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Clonal distribution and associated characteristics of *Escherichia coli* clinical and surveillance isolates from a military medical center

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ABSTRACT

Antimicrobial-resistant *Escherichia coli* are a concern for military health services. We studied 100 extended-spectrum beta-lactamase (ESBL)-producing and non-producing *E. coli* clinical and surveillance isolates from military personnel and civilians at Brooke Army Medical Center (2007–2011). Major *E. coli* lineages, most prominently ST10 (24%), ST131 (16%), and ST648 (8%), were distributed much as reported for other North American populations. ST131, represented mainly by its resistance-associated ST131-*H*30 clonal subset, was uniquely associated with a clinical origin, regardless of ESBL status. Thus, clonal background predicted resistance phenotype and clinical versus surveillance origin, and these findings could assist military clinicians and epidemiologists.

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1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) cause most community and hospital-associated extraintestinal *E. coli* infections (Johnson and Russo, 2005). Specific ExPEC lineages, or sequence types (STs), are commonly multidrug-resistant (MDR), and have been associated with international travel and medical care. The acquisition of difficult-to-treat, antimicrobial-resistant ExPEC is therefore a concern for military health services.

In this study, we examined the distribution of *E. coli* genotypes identified among clinical and surveillance (e.g. skin colonization) isolates from deployed and non-deployed active duty US military personnel and civilians, collected as part of routine medical care at a USmilitary medical center. Stool surveillance isolates were not included in this study. We sought to determine whether military personnel (especially those with a history of international deployment or injury), when infected or colonized cutaneously with *E. coli*, were at an increased risk of having specific extensively drug-resistant major lineages of ExPEC. In addition, we attempted to define the characteristics of these lineages as they occur among military personnel. We focused specifically on the presence of sequence type 131 (ST131) and its resistance-associated *H*30 and *H*30Rx subclones within this population, and compared these strains" prevalence with data collected from veterans receiving care within the Veterans Health Administration in 2011 (Colpan et al., 2013).

2. Materials and methods

2.1. Isolates and subjects

The study isolates included 100 de-identified archival *E. coli* clinical and surveillance isolates, which were obtained along with associated information (specimen type, collection date, collection site, isolate source, injury during deployment, etc.) from the Molecular Biology Laboratory at Brooke Army Medical Center, JBSA Fort Sam Houston, TX. The isolates had been recovered

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Table 1
Extraintestinal pathogenic *Escherichia coli* source and characteristics.

Prevalence of trait, No. of isolates (%)						
		Clinical		Surveillance		
Host characteristic, phylogroup, or ST[a]	Total ($n = 100$)	ESBL-producing ($n = 25$)	Non-ESBL-producing ($n = 25$)	ESBL-producing ($n = 25$)	Non-ESBL-producing ($n = 25$)	*P* value[b]
Active duty	84 (84)	18 (72)	20 (80)	22 (88)	24 (96)	0.11
Deployment injury	65 (65)	11 (44)	1 (4)	19 (76)	18 (72)	<0.001
Group A	30 (30)	8 (32)	4 (16)	10 (40)	8 (32)	0.31
Group B1	13 (13)	2 (8)	3 (12)	3 (12)	5 (20)	0.73
Group B2	31 (31)	8 (32)	14 (56)	2 (8)	7 (28)	0.003
Group C	2 (2)	1 (4)	1 (4)	0 (0)	0 (0)	n.a.[c]
Group D	16 (16)	5 (20)	1 (4)	5 (20)	5 (20)	0.26
Group F	8 (8)	1 (4)	2 (8)	5 (20)	0 (0)	0.08
ST10 (group A)	24 (24)	8 (32)	1 (4)	7 (28)	8 (32)	0.06
ST131 (group B2)	16 (16)	8 (32)	6 (24)	1 (4)	1 (4)	0.01
ST131 *H*30	14 (14)	6 (24)	6 (24)	1 (4)	1 (4)	0.60
ST131 *H*30Rx	8 (8)	4 (16)	3 (12)	0 (0)	1 (4)	n.a.[c]
ST131 non-*H*30	2 (2)	2 (8)	0 (0)	0 (0)	0 (0)	n.a.[c]
ST648 (group F)	8 (8)	1 (4)	2 (8)	5 (20)	0 (0)	n.a.[c]

[a] ST = sequence type. Only the three most prevalent STs are shown. Other STs that accounted for ≥3% of isolates each, in order of descending overall prevalence (no. of isolates), included: ST38 (5%), ST95 (5%), ST69 (4%), ST405 (4%), and ST73 (3%).
[b] By Fisher's exact test (two-tailed), for four-group comparisons.
[c] n.a., not applicable (number too small for statistical analysis).

between 2007 and 2011 from 100 unique military personnel and civilians attending the same military medical center (Table 1) and had been tested for extended-spectrum beta-lactamase (ESBL) production, as described below. The archival collections included isolates from patients with a suspected infection (clinical) or who were being screened for antimicrobial-resistant *E. coli* from nares or groin skin sites (surveillance). The 100 study isolates were selected randomly as four groups of 25 isolates each: (i) ESBL-producing clinical isolates, (ii) non-ESBL-producing clinical isolates, (iii) ESBL-producing surveillance isolates, and (iv) non-ESBL-producing surveillance isolates (Table 1). Specimen type and the subject's military versus civilian status and deployment history did not influence isolate selection.

The study was approved by research oversight committees at the participating institutions. It did not qualify for review by the respective institutional review boards due to its use of archived isolates that had been collected for routine monitoring purposes, along with generic deidentified epidemiological data.

2.2. Laboratory analysis

Antimicrobial susceptibilities and ESBL production was determined using the BD Phoenix Automated Microbiology System (Becton Dickinson and Company, Sparks, MD, USA) per manufacturer guidelines utilizing NMIC/ID-123 panels. Minimum inhibitory concentration (MIC) results were interpreted according to the Clinical Laboratory and Standards Institute criteria (Clinical and Laboratory Standards Institute, 2013). In addition, isolates were assessed molecularly for $bla_{\text{CTX-M-15}}$, major *E. coli* phylogenetic group (phylogroup) (Clermont et al., 2013), ST or ST complex (STc; as determined by *fumC-fimH* typing (Weissman et al., 2012), full or partial MLST (Maiden et al., 1998), or STc-specific PCR assays (Clermont et al., 2014; Johnson et al., 2009; Matsumura et al., 2012)), membership in the ST131-*H*30 clonal subset or its sublineage ST131-*H*30Rx (Banerjee et al., 2013), O type (O16 and O25b only) (Johnson et al., 2014), and extended virulence genotype (for 50 markers) (Johnson et al., 2015). Resistance scores were defined as the number of antibiotic classes to which an isolate exhibited resistance. Virulence scores were defined based on the raw count of detected virulence genes.

2.3. Statistical analysis

Comparisons of proportions were tested using Fisher's exact or chi-squared tests. Comparisons of scores were tested using the Wilcoxon rank-sum test.

3. Results

3.1. Clinical and epidemiological data

The 100 source subjects included 84 (84%) active duty military personnel and 16 (16%) civilians receiving care at Brooke Army Medical Center (Table 1). Of 65 subjects with a known injury, 49 (75%) were injured during military deployment, which occurred exclusively in Iraq and/or Afghanistan. *E. coli* isolates recovered from these 49 deployed and injured military members included 10 isolates from wound sites, 2 from blood and 37 collected from the groin area, as part of surveillance sampling. The 50 clinical isolates were from urine ($n = 18$), blood ($n = 8$), wound ($n = 23$), and body fluid ($n = 1$). The 50 surveillance isolates were from groin ($n = 49$) and nares ($n = 1$).

3.2. Phylogroups

The 4 most common *E. coli* phylogroups overall, in descending frequency, were B2 (31%), A (30%), D (16%), and B1 (13%) (Table 1). Phylogroup B2 isolates were uniquely over-represented among the clinical isolates. Phylogroup A isolates (specifically ST10), although somewhat more common among surveillance than clinical isolates, were more common among clinical isolates than expected based on prior work (Salipante et al., 2015).

3.3. Sequence Types

The 8 most common STs, in descending frequency, were ST10 (24%), ST131 (16%), ST648 (8%), ST38 (5%), ST95 (5%), ST69 (4%), ST405 (4%), and ST73 (3%). Collectively, these 8 predominant STs accounted for 69% of the population. No temporal prevalence trends were apparent for any specific STs (not shown).

Statistical comparisons were done only with the 3 most common STs. ST10 was under-represented among non-ESBL-producing clinical isolates, whereas ST648 was over-represented among ESBL-producing surveillance isolates and absent among non-ESBL surveillance isolates. The combined prevalence of the three most common STs (ST10, 131, and 648) varied by host group, i.e., 36/84 (43%) for active duty subjects (including 24/49 [49%] for subjects with deployment-related wounds), versus 12/16 (75%) for civilian subjects ($p = 0.02$).

ST131, the most prevalent ST among clinical isolates, was the only ST that was significantly over-represented among clinical isolates as