

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

Timothy T. O'Bryan (Veterans Affairs Medical Center; current affiliation, Gentra Systems, Minneapolis, MN) provided expert technical assistance, and Ann Emery (Veterans Affairs Medical Center) helped prepare the manuscript.

REFERENCES

1. **Arthur, M., C. E. Johnson, R. H. Rubin, R. D. Arbeit, C. Campanelli, C. Kim, S. Steinbach, M. Agarwal, R. Wilkinson, and R. Goldstein.** 1989. Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect. Immun. **57:**303–313.
2. **Blanco, M., J. E. Blanco, M. P. Alonso, and J. Blanco.** 1996. Virulence factors and O groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. Eur. J. Epidemiol. **12:**191–198.
3. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
4. **Duriez, P., O. Clermont, S. Bonacorsi, E. Bingen, A. Chaventre, J. Elion, B. Picard, and E. Denamur.** 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology **147:**1671–1676.
5. **Elo, J., L. G. Tallgren, V. Vaisanen, T. K. Korhonen, S. B. Svenson, and P. H. Makela.** 1985. Association of P and other fimbriae with clinical pyelonephritis in children. Scand. J. Urol. Nephrol. **19:**281–284.
6. **Enerbäck, S., A.-C. Larsson, H. Leffler, A. Lundell, P. de Man, B. Nilsson, and C. Svanborg-Edén.** 1987. Binding to galactoseα1$\rightarrow$4galactoseβ-containing receptors as potential diagnostic tool in urinary tract infection. J. Clin. Microbiol. **25:**407–411.
7. **Gander, R. M., V. L. Thomas, and M. Forland.** 1985. Mannose-resistant hemagglutination and P receptor recognition of uropathogenic Escherichia coli isolated from adult patients. J. Infect. Dis. **151:**508–513.
8. **Goluszko, P., S. L. Moseley, S. L. Truong, A. Kaul, J. R. Williford, R. Selvarangan, and S. Nowicki.** 1997. Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75:K5:H-bearing Dr fimbriae: mutation in the dra region prevented tubulointerstitial nephritis. J. Clin. Investig. **99:**1662–1672.
9. **Gunther, N. W., IV, V. Lockatell, D. E. Johnson, and H. L. T. Mobley.** 2001. In vivo dynamics of type 1 fimbria regulation in uropathogenic *Escherichia coli* during experimental urinary tract infection. Infect. Immun. **69:**2838–2846.
10. **Huang, S.-H., C. Wass, Q. Fu, N. V. Prasadarao, M. Stins, and K. S. Kim.** 1995. *Escherichia coli* invasion of brain microvascular endothelial cells in vitro and in vivo: molecular cloning and characterization of invasion gene *ibe10*. Infect. Immun. **63:**4470–4475.
11. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
12. **Johnson, J. R., J. J. Brown, U. B. Carlino, and T. A. Russo.** 1998. Colonization with and acquisition of uropathogenic *Escherichia coli* strains as revealed by polymerase chain reaction-based detection. J. Infect. Dis. **177:**1120–1124.
13. **Johnson, J. R., P. Delavari, and T. O'Bryan.** 2001. *Escherichia coli* O18:K1:H7 isolates from acute cystitis and neonatal meningitis exhibit common phylogenetic origins and virulence factor profiles. J. Infect. Dis. **183:**425–434.
14. **Johnson, J. R., S. Jelacic, L. M. Schoening, C. Clabots, H. L. T. Mobley, and P. I. Tarr.** 2005. The IrgA homologue adhesin (Iha) is an *Escherichia coli* virulence factor in murine urinary tract infection. Infect. Immun. **73:**965–971.
15. **Johnson, J. R., M. Kuskowski, E. Denamur, J. Elion, and B. Picard.** 2000. Clonal origin, virulence factors, and virulence. Infect. Immun. **68:**424–425.
16. **Johnson, J. R., M. A. Kuskowski, K. Owens, S. Soto, J. P. Horcajada, M. T. Jimenez de Anta, and J. Vila.** 2005. Extended virulence genotypes of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. J. Infect. Dis. **191:**46–50.
17. **Johnson, J. R., A. R. Manges, T. T. O'Bryan, and L. R. Riley.** 2002. A disseminated multi-drug resistant clonal group of extraintestinal pathogenic *Escherichia coli* as a cause of pyelonephritis. Lancet **359:**2249–2251.
18. **Johnson, J. R., S. Moseley, P. Roberts, and W. E. Stamm.** 1988. Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. Infect. Immun. **56:**405–412.
19. **Johnson, J. R., A. C. Murray, M. A. Kuskowski, S. Schubert, M.-F. Prere, B. Picard, R. Colodner, R. Raz, and Trans-Global Initiative for Antimicrobial Resistance Analysis (TIARA) Investigators.** 2005. Distribution and characteristics of *Escherichia coli* clonal group A. Emerg. Infect. Dis. **11:**141–145.
20. **Johnson, J. R., T. T. O'Bryan, P. Delavari, M. Kuskowski, A. Stapleton, U. Carlino, and T. Russo.** 2001. Clonal relationships and extended virulence genotypes among *Escherichia coli* isolates from women with first episode or recurrent cystitis. J. Infect. Dis. **183:**1508–1517.
21. **Johnson, J. R., T. A. Russo, P. I. Tarr, U. Carlino, S. S. Bilge, J. C. J. Vary, and A. L. Stell.** 2000. Molecular epidemiological and phylogenetic associations of two novel putative virulence genes, *iha* and $iroN_{E.\ coli}$, among *Escherichia coli* isolates from patients with urosepsis. Infect. Immun. **68:**3040–3047.
22. **Johnson, J. R., F. Scheutz, F. Ulleryd, M. Kuskowski, T. T. O'Bryan, and T. Sandberg.** 2005. Phylogenetic and pathotypic comparison of concurrent urine and rectal *Escherichia coli* isolates from men with febrile urinary tract infection. J. Clin. Microbiol. **43:**3895–3900.
23. **Johnson, J. R., F. Scheutz, P. Ulleryd, M. A. Kuskowski, T. T. O'Bryan, and T. Sandberg.** 2005. Host-pathogen relationships among *Escherichia coli* isolates from men with febrile urinary tract infection. Clin. Infect. Dis. **40:**813–822.
24. **Johnson, J. R., and A. L. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
25. **Johnson, J. R., A. L. Stell, F. Scheutz, T. T. O'Bryan, T. A. Russo, U. B. Carlino, C. C. Fasching, J. Kavle, L. van Dijk, and W. Gaastra.** 2000. Analysis of F antigen-specific *papA* alleles of extraintestinal pathogenic *Escherichia coli* using a novel multiplex PCR-based assay. Infect. Immun. **68:**1587–1599.
26. **Johnson, J. R., S. J. Weissman, A. L. Stell, E. Tritchina, D. E. Dykhuizen, and E. V. Sokurenko.** 2001. Clonal and pathotypic analysis of archetypal *Escherichia coli* cystitis isolate NU14. J. Infect. Dis. **184:**1556–1565.
27. **Källenius, G., R. Mollby, S. B. Svenson, I. Helin, H. Hultberg, B. Cedergren, and J. Winberg.** 1981. Occurrence of P-fimbriated Escherichia coli in urinary tract infection. Lancet **ii:**1369–1372.
28. **Kanamura, S., H. Kurazono, S. Ishitoya, A. Terai, T. Habuchi, M. Nakano, O. Ogawa, and S. Yamamoto.** 2003. Distribution and genetic association of putative uropathogenic virulence factors *iroN*, *iha*, *kpsMT*, *ompT* and *usp* in *Escherichia coli* isolated from urinary tract infections in Japan. J. Urol. **170:**2490–2493.
29. **Li, G., C. Laturnus, C. Evers, and L. H. Wieler.** 2005. Identification of genes required for avian *Escherichia coli* septicemia by signature-tagged mutagenesis. Infect. Immun. **73:**2818–2827.
30. **Manges, A. R., J. R. Johnson, B. Foxman, T. T. O'Bryan, K. E. Fullerton, and L. W. Riley.** 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N. Engl. J. Med. **345:**1007–1013.
31. **Marre, R., and J. Hacker.** 1987. Role of S- and common-type 1-fimbriae of *Escherichia coli* in experimental upper and lower urinary tract infection. Microb. Pathog. **2:**223–226.
32. **Muhldorfer, I., G. Blum, A. Donohue-Rolfe, H. Heier, T. Olschlager, H. Tschape, U. Wallner, and J. Hacker.** 1996. Characterization of *Escherichia coli* strains isolated from environmental water habitats and from stool samples of healthy volunteers. Res. Microbiol. **147:**625–635.
33. **O'Hanley, P.** 1996. Prospects for urinary tract infection vaccines, p. 405–425. *In* H. L. T. Mobley and J. W. Warren (ed.), Urinary tract infections: molecular pathogenesis and clinical management. ASM Press, Washington, D.C.
34. **O'Hanley, P., D. Low, I. Romero, D. Lark, K. Vosti, S. Falkow, and G. Schoolnik.** 1985. Gal-Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. N. Engl. J. Med. **7:**414–420.
35. **Orskov, I., F. Orskov, A. Birch-Andersen, M. Kanamori, and C. Svanborg Eden.** 1982. O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scand. J. Infect. Dis. **33:**18–25.
36. **Picard, B., N. Picard-Pasquier, R. Krishnamoorthy, and P. Goullet.** 1991. Characterization of highly virulent *Escherichia coli* strains by ribosomal DNA restriction fragment length polymorphism. FEMS Microbiol. Lett. **82:**183–188.
37. **Picard, B., J. Sevali Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.
38. **Roberts, J. A., B.-I. Marklund, D. Ilver, D. Haslam, M. B. Kaack, G. Baskin, M. Louis, R. Möllby, J. Winberg, and S. Normark.** 1994. The Gal(α1–4)Gal-specific tip adhesin of *Escherichia coli* P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc. Natl. Acad. Sci. USA **91:**11889–11893.
39. **Ruiz, J., K. Simon, J. P. Horcajada, M. Velasco, M. Barranco, G. Roig, A. Moreno-Martinez, J. A. Martinez, T. Jimenez de Anta, J. Mensa, and J. Vila.** 2002. Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men. J. Clin. Microbiol. **40:**4445–4449.
40. **Russo, T. A., and J. R. Johnson.** 2003. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: an overlooked epidemic. Microbes Infect. **5:**449–456.
41. **Russo, T. A., and J. R. Johnson.** 2000. A proposal for an inclusive designation for extraintestinal pathogenic *Escherichia coli*: ExPEC. J. Infect. Dis. **181:**1753–1754.
42. **Sannes, M. R., M. A. Kuskowski, and J. R. Johnson.** 2004. Antimicrobial resistance among *Escherichia coli* isolates from women with cystitis and pyelonephritis, healthy human volunteers, and domestic dog feces. J. Am. Vet. Med. Assoc. **225:**368–373.
43. **Sannes, M. R., M. A. Kuskowski, and J. R. Johnson.** 2004. Geographical distribution of antimicrobial resistance among *Escherichia coli* causing acute uncomplicated pyelonephritis in the United States. FEMS Immunol. Microbiol. **42:**213–218.
44. **Sannes, M. R., M. A. Kuskowski, K. Owens, A. Gajewski, and J. R. Johnson.** 2004. Virulence factor profiles and phylogenetic background of *Escherichia coli* isolates from veterans with bacteremia versus uninfected control patients. J. Infect. Dis. **190:**2121–2128.

45. **Sokurenko, E. V., M. Feldgarden, E. Trintchina, S. J. Weissman, S. Avagyan, J. Johnson, and D. E. Dykhuizen.** 2004. Selection footprint in the FimH adhesin shows pathogenicity-adaptive niche differentiation in *Escherichia coli*. Mol. Biol. Evol. **21:**1373–1383.
46. **Stamm, W. E., G. W. Counts, K. R. Running, S. Fihn, M. Turck, and K. K. Holmes.** 1982. Diagnosis of coliform infection in acutely dysuric women. N. Engl. J. Med. **307:**463–468.
47. **Stamm, W. E., T. M. Hooton, J. R. Johnson, C. Johnson, A. Stapleton, P. L. Roberts, S. L. Moseley, and S. D. Fihn.** 1989. Urinary tract infections: from pathogenesis to treatment. J. Infect. Dis. **159:**400–405.
48. **Talan, D. A., W. E. Stamm, T. M. Hooton, G. J. Moran, T. Burke, A. Iravani, J. Reuning-Scherer, L. Faulkner, and D. Church.** 2000. Comparison of ciprofloxacin (7 days) and trimethoprim-sulfamethoxazole (14 days) for acute uncomplicated pyelonephritis in women. JAMA **283:** 1583–1590.
49. **Welch, R., V. Burland, G. Plunkett III, P. Redford, P. Roesch, D. Rasko, E. L. Buckles, S. R. Liou, A. Boutin, J. Hackett, D. Stroud, G. F. Mayhew, D. J. Rose, S. Zhou, D. C. Schwartz, N. T. Perna, H. L. Mobley, M. S. Connenberg, and F. R. Blattner.** 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA **99:**17020–17024.
50. **Zhang, L., B. Foxman, and C. F. Marrs.** 2002. Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J. Clin. Microbiol. **40:**3951–3955.