

Host Characteristics and Bacterial Traits Predict Experimental Virulence for *Escherichia coli* Bloodstream Isolates From Patients With Urosepsis

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Background. Extraintestinal *Escherichia coli* infections are common, costly, and potentially serious. A better understanding of their pathogenesis is needed.

Methods. Sixty-seven *E coli* bloodstream isolates from adults with urosepsis (Seattle, WA; 1980s) underwent extensive molecular characterization and virulence assessment in 2 infection models (murine subcutaneous sepsis and moth larval lethality). Statistical comparisons were made among host characteristics, bacterial traits, and experimental virulence.

Results. The 67 source patients were diverse for age, sex, and underlying medical and urological conditions. The corresponding *E coli* isolates exhibited diverse phylogenetic backgrounds and virulence profiles. Despite the *E coli* isolates' common bloodstream origin, they exhibited a broad range of experimental virulence in mice and moth larvae, in patterns that (for the murine model only) corresponded significantly with host characteristics and bacterial traits. The most highly mouse-lethal strains were enriched with classic "urovirulence" traits and typically were from younger women with anatomically and functionally normal urinary tracts. The 2 animal models corresponded poorly with one another.

Conclusions. Host compromise, including older age and urinary tract abnormalities, allows comparatively low-virulence *E coli* strains to cause urosepsis. Multiple *E coli* traits predict both experimental and epidemiological virulence. The larval lethality model cannot be a substitute for the murine sepsis model.

Keywords. *Escherichia coli* infections; *Galleria mellonella*; host compromise; insect model; mouse model; phylogenetic group urosepsis; sepsis; virulence; virulence factors; virulence genes.

Extraintestinal infections caused by *Escherichia coli* cause considerable morbidity, mortality, and economic loss [1]. Most such infections are due to distinctive strains termed extraintestinal pathogenic *E coli* (ExPEC), or uropathogenic *E coli* (UPEC), because of their heightened ability to cause infections outside the intestinal tract, including

in the urinary tract [2]. Effective preventive measures are needed against such infections, development of which will require a better understanding of the relevant bacterial traits and the host defense defects that predispose individuals to such infections.

A traditional approach to elucidating such host-pathogen interactions is epidemiological. For this, *E coli* isolates from different clinical syndromes (eg, asymptomatic bacteriuria, cystitis, pyelonephritis, and urosepsis) and fecal isolates from healthy individuals are assessed for clonal background and accessory traits ("virulence factors"), and then are compared by source group [3]. This approach presumes that clinical context reliably reflects a strain's intrinsic virulence, a problematical notion

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both because the fecal microbiota is the reservoir for the ExPEC (UPEC) strains that cause extraintestinal infections [4] and because host compromise presumably allows even comparatively low-virulence strains to cause severe infections [5–7]. Indeed, the latter relationship has been used to infer (1) which bacterial traits are most important in allowing *E coli* to overcome host defenses and cause severe infections and (2) which host defenses are most important in protecting against a particular syndrome [6, 7].

A limitation of these epidemiological approaches is that, rather than measuring intrinsic virulence directly, they instead infer virulence from clinical source or host compromise status. Laboratory studies that directly assessed extraintestinal virulence in murine models have shown that experimental virulence corresponds with many of the same bacterial traits that epidemiological studies associate with clinical source and host compromise [8–11]. However, to date, no study has used the same strain set to directly compare experimental virulence with both host characteristics and bacterial traits. In addition, because standard murine infection models pose both ethical and financial challenges, validation of a suitable invertebrate model, such as a recently described moth larva lethality model [12], against an established murine model could facilitate future studies in this area by simplifying the direct virulence assessment of *E coli* isolates.

Accordingly, we took advantage of a well characterized collection of *E coli* bloodstream isolates from patients with urosepsis in Seattle, Washington (1980s), for which associated host characteristics were known [6, 7, 13–18]. We further characterized these isolates using an updated *E coli* phylotyping method [19], multilocus sequence typing (MLST), and an expanded virulence gene polymerase chain reaction (PCR) panel, and then we assessed their virulence experimentally using both a murine subcutaneous sepsis model [10] and a wax moth larval lethality model [12]. Next, we sought associations between host characteristics, experimental virulence, and bacterial traits, and we compared the results of the murine and moth larval models.

MATERIALS AND METHODS

Isolates

The 67 *E coli* urosepsis study isolates were cultured from the bloodstream of adult patients in the mid-1980s at 4 hospitals in Seattle, Washington [6, 7, 13–18]. They represented both community-onset and hospital-onset infections and diverse clinical presentations and hosts. Previous reports describe their virulence gene profiles and expression, antimicrobial resistance, O:K:H serotypes, carboxylesterase B phenotype, and associated medical and urological host conditions [6, 7, 13–18].

Virulence Genotyping

The isolates were newly characterized for 57 putative virulence genes of ExPEC, using the primers and PCR conditions listed in

the [Supplementary Data](#). The virulence score was the number of virulence genes detected, adjusted for multiple detection of the *pap* (P fimbriae), *sfa/foc* (S and F1C fimbriae), *kps* II (group 2 capsule), and *clb* (colibactin) operons. To allow a comparison of 2 established molecular definitions of extraintestinal pathotypes (ExPEC and UPEC) for their ability to predict experimental systemic virulence, isolates were classified accordingly [20, 21]. They were designated presumptively as ExPEC if positive for ≥ 2 of 5 markers, including *papAH* and/or *papC*, *sfa/focDE*, *afa/draBC* (Dr antigen-binding adhesins), *kpsM* II, and *iutA* (aerobactin receptor) [20], and as UPEC if positive for ≥ 3 of 4 markers, including *chuA* (heme binding), *fyuA* (yersiniabactin receptor), *vat* (vacuolating toxin), and *yfcV* (fimbrial subunit) [21].

Phylotyping, Multilocus Sequence Typing, and Phylogenetic Analysis

Phylogenetic group was determined by the revised Clermont method [19]. Multilocus sequence typing was done per the Achtman schema (mlst.warwick.ac.uk/). Sequence types were grouped into clonal complexes (CCs) based on identity at ≥ 6 of the 7 MLST loci.

Murine Sepsis Model

The murine subcutaneous sepsis model has been described previously [8–11]. Test strains were grown overnight at 37°C on Luria-Bertani agar plates. The following morning, colonies were used to inoculate Luria-Bertani broth, which was incubated at 37°C for 4 hours to yield log-phase growth. Cells were pelleted, washed twice in phosphate-buffered saline (PBS), and resuspended in PBS to an OD₅₉₀ of 1.0, corresponding with approximately 1.5×10^9 colony-forming units (CFU)/mL. From these stocks, 200 μ L aliquots (containing approximately 3×10^8 CFU) were injected subcutaneously into female outbred Swiss-Webster mice (Harlan, Indianapolis, IN), using 5 mice initially, with 5–10 additional mice used if the initial standard error exceeded 20%. In parallel, laboratory strain MG1655 (group A) and pyelonephritis isolate CFT073 (group B2) were injected into 5 mice each as negative and positive controls, respectively [8–10].

Postinoculation, mice were observed for 72 hours and were scored daily for illness severity, using a scale ranging from 1 (healthy) to 5 (dead). Mice were euthanized upon reaching stage 4 or surviving 72 hours. Among the mice challenged with a given strain, illness scores were averaged to give the strain's overall illness score, and the proportion that died or reached stage 4 was the strain's lethality score.

Moth Larval Model

The moth larval lethality model was largely as described [12]. Weekly, a new batch of *Galleria mellonella* moth larvae ($n = 500$) was purchased from a local supplier (Vados Bait, Spring Lake Park, MN). The 300 most active and uniform-in-size

larvae were used. Inoculum suspensions were prepared as described for the murine model. From these stocks, 20 μL aliquots containing approximately 1.7×10^6 CFU were inoculated into the last left proleg of 10 larvae each. Strains MG1655 and CFT073 were used in parallel as negative and positive controls, respectively [12].

Postinoculation, larvae were incubated at 37°C and were scored at 24 hours for lethality, as indicated by absence of movement when touched. Among the larvae challenged with a given strain, the proportion that died was the strain's lethality score. Controls receiving proleg puncture alone, or inoculation with sterile PBS, exhibited no lethality up to 48 hours.

Ethical Considerations

This study was approved by the Minneapolis VA Medical Center Institutional Animal Care and Use Committee (animal experiments) and the University of Washington Institutional Review Board (medical record review).

Statistical Methods

Comparisons involving proportions were tested using a χ^2 test with "(N-1)/N" correction [22]. Correlation between continuous variables was assessed using Spearman rho. Associations of independent predictor variables with continuous outcome variables were assessed using simple linear regression. For simplified assessments of similarity relationships among variables, the extensive dataset was summarized using principal components analysis.

RESULTS

Host Characteristics

The 67 source patients with urosepsis were highly diverse with respect to age, sex, and underlying conditions (Supplementary Table 1). Ages ranged from 20 to 92 years (median, 62), 57% were male, 37% had a compromising medical condition, 52% had a compromising urinary tract condition, and 31% had a history of alcohol abuse.

Associations Among Host Characteristics

Host characteristics were highly interrelated. Males tended to be older than females (median, 64.5 vs 56 years; $P = .007$) and more commonly had urinary tract abnormalities (48% vs 28%; $P = .01$) or any form of urinary tract compromise (63% vs 38%; $P = .04$). Age was associated positively with urinary tract abnormalities (median age if present, 64.5 vs 61.0 years; $P = .03$) and negatively with alcohol abuse (median age if present, 59 vs 64 years; $P = .006$). Alcohol abuse was associated negatively with urinary tract abnormalities (14% vs 27%; $P < .001$), urinary tract instrumentation (5% vs 33%; $P = .01$), any urinary tract compromise (19% vs 67%; $P < .001$), and compromising medical conditions (19% vs 46%; $P = .04$).

Host Characteristics Versus Experimental Virulence

In the murine subcutaneous sepsis model, the 67 *E coli* study isolates exhibited a wide range of illness severity and lethality, despite all deriving from blood cultures (Figure 1). Illness severity was fairly evenly distributed across the spectrum, whereas lethality was strongly bimodal, with high peaks at either extreme of the scale.

Experimental virulence in mice corresponded significantly with multiple patient characteristics, in patterns suggesting that host compromise allowed low-virulence strains to cause bacteremia (Table 1). In particular, mouse illness severity and lethality were associated negatively with urinary tract abnormalities, urinary tract compromise, and presence of a medical or urological compromising condition and exhibited a borderline negative association with males. They also were correlated negatively with advancing host age (for illness severity, $\text{Rho} = -0.257$, $P = .036$; for lethality, $\text{Rho} = -0.244$, $P = .047$). In contrast, virulence outcomes were strongly positively associated with alcohol abuse history. Virulence outcomes were not significantly associated with urinary tract instrumentation or medical illnesses.

Bacterial Characteristics

The 67 *E coli* urosepsis isolates were highly diverse with respect to O:K:H serotype, phylogenetic group, clonal group, and virulence gene content (Supplementary Table 2). Most isolates were from phylogroup B2 (67%), with clonal groups CC73 (25%) and CC95 (18%) predominating. Virulence genes that occurred in $\geq 75\%$ of isolates included, by descending prevalence, *fimH* (type 1 fimbriae: 99%), *fyuA* (yersiniabactin: 94%), *ompT* (outer membrane protease: 87%), *chuA* (heme uptake: 85%), *papAHCEFG* (P fimbriae: 79%–82%), *iutA* (aerobactin system: 78%), and *yfcV* (fimbrial adhesin: 75%). According to the molecular criteria specified in Materials and Methods, 88% of isolates qualified as ExPEC and 75% qualified as UPEC.

Host Characteristics Versus Bacterial Characteristics

Each of the studied host characteristics except diabetes corresponded significantly with multiple bacterial characteristics (Table 2). Except for the associations involving alcoholism, most such associations were negative, implying a reduced requirement for the particular trait in the presence of the compromising host condition. Most of the positive associations, again excepting those involving alcoholism, involved bacterial traits that are less common, less well known, and/or less well established as virulence genes.

Bacterial Characteristics Versus Experimental Virulence

Outcomes in the murine sepsis model varied significantly in relation to strain background and virulence gene content (Table 3). In particular, mouse illness severity and/or lethality were associated positively with group B2, CC95, the F11 and

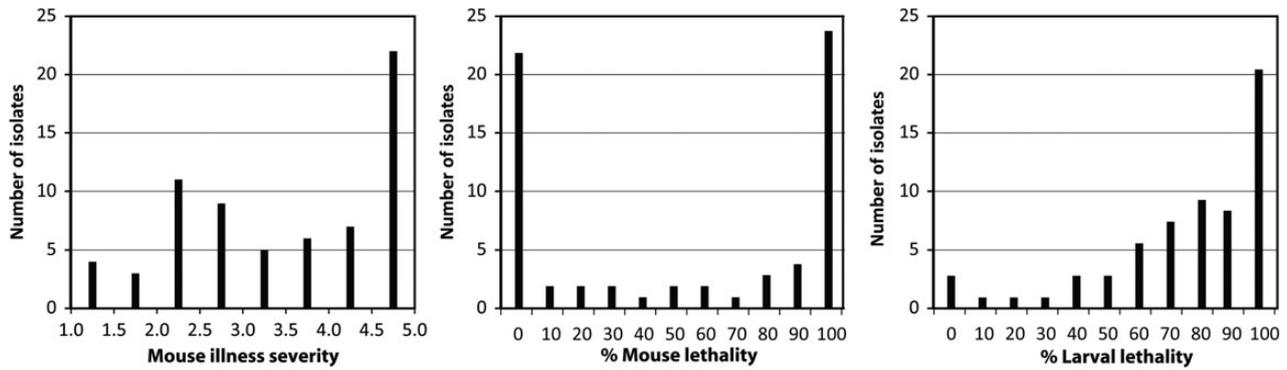


Figure 1. Experimental virulence of 67 *Escherichia coli* urosepsis isolates in a murine subcutaneous sepsis model and a moth larval lethality model. Results shown are based on the total number of mice (≥ 5) or larvae (≥ 10) challenged with a given isolate. Results are grouped by score stratum (1.0–1.4, 1.5–1.9, etc) for mouse illness severity and by decile (0%–9%, 10%–19%, etc) for percentage of mouse and larval lethality.

F12 *papA* alleles, *papAHCEFG*, *papG* allele II, *hlyA*, *fyuA*, *vat*, *kpsM* II, K1 capsule, *usp*, H7 *fliC*, *malX*, *clbB/N*, *chuA*, *yfcV*, and both EXPEC and UPEC status. They also were correlated closely with virulence score (for illness severity, $Rho = 0.53$; for lethality, $Rho = 0.5$; $P < .001$ for each). In contrast, they were associated negatively with group C, CC23, CC14, *afaE8*, *bmaE*, and *tsh*.

Larval Lethality Versus Murine Virulence

In the *Galleria* model the 67 *E coli* study isolates likewise exhibited a broad range of virulence (Figure 1). However, in contrast to the murine model, the distribution was skewed unimodally toward the high end. Larval lethality was not correlated significantly with either mouse illness severity or mouse lethality (data not shown).

Larval lethality was not significantly associated with any host characteristic (data not shown), and it was associated positively with only 8 bacterial variables, each of which had negative or indifferent virulence associations in mice (Table 3; Supplementary Table 3). The only bacterial trait that was associated negatively with larval lethality (*kpsM* II) was associated positively with virulence in mice.

Principal Components Analysis

To display graphically the similarity relationships among the study variables, principal components analysis was used with the main assessed bacterial traits. In a plot of the first 2 principal components, murine lethality and illness severity were strongly positive on the first component and moderately positive on the second component (Figure 2). In contrast, larval lethality was

Table 1. Experimental Virulence in Mice in Relation to Host Characteristics Among 67 *Escherichia coli* Urosepsis Isolates

Host Characteristic ^a	Mean Illness Severity Score ^b					Mean Lethality Proportion ^b			
	From Host Without Characteristic		From Host With Characteristic		<i>P</i> Value ^a	From Host Without Characteristic		From Host With Characteristic	
	No. (%) of 67)	Median (Range)	No. (%) of 67)	Median (Range)		Median (Range)	Median (Range)	<i>P</i> Value ^a	
Male	29 (43)	4.2 (1.8–5.0)	38 (57)	3.0 (1.0–5.0)	.06	0.90 (0–1.0)	0.27 (0–1.0)	.10	
Urinary tract abnormality	37 (55)	4.4 (1.8–5.0)	30 (45)	2.9 (1.0–5.0)	<.001	1.0 (0–1.0)	0 (0–1.0)	.003	
Urinary tract instrumentation	51 (76)	3.8 (1.1–5.0)	16 (24)	3.3 (1.0–5.0)		0.8 (0–1.0)	0.42 (0–1.0)		
Any urinary tract compromise	32 (48)	4.4 (1.8–4.8)	35 (52)	3.0 (1.0–5.0)	.01	0.95 (0–1.0)	0.30 (0–1.0)	.02	
Any medical illness ^c	42 (63)	3.6 (1.0–5.0)	25 (37)	3.0 (1.1–5.0)		0.65 (0–1.0)	0.10 (0–1.0)		
Any compromising condition ^d	21 (31)	4.4 (2.0–4.7)	46 (69)	3.0 (1.0–5.0)	.01	1.0 (0–1.0)	0.25 (0–1.0)	.008	
Alcohol abuse	46 (69)	3.1 (1.0–5.0)	21 (31)	4.2 (2.0–5.0)	.04	0.37 (0–1.0)	0.90 (0–1.0)	.09	

^a *P* values (by the Mann-Whitney *U* test) are shown where $P \leq .10$.

^b Among the 5–10 mice per isolate. Illness severity ranged from 1 (healthy) to 5 (dead). Lethality ranged from 0 (none) to 1.0 (100%).

^c Medical illnesses included diabetes, cancer, immunosuppression, and uremia. No individual condition was significantly associated with either virulence outcome.

^d Any urological condition or medical illness (excluding alcohol abuse).

Table 2. Association of Host Characteristics With Bacterial Traits Among 67 *Escherichia coli* Urosepsis Isolates

Host Characteristic ^a	No. (% of 67) ^b	Bacterial Trait ^c	Prevalence of Trait, Overall and in Relation to Host Characteristic ^d			P Value ^c
			Overall (% of 67)	Characteristic Absent ^d	Characteristic Present ^d	
Male sex	38 (56)	<i>ibeA</i>	4 (9)	4 (14)	0 (0)	.02
		<i>sfaS</i>	3 (4)	3 (10)	0 (0)	.04
		<i>astA</i>	3 (4)	3 (10)	0 (0)	.04
Urinary tract abnormality	30 (45)	group B2	45 (67)	29 (78)	16 (53)	.03
		group C/CC23	7 (10)	1 (3)	6 (20)	.02
		F7-2 <i>papA</i>	12 (18)	10 (27)	2 (7)	.03
		<i>papAH</i>	53 (79)	33 (89)	20 (67)	.02
		<i>papC</i>	54 (81)	34 (92)	20 (67)	.009
		<i>papEFG</i>	53 (79)	34 (92)	19 (63)	.004
		<i>papG</i> II	49 (73)	32 (87)	17 (57)	.01
		<i>hra</i>	18 (27)	6 (16)	12 (40)	.03
		<i>vat</i>	43 (64)	29 (78)	14 (48)	.01
		<i>kpsM</i> II	56 (84)	35 (95)	21 (70)	.009
		<i>usp</i>	45 (67)	29 (78)	16 (53)	.03
		<i>ompT</i>	58 (87)	35 (95)	23 (77)	.03
<i>tsh</i>	3 (4)	0 (0)	3 (10)	.047		
Urinary instrumentation	16 (24)	<i>papC</i>	54 (81)	44 (86)	10 (63)	.03
		<i>papG</i> II	49 (73)	41 (80)	8 (50)	.02
		<i>iha</i>	37 (55)	33 (65)	4 (25)	.005
		<i>sat</i>	35 (52)	31 (61)	4 (25)	.01
Cancer	13 (19)	group D	6 (9)	3 (6)	3 (23)	.045
		F14 <i>papA</i>	6 (9)	3 (6)	3 (23)	.045
		<i>papG</i> II	49 (73)	42 (78)	7 (54)	.02
		<i>papG</i> III	5 (7)	2 (4)	3 (23)	.02
		<i>iha</i>	37 (55)	33 (61)	4 (31)	.047
		<i>hlyF</i>	9 (13)	5 (9)	4 (31)	.04
		<i>kpsMT</i> III	1 (1.5)	0 (0)	1 (8)	.04
		<i>ompT</i>	58 (86)	49 (91)	9 (69)	.04
<i>tsh</i>	3 (4)	1 (2)	2 (15)	.02		
Immunosuppression	13 (19)	<i>sfa/focDE</i>	18 (27)	11 (20)	7 (54)	.01
		<i>sfaS</i>	3 (4)	1 (2)	2 (15)	.03
		<i>iroN</i>	26 (39)	17 (32)	6 (69)	.01
Uremia	8 (12)	F11 <i>papA</i>	15 (22)	11 (19)	4 (50)	.04
		<i>traT</i>	43 (64)	35 (59)	8 (100)	.02
		<i>ompT</i>	58 (86)	53 (89)	5 (63)	.03
Alcohol abuse	21 (31)	F8 <i>papA</i>	2	0 (0)	2 (10)	.03
		<i>papAH</i>	53	33 (72)	20 (95)	.03
		<i>papC</i>	54	34 (74)	20 (95)	.04
		<i>papG</i> II	49	30 (65)	19 (91)	.03
		<i>afadraBC</i>	4	0 (0)	4 (4)	.002
		<i>iha</i>	37	21 (46)	16 (76)	.02
		<i>traT</i>	43	25 (54)	18 (88)	.01
		ExPEC	59	38 (83)	21 (100)	.049

^a Diabetes (n = 8 patients) had no significant associations with bacterial traits but exhibited a trend toward a negative association with *chuA* (88% if no diabetes, 63% if diabetes; *P* = .054).

^b Prevalence of host characteristic.

^c Bacterial traits shown are those that yielded *P* < .05 (using a χ^2 test with '(N-1)/N' adjustment). Parentheses indicate negative associations.

^d Prevalence of bacterial trait among isolates from patients with or without the indicated host characteristic. Only traits with at least one significant association are shown.

Table 3. Experimental Virulence in Mice in Relation to Bacterial Characteristics Among 67 *Escherichia coli* Urosepsis Isolates

Category	Specific Trait ^a	Mean Illness Severity Score ^b				Mean Lethality ^b				
		Trait Absent		Trait Present		P Value ^c	Trait Absent		Trait Present	
		No. (% of 67)	Median (Range)	No. (% of 67)	Median (Range)		Median (Range)	Median (Range)	P Value ^c	
Phylogroup	Group B2	22	2.7 (1.3–4.7)	45	4.3 (1.–5.0)	<.001	0 (0–1)	1.0 (0–1)	<.001	
	Group C (=CC23)	60	3.8 (1.0–5)	7	2.3 (1.8–3.0)	(.006)	0.8 (0–1)	0 (0–0.2)	.003	
Clonal complex	CC14	63	3.8 (1.0–5)	4	2.1 (1.1–3.5)	.03	0.8 (0–1)	0.05 (0–0.6)		
	CC73	50	3.3 (1.1–5)	17	4.4 (1.0–4.7)	.099	0.42 (0–1)	1.0 (0–1)	.048	
	CC95	55	3.2 (1.0–5)	12	4.6 (2.4–4.8)	.01	0.4 (0–1)	1.0 (0–1)	.02	
Adhesins	<i>papA</i> F11 allele	52	3.3 (1.0–5)	15	4.7 (2.3–5)	.02	0.45 (0–1)	1.0 (0–1)	.09	
	<i>papA</i> F12 allele	64	3.5 (1–5)	3	4.7 (4.5–4.7)	.04	.55 (0–1)	1.0 (0–1)	.047	
	<i>papAHCEFG</i>	13–14	2.1 (1.1–4.8)	53–54	4.0–4.1 (1–5)	<.001	0 (0–0.8)	0.9 (0–1)	<.001	
	<i>papG</i> allele II	18	2.2 (1.1–5)	49	4.1 (1–5)	<.001	0 (0–0.8)	0.9 (0–1)	<.001	
	<i>afaE8</i>	63	3.7 (1.0–5)	4	2.8 (2.7–4.7)	(.02)	0.8 (0–1)	0 (0–1)	.02	
	<i>bmaE</i>	63	3.7 (1.0–5)	4	2.8 (2.7–4.7)	(.02)	0.8 (0–1)	0 (0–1)	.02	
	<i>yfcV</i>	17	2.3 (1.3–4.7)	50	4.2 (1–5)	<.001	0 (0–1)	0.9 (0–1)	<.001	
	<i>tsh</i>	64	3.7 (1.0–5)	3	2.0 (1.8–2.4)	(.03)	0.75 (0–1)	0 (0–0)	.04	
Toxins	<i>hlyD</i>	38	3.0 (1.0–4.8)	29	4.3 (1.0–5)	.03	0.2 (0–1)	1.0 (0–1)	.03	
	<i>vat</i>	24	2.6 (1.3–4.7)	43	4.4 (1.0–5)	<.001	0 (0–0.8)	1.0 (0–1)	<.001	
Iron acquisition	<i>fyuA</i>	4	1.9 (1.3–2.7)	63	3.8 (1.0–5)	.005	0 (0–0)	0.8 (0–1)	.02	
	<i>chuA</i>	10	2.4 (1.8–3.5)	57	4.0 (1.0–5)	.002	0 (0–0.5)	0.9 (0–1)	<.001	
Capsule	<i>kpsM</i> II	11	2.3 (1.3–3.8)	56	4.0 (1.0–5)	<.001	0 (0–0.8)	0.9 (0–1)	<.001	
	K1 variant	48	3.0 (1–5)	19	4.3 (2.4–4.8)	.009	0.25 (0–1)	1.0 (0–1)	.01	
Miscellaneous	<i>usp</i>	22	2.7 (1.3–4.7)	45	4.3 (1.0–5)	<.001	0 (0–1)	1.0 (0–1)	<.001	
	H7 <i>fliC</i> variant	54	3.1 (1.0–5)	13	4.7 (2.4–5)	.004	0.35 (0–1)	1.0 (0–1)	.01	
	<i>malX</i>	19	2.7 (1.3–4.7)	48	4.3 (1.0–5)	<.001	0 (0–1)	0.9 (0–1)	<.001	
	<i>clbB, clbN</i>	30	2.8 (1.3–4.8)	37	4.3 (1.0–5)	.005	0 (0–1)	0.9 (0–1)	.003	
Combination	ExPEC	8	2.0 (1.3–3.0)	59	3.8 (1–5)	<.001	0 (0–0.2)	0.8 (0–1)	.001	
	UPEC	17	2.3 (1.3–4.7)	50	4.2 (1.0–5)	<.001	0 (0–1)	0.9 (0–1)	<.001	

^a Traits shown are those that yielded $P < .05$ for illness severity and/or lethality.

^b Among the 5–10 mice tested per isolate. Mean illness severity per isolate ranged from 1 (healthy) to 5 (dead); median 3.5. Mean lethality per isolate ranged from 0 (none) to 1.0 (100%); median 60%.

^c P values (by the Mann-Whitney U test) are shown where $P < .10$. Parentheses indicate negative associations.

close to the origin on both components, well removed from the murine endpoints. This illustrates the marked disconnect between the 2 animal models.

Host characteristics were mostly neutral to negative on the first component, and therefore distant from murine lethality and illness. Urinary tract abnormalities, age, and male sex were even more distant from the murine model endpoints due to their negative values on the second component. Alcoholism, with its uniquely positive value on the first component, stood apart from the other host variables, closest to the murine model outcomes. This result illustrates the disconnect between the other host conditions and both alcoholism and murine virulence.

The bacterial traits nearest the murine model endpoints were group B2, ExPEC, UPEC, and the individual virulence traits most closely associated with murine virulence in univariable

analyses. Distant from the murine endpoints, with negative values on the first component, were the non-B2 phylogenetic groups, CC23, and multiple other individual virulence traits. Moderately positive on the first component, but with extreme positive versus negative values on the second component, were (group B2-derived) CC95 and CC73, each surrounded by its characteristic virulence traits. Collectively, these findings illustrate the interdependence of host and bacterial characteristics in determining experimental virulence in the murine sepsis model, the phylogenetic linkage of virulence-associated *E. coli* traits, and the nonequivalence of the murine and larval models.

DISCUSSION

In this study, we further analyzed a well characterized panel of 67 *E. coli* urosepsis isolates according to strain background,

were depleted of these traits, but they were enriched with other lineages, for example, phylogroups C and D and clonal groups CC23 and CC14, plus distinctive accessory traits (eg, *papG* allele III, *bmaE*, *gafD*, *afaE8*, and *tsh*).

The opposed associations of urinary tract abnormalities and alcohol abuse with virulence in mice and bacterial traits probably results from confounding, rather than alcoholism per se selecting for infections caused by extra-virulent *E coli* strains. The latter would imply that alcohol abuse somehow strengthens host defenses against urosepsis, which seems implausible. A more likely explanation is the negative associations of alcohol abuse with multiple host factors that predispose to infections with less virulent *E coli*, including older age, male sex, and urinary tract abnormalities. We suspect that a “competing hazards” situation exists, whereby hosts with significant alcoholism may tend to die at an earlier age, before accumulating the acquired urinary tract abnormalities and other host defense defects that often accompany older age.

The dominant effect of urinary tract abnormalities in determining the urosepsis isolates’ experimental virulence, phylogenetic background, and accessory trait profiles is unsurprising, given the critical importance of urinary tract defenses in the pathogenesis of urosepsis [23–25]. Less obvious is how the murine sepsis model, which completely bypasses the urinary tract, could discern differences involving virulence traits such as adhesins (eg, P fimbriae) that are classically regarded as important for urovirulence, not systemic infection. Likewise, the composite variable UPEC, which was derived with a focus on urinary tract infection [21], here was highly predictive of systemic virulence in the murine sepsis model. In the study that derived the UPEC definition, molecular UPEC status could not differentiate neonatal meningitis isolates from urinary tract infection isolates [21]. These observations support a broader view of the syndrome capabilities of what traditionally have been called uropathogenic *E coli*, and their virulence factors, that acknowledges these organisms’ ability to infect diverse (including nonurinary) sites, as reflected in the more inclusive designation extraintestinal pathogenic *E coli* [2].

The marked discrepancies we found between the larval lethality and murine sepsis models—including divergent associations with host and bacterial characteristics and noncorrelation with one another—suggest that they are not directly interchangeable for studying extraintestinal *E coli* infections. Which model is more valid or useful may depend on the study question. Because only the murine model’s results corresponded in biologically plausible ways with host characteristics and bacterial traits, it seems more likely to mimic the pathogenesis of *E coli* sepsis in humans.

This study has several limitations. First, the strain collection is nearly 30 years old; confirmation using contemporary isolates would be desirable. Second, only presence/absence of virulence genes, not level of expression, was assessed. Third, the animal models are of uncertain physiological relevance to human

infections. Fourth, the host data were from retrospective medical record review and were analyzed according to presence/absence and broad categories, thereby possibly missing associations that would be apparent with a more granular or quantitative analysis. It also has notable strengths; these include attention to 3 key domains (host characteristics, bacterial traits, and experimental virulence), extensive characterization of the isolates, and assessment of an insect model as a proxy for the murine model.

CONCLUSIONS

In summary, in both a murine subcutaneous sepsis model and a moth larval lethality model, we observed a wide range of experimental virulence among the 67 *E coli* urosepsis bloodstream isolates. Virulence in the murine model, which bypassed the urinary tract, corresponded positively with classical urovirulence traits and negatively with compromising host conditions, notably urinary tract abnormalities, old age, and male sex. In contrast, larval lethality corresponded poorly with murine model outcomes, bacterial traits, and host characteristics. These findings illustrate the interdependence of host and bacterial characteristics in determining the occurrence of urosepsis, demonstrate that bloodstream origin does not guarantee that an *E coli* strain is highly virulent in mammals, and challenge the suitability of the larval lethality model as a surrogate for the murine sepsis model or naturally occurring human sepsis.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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