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Phenotypic and Genotypic Changes over Time and across Facilities of Serial Colonizing and Infecting *Escherichia coli* Isolates Recovered from Injured Service Members

Katrin Mende,[a,b] Miriam L. Beckius,[a] Wendy C. Zera,[a,b] Xin Yu,[a] Kristelle A. Cheatle,[a] Deepak Aggarwal,[b] Ping Li,[b] Bradley A. Lloyd,[c]* David R. Tribble,[b] Amy C. Weintrob,[b,d] Clinton K. Murray[a]

San Antonio Military Medical Center, JBSA Fort Sam Houston, Texas, USA[a]; Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA[b]; Landstuhl Regional Medical Center, Landstuhl, Germany[c]; Walter Reed National Military Medical Center, Bethesda, Maryland, USA[d]

***Escherichia coli* is the most common colonizing and infecting organism isolated from U.S. service members injured during deployment. Our objective was to evaluate the phenotypic and genotypic changes of infecting and colonizing *E. coli* organisms over time and across facilities to better understand their transmission patterns. *E. coli* isolates were collected via surveillance cultures and infection workups from U.S. military personnel injured during deployment (June 2009 to May 2011). The isolates underwent antimicrobial susceptibility testing, pulsed-field gel electrophoresis, and multiplex PCR for phylotyping to determine their resistance profiles and clonality. A total of 343 colonizing and 136 infecting *E. coli* isolates were analyzed, of which 197 (57%) and 109 (80%) isolates, respectively, produced extended-spectrum β-lactamases (ESBL). Phylogroup A was predominant among both colonizing (38%) and infecting isolates (43%). Although 188 unique pulsed-field types (PFTs) were identified from the colonizing isolates, and 54 PFTs were identified from the infecting isolates, there was a lack of PFT overlap between study years, combat zones, and military treatment facilities. On a per-subject basis, 26% and 32% of the patients with serial colonizing isolates and 10% and 21% with serial infecting isolates acquired changes in their phylogroup and PFT profiles, respectively, over time. The production of ESBL remained high over time and across facilities, with no substantial changes in antimicrobial susceptibilities. Overall, our results demonstrated an array of genotypic and phenotypic differences for the isolates without large clonal clusters; however, the same PFTs were occasionally observed in the colonizing and infecting isolates, suggesting that the source of infections may be endogenous host organisms.**

Infections caused by multidrug-resistant organisms (MDROs), including extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, are a well-recognized and increasing global health problem that affect both civilian (1–6) and military (7–13) populations. In general, ESBL-producing *E. coli* isolates are associated with nosocomial infections; however, the organism has also been linked with community-acquired infections in recent years, adding to the concern over the potential spread of this MDRO (1). Specifically, the transmission of genotypically related ESBL-producing *E. coli* isolates may occur between patients and hospitals, as well as between cities and countries, due to the high prevalence of foreign travel (5). Although it has been established that *E. coli* has a high degree of genomic diversity (14, 15), further information on the genotypic and phenotypic changes across time and location is required to better understand the transmission patterns of this organism.

As a result of the increased rate of infections associated with Gram-negative MDROs among U.S. military personnel injured during the recent conflicts in Iraq and Afghanistan (7–13), several infection control practices were implemented at military treatment facilities (MTFs) that received personnel wounded in combat in an effort to standardize screening procedures, including the Department of Defense (DoD)-mandated active surveillance cultures for asymptomatic inguinal colonization (i.e., groin swabs) at admission and the institution of preemptive contact isolation (11, 16, 17). Various cultures were also obtained from wounded service members based upon clinical indications during their period of hospitalization. These cultures have primarily reported Gram-negative bacterial growth, with *E. coli* being one of the most commonly isolated organisms (8, 10, 13, 17, 18).

Per infection control procedures, if a culture indicates MDRO growth, the patient remains under contact isolation (19, 20). Nonetheless, it is unclear if this procedure is effective at lessening the risk of MDRO nosocomial transmission, because the genotypic patterns of the isolates have not been examined. It is also not known to what extent colonization is predictive of infection with the same organism (11, 17, 18). Patients may also have relapsed infections or develop serial infections with the same organism, but it is not definitively known whether the organism is truly the same or a new strain of the same species has been acquired.

The relatedness of Gram-negative MDROs among military personnel has been examined within U.S. MTFs (9, 17, 18, 21–23) but generally not across facilities. Although these analyses have isolated multiple distinct *E. coli* strains from deployed military personnel, occurrences of related *E. coli* strains or overlapping

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Address correspondence to Katrin Mende, katrin.mende.ctr@mail.mil.
* Present address: Bradley A. Lloyd, San Antonio Military Medical Center, JBSA Fort Sam Houston, Texas, USA

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pulsed-field gel electrophoresis patterns between the isolates collected from deployed and nondeployed patients within the same facility have not been frequently reported (17, 18). Overall, the results of these studies demonstrated the genotypic diversity of *E. coli*, with only a small number of subjects at the same facility colonized with similar strains; however, the relatedness of these strains to those collected from injured service members across different MTFs is uncertain.

The objective of our analysis was to compare the phenotypic and genotypic changes of colonizing and infecting *E. coli* isolates both within individual patients and across all subjects with regard to time (i.e., study years), MTFs, and combat zones. These data will support a better understanding of transmission patterns and may allow for improvements to infection control strategies.

MATERIALS AND METHODS

As part of the Trauma Infectious Disease Outcomes Study (TIDOS), an ongoing observational cohort study of short- and long-term infectious complications among military personnel injured during deployment in Iraq (Operations Iraqi Freedom and New Dawn) and Afghanistan (Operation Enduring Freedom) (8), data on trauma history and inpatient care were obtained through the use of a trauma registry and supplemented by the TIDOS infectious disease module. Trauma patients were eligible for inclusion in TIDOS if they were active-duty personnel or DoD beneficiaries, ≥18 years of age, and sustained deployment-related injuries requiring medical evacuation through Landstuhl Regional Medical Center (LRMC) (Germany) before subsequent transfer to a participating U.S. MTF (Brooke Army Medical Center, National Naval Medical Center, or Walter Reed Army Medical Center). In addition, a bacterial repository was established to store the isolates collected at the MTFs obtained from asymptomatic surveillance cultures and clinical cultures.

E. coli colonizing and infecting isolates were collected from wounded military personnel who were admitted to LRMC before being transferred to a participating U.S. MTF between June 2009 and May 2011. The isolates were obtained from surveillance groin swabs at admission, per DoD mandate for facilities receiving combat casualties (11, 16, 17), and inpatient clinical cultures performed at all facilities. The information captured through the TIDOS infectious disease module indicated whether the isolates were associated with colonization or infection (i.e., linked to an infectious disease event, as determined through a review of medical records for clinical findings and laboratory test results; these were classified using the standardized definitions of the National Healthcare Safety Network for nosocomial infections) (8, 24).

The antimicrobial susceptibilities and ESBL production of the isolates were determined using the BD Phoenix automated microbiology system (Becton, Dickinson and Company, Sparks, MD), as per the manufacturer guidelines, utilizing NMIC/ID-123 panels, and interpreted according to the Clinical Laboratory and Standards Institute criteria (25). The ESBL phenotypes of the colonizing and infecting *E. coli* isolates were compared across the participating MTFs, combat zones (i.e., Iraq and Afghanistan), and study years (i.e., June 2009 to May 2010 and June 2010 to May 2011). A change observed in the ESBL classification was defined by the identification of a different phenotype among subsequent serial isolates within the same subject, while the same ESBL classification detected in subsequent isolates was referred to as stable. Individuals could also have multiple isolates that met the ESBL classification of stable and change.

In accordance with the U.S. Food and Drug Administration protocol for Gram-negative rods utilizing the XbaI endonuclease, pulsed-field gel electrophoresis was used to evaluate genotypic patterns among the isolates, and the resulting patterns were grouped in pulsed-field types (PFTs). Clonality was assessed using the commercial software BioNumerics (Applied Maths, Inc., Austin, TX) and defined by 85% similarity using the band-based Dice coefficient, with 1.5% optimization and 1% tolerance, as well as an unweighted-pair group mean average cluster analysis. Multiplex PCRs were performed to determine the phylogenetic groups (A, B1, B2, C, D, E, F, and clade I) of the *E. coli* isolates, per the newly revised Clermont method (26). First, a quadruplex PCR was conducted to determine the quadruplex genotypes corresponding to the presence/absence of the four genes *arpA*, *chuA*, *yjaA*, and TspE4.C2. Based on the quadruplex phenotype, the isolates either were immediately assigned a phylogroup or required additional PCR testing to distinguish between A and C, E and D, or E and clade I, as described previously (26, 27).

The relatedness of the colonizing and infecting *E. coli* isolates (independently and combined) across different MTFs, combat zones, and study years was examined. Serial isolates collected from the subjects were examined for changes in their PFT and phylogroup profiles (i.e., detection of a different PFT or phylogroup in subsequent serial isolates compared to an earlier isolate) or stability (i.e., same PFT or phylogroup detected in subsequent isolates). Individuals could also have multiple isolates that met the definition of stable and changed PFT or phylogroup profiles, respectively.

Tests of association were conducted using a generalized estimating equation method with PROC GENMOD SAS version 9.3 (SAS, Cary, NC). Statistical significance was defined as a P value of <0.05.

TABLE 1 Baseline characteristics of U.S. military personnel subjects with colonizing or infecting *E. coli* isolates

Baseline characteristic[a]	Patient data ($n = 149$)
Male	143 (96.0)
Age (median [IQR]) (yr)	24.0 (22.1–29.3)
Branch of service	
Army	87 (58.4)
Marine	42 (28.2)
Navy	6 (4.0)
Air Force	6 (4.0)
Missing branch information	8 (5.4)
Rank	
Enlisted	124 (83.2)
Officer	11 (7.4)
Warrant	1 (0.7)
Missing rank information	13 (8.7)
Injury severity score (median [IQR])[b]	14.0 (10.0–21.0)
Theater of operation	
Operation Iraqi Freedom/Operation New Dawn (Iraq)	12 (8.1)
Operation Enduring Freedom (Afghanistan)	131 (87.9)
Missing theater of operation information	6 (4.0)
Mechanism of injury	
Blast	106 (71.2)
Nonblast	37 (24.8)
Missing information	6 (4.0)

[a] All data are given as no. (%), unless otherwise indicated. IQR, interquartile range.
[b] A summary of the injury scores determined for multiple body regions is given in Linn (28).

RESULTS

Study population. Between June 2009 and May 2011, 2,943 wounded military personnel were admitted to LRMC, of which approximately half transferred to one of the participating U.S. MTFs. Among these wounded service members, a total of 857 infectious disease events (387 patients) linked to cultures with

TABLE 2 Description of *E. coli* isolates over time and across military treatment facilities[a]

Isolates by ESBL production[b]	Total isolates	Study yr		Combat zone			Military treatment facility[c]			
		Yr 1[d]	Yr 2[e]	Iraq	Afghanistan	Unknown[f]	LRMC	WRAMC	NNMC	BAMC
Colonizing isolates	343	127	216	27	302	14	108	101	66	68
ESBL producing	197 (57.4)	86 (67.7)	111 (51.4)[g]	14 (51.9)	176 (58.3)	7 (50.0)	63 (58.3)	54 (53.5)	40 (60.6)	40 (58.8)
Non-ESBL producing	146 (42.6)	41 (32.3)	105 (48.6)	13 (48.1)	126 (41.7)	7 (50.0)	45 (41.7)	47 (46.5)	26 (39.4)	28 (41.2)
Infecting isolates	136	43	93	11	125	0	9	42	66	19
ESBL producing	109 (80.1)	33 (76.7)	76 (81.7)	5 (45.5)	104 (83.2)	0	7 (77.8)	35 (83.3)	53 (80.3)	14 (73.7)
Non-ESBL producing	27 (19.9)	10 (23.3)	17 (18.3)	6 (54.5)	21 (16.8)	0	2 (22.2)	7 (16.7)	13 (19.7)	5 (26.3)
Total	479	170	309	38	427	14	117	143	132	87

[a] All data are presented as no. (%).
[b] ESBL, extended-spectrum β-lactamase.
[c] LRMC, Landstuhl Regional Medical Center; WRAMC, Walter Reed Army Medical Center; NNMC, National Naval Medical Center; BAMC, Brooke Army Medical Center.
[d] Dates of culture: June 2009 to May 2010.
[e] Dates of culture: June 2010 to May 2011.
[f] Military operation data are missing for six subjects.
[g] ESBL production between the study years for the colonizing isolates is significantly different ($P = 0.01$).

growth were diagnosed. Of these, there were 157 (18.3%) inpatient infections (from 90 patients) associated with *E. coli*.

The study population included 149 patients with colonizing and/or infecting *E. coli* isolate data and primarily consisted of young men serving in the U.S. Army (Table 1). Due to declining combat operations in Iraq during the study years, the majority of the personnel sustained injuries in Afghanistan (88%). Injury severity was moderate to high (median injury severity score [i.e., a summary of injury scores determined for multiple body regions {28}], 14) and likely the result of a predominance of blast injuries (71%).

***E. coli* isolates.** Overall, a total of 2,604 and 1,388 colonization surveillance cultures were obtained from wounded personnel at admission to LRMC and the participating U.S. MTFs, respectively. From the study population of 149 patients, 479 *E. coli* isolates were collected, of which 343 (from 148 patients) and 136 (from 43 patients) were identified as colonizing and infecting, respectively (Table 2). The *E. coli* colonizing isolates were all collected from groin surveillance cultures and primarily obtained from military personnel injured serving in the Afghanistan combat theater (88%), which corresponded to the decline in military personnel deployed in support of operations in Iraq. Across the MTFs, the cultures performed at LRMC contributed the largest number of colonizing isolates used in the analysis (32% of 343 isolates, which is 4% of all the surveillance cultures performed at LRMC). In addition, *E. coli* isolates were identified from 17% of the admission surveillance cultures from service members after transition to one of the U.S. MTFs.

Infecting *E. coli* isolates were collected from wound (65%), blood (13%), urine (4%), tissue (4%), sputum (3%), tracheal aspirate (3%), bone (3%), and other/nonspecified cultures (5%). Regarding the comparison groups (Table 2), the proportions collected from the study years (68% from the second year) and combat zones (92% from personnel serving in Afghanistan) were similar to the distribution of colonizing isolates. Due to the short duration of hospitalization of the wounded personnel at LRMC

TABLE 3 Description of antimicrobial resistance of *E. coli* isolates across military treatment facilities[a]

Resistances by isolate type	Total	Study yr		Combat zone			Military treatment facility[b]			
		Yr 1[c]	Yr 2[d]	Iraq	Afghanistan	Unknown[e]	LRMC	WRAMC	NNMC	BAMC
Colonizing isolates	343	127	216	27	302	14	108	101	66	68
Aminoglycosides	0	0	0	0	0	0	0	0	0	0
β-Lactams	197 (57.4)	86 (67.7)	111 (51.4)	14 (51.9)	176 (58.3)	7 (50.0)	63 (58.3)	54 (53.5)	40 (60.6)	40 (58.8)
Carbapenems	0	0	0	0	0	0	0	0	0	0
Fluoroquinolones	211 (61.5)	86 (67.7)	125 (57.9)	13 (48.1)	189 (62.6)	9 (64.3)	54 (50.0)	66 (65.4)	46 (69.7)	45 (66.2)
Infecting isolates	136	43	93	11	125	0	9	42	66	19
Aminoglycosides	1 (0.7)	0	1 (1.1)	0	1 (0.8)	0	0	1 (2.4)	0	0
β-Lactams	109 (80.1)	33 (76.7)	76 (81.7)	5 (45.5)	104 (83.2)	0	7 (77.8)	35 (83.3)	53 (80.3)	14 (73.7)
Carbapenems	0	0	0	0	0	0	0	0	0	0
Fluoroquinolones	109 (80.1)	31 (72.1)	78 (83.9)	6 (54.5)	103 (82.4)	0	6 (66.7)	39 (92.9)	53 (80.3)	11 (57.9)
Total	479	170	309	38	427	14	117	143	132	87

[a] All data are presented as no. (%).
[b] LRMC, Landstuhl Regional Medical Center; WRAMC, Walter Reed Army Medical Center; NNMC, National Naval Medical Center; BAMC, Brooke Army Medical Center.
[c] Dates of culture: June 2009 to May 2010.
[d] Dates of culture: June 2010 to May 2011.
[e] Military operation data are missing for six subjects.

(average, 2 days) before transfer to the United States, a lower number of infecting isolates was identified from the LRMC cultures (7% of 136 isolates) than that identified from the U.S. MTFs.

Extended-spectrum β-lactamase production and multidrug resistance. In general, a higher proportion of infecting isolates (80%) was classified as ESBL producers compared with 57% of the colonizing isolates (Table 2). The proportion of colonizing ESBL-producing isolates was significantly different across the study years ($P = 0.01$) but not combat zones or MTFs. There was no significant difference in ESBL production in the infecting isolates. Regarding the colonizing isolates, ESBL-producing *E. coli* was detected in 2% and 10% of the overall number of surveillance cultures performed at LRMC and the U.S. MTFs, respectively. Multidrug resistance was also examined, and the proportion of resistant isolates was comparable to the ESBL production data. In general, resistance to β-lactams and fluoroquinolones was high for both the colonizing and infecting isolates, while the isolates from both groups remained susceptible to aminoglycosides and carbapenems (Table 3).

Changes in the ESBL phenotype of the *E. coli* serial isolates from patients were assessed (Table 4). Overall, the majority of the subjects with serial colonizing isolates demonstrated a stable ESBL classification over time (85%). Similar results were seen in the patients with serial infecting isolates (90% stable) and both colonizing and infecting isolates (95% stable). When the data were examined within patients across the MTFs, 17% of the patients with colonizing organisms isolated at different MTFs demonstrated the acquisition of a new ESBL phenotype. Four patients with serial infecting isolates were also assessed, and one demonstrated a change in his/her ESBL phenotype across the MTFs. Among the patients with both colonizing and infecting isolates, 22% had a change in their ESBL phenotype.

Pulsed-field gel electrophoresis types and phylogroups. (i)Colonizing *E. coli* isolates. The *E. coli* PFT and phylogroup data related to changes over time, combat zones, and across MTFs are reported in Tables 5 and 6, respectively. Overall, there were 188 unique or different PFTs identified from the colonizing *E. coli* isolates, and the most common were PFTs 9, 25, 154, 16, and 122. In general, no large PFT clusters were seen, and notable differences in the PFT profiles were observed when the data were compared across the groups. Regarding the phylogroups, A was predominant (38%), followed by B2 (20%) and D (20%). When the two most common phylogroups (A and B2) were compared, there was no significant difference across the study years, combat zones, or U.S. MTFs.

The PFT and phylogroup profiles from the patients with serial isolates were also examined for changes over time and across facilities (Table 4). A total of 128 patients with serial colonizing isolates were identified, of which 32% acquired a change in their PFT profile and 26% a change in their phylogroups. Among the 92 patients with serial isolates collected across the MTFs, the majority (76% and 79%) exhibited stable PFT and phylogroup profiles, respectively, while 38% and 30% had a change in their PFT and phylogroup profiles, respectively.

(ii) Infecting *E. coli* isolates. Fifty-four different PFTs were identified from the infecting *E. coli* isolates, with PFTs 192, 211, 45, 209, and 218 being the most frequently recorded (Table 5). As with the colonizing isolates, there were no substantial PFT clusters among the infecting isolates, with the PFT profiles differing between the MTFs, combat zones, and study years. The infecting isolate phylogroup profile (Table 6) was also similar to that of the colonizing isolates, with A contributing the majority (43%), followed by D (19%) and B2 (15%). In addition, phylogroup A was found in isolates collected from all anatomic sites, except from urine (Table 7).

An evaluation of the PFT profiles from the 29 patients with serial infecting isolates (Table 4) found that 21% and 10% acquired a change in their PFT and phylogroup profiles, respectively, over time. Moreover, four subjects with serial infecting isolates collected across multiple MTFs were identified, and half reported a change in their PFT profile, while 25% had a change in their phylogroup profile.

TABLE 4 Observations from subjects with serial *E. coli* isolates[a]

Data by isolate type[b]	No. (%) for group: Overall	Subjects with serial isolates across MTFs
Colonization surveillance[c]		
Total subjects	128	92
ESBL classification		
Stable	109 (85.2)	84 (91.3)
Change	19 (14.8)	16 (17.4)
Pulsed-field type		
Stable	87 (68.0)	70 (76.1)
Change	41 (32.0)	35 (38.0)
Phylogroup		
Stable	95 (74.2)	73 (79.3)
Change	33 (25.8)	28 (30.4)
Infection cases[d]		
Total subjects	29	4
ESBL classification		
Stable	26 (89.7)	3 (75.0)
Change	3 (10.3)	1 (25.0)
Pulsed-field type		
Stable	23 (79.3)	3 (75.0)
Change	6 (20.7)	2 (50.0)
Phylogroup		
Stable	26 (89.7)	4 (100)
Change	3 (10.3)	1 (25.0)
Colonization with subsequent infection		
Total subjects	37	18
ESBL classification		
Stable	35 (94.6)	18 (100)
Change	4 (10.8)	4 (22.2)
Pulsed-field type		
Stable	28 (75.7)	14 (77.8)
Change	13 (35.1)	10 (55.6)
Phylogroup		
Stable	32 (86.5)	15 (83.3)
Change	9 (24.3)	7 (38.9)

[a] All data are presented as no. (%).
[b] Stable is defined by the occurrence of the same extended-spectrum β-lactamase (ESBL) phenotype, pulsed-field type (PFT), or phylogroup across serial isolates. Change indicates that at least one serial isolate acquired a different ESBL phenotype, PFT, or phylogroup compared to that of the initial isolate. A subject may be counted in both categories if they have different serial isolates that meet the classifications of stable and change.
[c] Overall number of subjects with colonizing isolates (single and serial) is 148.
[d] Overall number of subjects with infecting isolates (single and serial) is 43.

TABLE 5 *E. coli* PFTs over time and across combat zones and military treatment facilities[a]

PFT data by isolate type	Study yr		Combat zone		MTF[b]				
	Yr 1[c]	Yr 2[d]	Iraq[e]	Afghanistan[e]	All	LRMC	WRAMC	NNMC	BAMC
Overall unique PFTs[f]	85	139	21	187	209	98	84	65	47
Colonizing isolates									
Unique PFTs	80	123	20	167	188	95	78	53	44
Most common PFTs									
9	0	8 (3.7)	0	6 (1.9)	8 (2.3)	4 (3.7)	1 (0.9)	0	3 (4.4)
25	6 (4.7)	1 (0.5)	0	6 (1.9)	7 (2.0)	3 (2.7)	1 (0.9)	1 (1.5)	2 (2.9)
154	3 (2.3)	4 (1.9)	0	7 (2.3)	7 (2.0)	1 (0.9)	0	4 (6.0)	2 (2.9)
16	0	6 (2.8)	0	6 (1.9)	6 (1.7)	2 (1.8)	1 (0.9)	0	3 (4.4)
122	0	5 (2.3)	0	5 (1.6)	5 (1.4)	1 (0.9)	0	4 (6.0)	0
138	1 (0.7)	3 (1.4)	0	4 (1.3)	4 (1.1)	0	4 (3.9)	0	0
193	4 (3.1)	0	0	4 (1.3)	4 (1.1)	0	4 (3.9)	0	0
120	3 (2.3)	1 (0.5)	0	4 (1.3)	4 (1.1)	2 (1.8)	1 (0.9)	1 (1.5)	0
195	0	4 (1.9)	0	4 (1.3)	4 (1.1)	0	0	0	4 (5.8)
190	4 (3.1)	0	1 (3.7)	0	4 (1.1)	0	4 (3.9)	0	0
124	2 (1.5)	2 (0.9)	2 (7.4)	2 (0.6)	4 (1.1)	2 (1.8)	1 (0.9)	0	1 (1.4)
125	4 (3.1)	0	0	4 (1.3)	4 (1.1)	0	4 (3.9)	0	0
45	2 (1.5)	2 (0.9)	0	2 (0.6)	4 (1.1)	0	1 (0.9)	0	3 (4.4)
29	0	4 (1.9)	0	4 (1.3)	4 (1.1)	1 (0.9)	0	1 (1.5)	2 (2.9)
71	1 (0.7)	3 (1.4)	0	4 (1.3)	4 (1.1)	2 (1.8)	2 (1.9)	0	0
Total colonizing isolates[g]	127	216	27	302	343	108	101	66	68
Infecting isolates									
Unique PFTs	19	35	5	49	54	7	18	23	10
Most common PFTs									
192	0	10 (10.8)	0	10 (8.0)	10 (7.3)	0	6 (14.2)	4 (6.0)	0
211	0	9 (9.7)	0	9 (7.2)	9 (6.6)	0	0	9 (13.6)	0
45	8 (18.6)	0	0	8 (6.4)	8 (5.8)	0	7 (16.6)	0	1 (5.2)
209	0	7 (7.5)	0	7 (5.6)	7 (5.1)	0	0	7 (10.6)	0
218	0	5 (5.4)	0	5 (4.0)	5 (3.6)	0	0	5 (7.5)	0
3	0	5 (5.4)	0	5 (4.0)	5 (3.6)	0	0	5 (7.5)	0
215	0	4 (4.3)	0	4 (3.2)	4 (2.9)	0	0	4 (6.0)	0
191	4 (9.3)	0	0	4 (3.2)	4 (2.9)	0	0	4 (6.0)	0
142	0	4 (4.3)	0	4 (3.2)	4 (2.9)	0	0	4 (6.0)	0
13	0	4 (4.3)	4 (36.3)	0	4 (2.9)	0	0	0	4 (21.0)
178	3 (6.9)	0	0	3 (2.4)	3 (2.2)	3 (33.3)	0	0	0
Total infecting isolates[g]	43	93	11	125	136	9	42	66	19

[a] All data are presented as no. (%).
[b] LRMC, Landstuhl Regional Medical Center; WRAMC, Walter Reed Army Medical Center; NNMC, National Naval Medical Center; BAMC, Brooke Army Medical Center.
[c] Dates of culture: June 2009 to May 2010.
[d] Dates of culture: June 2010 to May 2011.
[e] Among the 14 colonizing isolates from unknown military operations, there were 8 unique PFTs.
[f] Number of overall unique colonizing and infecting PFTs. Some PFTs may be the same between the colonizing and infecting isolates.
[g] Total for each column includes isolates not incorporated as part of the most common PFTs; therefore, the sum of the isolates listed for each column is less than the total.

(iii) Patients with both colonizing and infecting *E. coli* isolates. Thirty-seven patients with both colonizing and infecting isolates were assessed, and changes in their PFT and phylogroup profiles over time were observed in 35% and 24% of the subjects, respectively (Table 4). There were also 18 patients with both colonizing and infecting isolates collected across MTFs, of which 56% and 39% reported a PFT and phylogroup profile change, respectively.

DISCUSSION

In our analysis, *E. coli* colonizing and infecting isolates collected from wounded military personnel demonstrated a large variety of genotypic differences without a substantial overlap of the corresponding PFTs across the study years, combat zones, and between different MTFs. There were no sizeable PFT clusters among the colonizing and infecting isolates, and the number of multiple PFTs within patients increased as the amount of serial isolates grew. As with prior data collected from various human populations (26, 29), phylogroup A was predominant in our study group. In general, the *E. coli* phenotypes and genotypes were mostly consistent across time and between MTFs on a per-patient basis. A minority of the patients had changes in their PFT profiles, phylogroups, and ESBL phenotypes, indicating the possibility of colonization by different or newly acquired *E. coli* strains, perhaps due to enhanced antimicrobial pressure caused by the administration of antimicrobials as either prophylaxis or treatment. It is also feasible that the PFT and phylogroup changes may be related to the natural wide-ranging genetic diversity of *E. coli* within patients (30–33), as genetic variations over time have been shown to occur in healthy subjects with intestinal *E. coli* infections (30–32). More-

TABLE 6 *E. coli* phylogroups over time and across combat zones and military treatment facilities[a]

Phylogroup by isolate type	Total isolates	Study yr		Combat zone			Military treatment facility[b]			
		Yr 1[c]	Yr 2[d]	Iraq	Afghanistan	Unknown[e]	LRMC	WRAMC	NNMC	BAMC
Colonizing	343	127	216	27	302	14	108	101	66	68
A[f]	129 (37.6)	52 (40.9)	77 (35.6)	10 (37.0)	112 (37.1)	7 (50.0)	43 (39.8)	42 (41.6)	24 (36.4)	20 (29.4)
B1	42 (12.2)	21 (16.5)	21 (9.7)	2 (7.4)	38 (12.6)	2 (14.3)	13 (12.0)	13 (12.9)	8 (12.1)	8 (11.8)
B2[f]	68 (19.8)	24 (18.9)	44 (20.4)	5 (18.5)	58 (19.2)	5 (35.7)	24 (22.2)	23 (22.8)	8 (12.1)	13 (19.1)
C	5 (1.5)	1 (0.8)	4 (1.9)	1 (3.7)	4 (1.3)	0	3 (2.8)	1 (1.0)	1 (1.5)	0
D	67 (19.5)	17 (13.4)	50 (23.2)	8 (29.6)	59 (19.5)	0	18 (16.7)	17 (16.8)	17 (25.8)	15 (22.1)
E	5 (1.5)	3 (2.4)	2 (0.9)	1 (3.7)	4 (1.3)	0	3 (2.8)	1 (1.0)	0	1 (1.5)
F	26 (7.6)	8 (6.3)	18 (8.3)	0	26 (8.6)	0	4 (3.7)	4 (4.0)	7 (10.6)	11 (16.2)
Clade I	1 (0.3)	1 (0.8)	0	0	1 (0.3)	0	0	0	1 (1.5)	0
Infecting	136	43	93	11	125	0	9	42	66	19
A[g]	59 (43.4)	26 (60.5)	33 (35.5)	4 (36.4)	55 (44.0)	0	4 (44.4)	24 (57.1)	29 (43.9)	2 (10.5)
B1	17 (12.5)	5 (11.6)	12 (12.9)	0	17 (13.6)	0	0	3 (7.1)	10 (15.2)	4 (21.1)
B2[g]	20 (14.7)	3 (7.0)	17 (18.3)	4 (36.4)	16 (12.8)	0	4 (44.4)	6 (14.3)	4 (6.1)	6 (31.6)
C	2 (1.5)	0	2 (2.2)	0	2 (1.6)	0	0	0	2 (3.0)	0
D	26 (19.1)	3 (7.0)	23 (24.7)	3 (27.2)	23 (18.4)	0	1 (11.1)	9 (21.4)	13 (19.7)	3 (15.8)
E	0	0	0	0	0	0	0	0	0	0
F	12 (8.8)	6 (13.9)	6 (6.5)	0	12 (9.6)	0	0	0	8 (12.1)	4 (21.1)
Clade I	0	0	0	0	0	0	0	0	0	0

[a] All data are presented as no. (%).
[b] LRMC, Landstuhl Regional Medical Center; WRAMC, Walter Reed Army Medical Center; NNMC, National Naval Medical Center; BAMC, Brooke Army Medical Center.
[c] Dates of culture: June 2009 to May 2010.
[d] Dates of culture: June 2010 to May 2011.
[e] Military operation data are missing for six subjects.
[f] Colonizing phylogroups A and B2 were compared using a generalized estimating equation model. There were no significant differences across study years, combat zones, and U.S. military treatment facilities.
[g] Infecting phylogroups A and B2 were compared using a generalized estimating equation model; however, the model failed to produce valid *P* values.

over, both colonizing and infecting isolates were frequently classified as ESBL producing. In particular, 10% of colonizing *E. coli* isolates of the total number of surveillance cultures performed at the U.S. MTFs were classified as ESBL producers. The high proportion of colonizing *E. coli* isolates classified as ESBL producers was surprising and may have been the result of antimicrobial pressure. On the whole, the proportion of ESBL-producing isolates remained fairly consistent across the MTFs for both the colonizing and infecting isolates, with no considerable changes in the antimicrobial susceptibilities observed over time among patients within and across facilities.

Presently, limited data are available in the scientific literature on the genotypic characteristics of *E. coli* isolates collected from wounded military personnel. In a prospective analysis of injured service members admitted to Walter Reed Army Medical Center, 11 unique PFTs were identified from 42 colonizing ESBL-producing *E. coli* isolates, which were collected from 9 deployed patients. Furthermore, one of the colonizing *E. coli* strains was recovered from eight different deployed patients, and one nondeployed subject was found to be colonized with a strain of ESBL-producing *E. coli* highly related to a strain isolated from a deployed patient (17). An additional analysis assessing the military personnel at Brooke Army Medical Center reported 11 unique colonizing multidrug-resistant *E. coli* PFTs collected from 11 patients who had been

TABLE 7 *E. coli* phylogroups among colonizing and infecting isolates by site of collection

Phylogroup	No. (%) of colonizing isolates[a]	No. (%) of infecting isolates collected from:							
		Wound	Blood	Urine	Tissue	Sputum	Tracheal aspirate	Bone	Other[b]
A	129 (37.6)	45 (50.6)	5 (29.4)	0	1 (20.0)	1 (25.0)	2 (50.0)	4 (100)	1 (14.3)
B1	42 (12.2)	8 (9.0)	4 (23.5)	0	2 (40.0)	0	0	0	3 (42.9)
B2	68 (19.8)	5 (5.6)	5 (29.4)	3 (50.0)	0	2 (50.0)	2 (50.0)	0	3 (42.9)
C	5 (1.5)	1 (1.1)	0	0	0	1 (25.0)	0	0	0
D	67 (19.5)	20 (22.5)	1 (5.9)	3 (50.0)	2 (40.0)	0	0	0	0
E	5 (1.5)	0	0	0	0	0	0	0	0
F	26 (7.6)	10 (11.2)	2 (11.8)	0	0	0	0	0	0
Clade I	1 (0.3)	0	0	0	0	0	0	0	0
Total isolates	343	89	17	6	5	4	4	4	7

[a] All colonizing isolates were collected from groin swabs.
[b] Other includes bronchoalveolar lavage fluid/bronchial wash, intravascular catheter tip, cerebrospinal fluid, intra-abdominal, peritoneal space, and other nonspecified specimens.

deployed to Afghanistan and were generally healthy. When the deployed patients were compared with the nondeployed military personnel, no overlap of the PFT profiles between the isolates was detected. The isolates collected from nondeployed patients were also significantly more susceptible to antimicrobials than those from deployed patients. Overall, the analysis determined that the deployed personnel had a 5.5-fold increased rate of community-acquired multidrug-resistant *E. coli* colonization compared to that of the nondeployed service members (18). While our data reported here do not include nondeployed personnel, the large variation in the PFT profiles between service members from the different time periods, combat zones, and MTFs is consistent with the large genotypic diversity of *E. coli* depicted in the scientific literature. The disparity in the PFT profiles between the combat zones also suggests that wounded service members likely acquired *E. coli* isolates prior to their arrival at LRMC. One possible explanation for the high levels of multidrug resistance among the deployed patients described in the earlier report, along with our data here, is antimicrobial pressure in treatment facilities; however, further analysis on the matter is required to determine the validity of this explanation.

Deployed military personnel may be exposed to *E. coli* through multiple sources, including the soil and water, as has been shown in the combat zones and in data from other countries (12, 34). Although data specific to *E. coli* are limited, the genotypic evaluations performed with other colonizing and infecting MDROs documented in wounded military personnel are useful for comparative purposes in relation to the identification of potential sources or risk factors. One MDRO, *Acinetobacter baumannii-A. calcoaceticus* complex (ABC), was commonly reported to be associated with service members who sustained injuries in Iraq but less among those wounded in Afghanistan (18). In an analysis of 102 ABC isolates collected from 59 U.S. service members injured in Iraq, 23 unique PFTs were identified. It is notable that six of the PFTs comprised approximately 80% of the isolates (35). Another analysis detected 66 PFTs from 170 ABC clinical isolates collected from patients injured in Iraq. Twenty-five PFTs were also recovered from 34 environmental isolates obtained from field hospitals. A comparison of the clinical and environmental isolates identified five cluster groups in which at least one environmental isolate was genetically related to a clinical isolate (9). In contrast to the ABC PFT data, the *E. coli* isolates reported here were not highly clonal, suggesting that the risk factors for colonization and infection in our cohort of wounded military personnel may be different than those for ABC (e.g., nosocomial transmission).

Unlike many of the prior studies, our analysis examined both colonizing and infecting isolates (independently and combined) among wounded military personnel. Generally, colonization is defined as the presence of bacteria on intact skin without initiation of a host response, while infection is a clinical diagnosis based upon clinical findings and laboratory test results. Presently, it is unknown if a direct link exists between colonization and eventual infection (11). Regarding trauma sustained during the conflicts in Iraq and Afghanistan, studies have reported differences in the bacteria collected at the time of injury, primarily that they are Gram positive, compared to the isolates recovered from infections occurring later during the course of hospitalization. The relevance of this observation, along with the possible contribution of nosocomial transmission to subsequent infections, is a subject of debate (13, 36, 37). In our analysis, it is of note that 14% and 33% of the subjects with colonizing and both colonizing and infecting isolates, respectively, had PFTs identified amid the colonizing isolates that were later associated with infecting isolates following the transition to a different MTF. Furthermore, the PFT patterns, phylogroups, and multidrug resistance types remained largely unchanged within the patients in our study, suggesting that subsequent infections may have been caused by isolates with the same or similar PFTs or phylogroups and susceptibilities as those found colonizing the subject.

There are limitations to our analysis that should be considered. First, our study population consisted of patients more severely injured than those who were transferred to other U.S. MTFs (8). Therefore, the applicability of the data reported in our analysis to all injured service members who had transitioned through LRMC is not certain. In addition, the initiation of the analysis coincided with the decline of combat operations in Iraq, which resulted in a lower number of isolates from personnel injured in support of these operations, and it limited the comparison of isolate data across combat zones. Lastly, surveillance cultures included only groin swabs and not perirectal or stool specimens, which may have impacted the degree of heterogeneity among the colonizing *E. coli* isolates.

The findings of our analysis highlight the importance of MDRO admission screening and inpatient surveillance, as the identification of diverse genotypes and phenotypes across time and location provides information that may be utilized to ascertain the effectiveness of infection control measures in relation to the possibility of nosocomial transmission among wounded warriors. A heightened awareness of colonization and infection patterns will also allow for improvements in patient care through better selection of empiric antibiotics, targeted infection control efforts, and feedback to the facilities involved in patient evacuation and initial treatment. Further analyses to evaluate whether a direct association exists between MDRO colonization and ensuing infection, an assessment of risk factors for infection, and an evaluation of *E. coli* virulence factors are warranted.

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