Association of *Enterococcus* spp. with Severe Combat Extremity Injury, Intensive Care, and Polymicrobial Wound Infection

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Abstract

Background: Combat-related extremity wound infections can complicate the recovery of injured military personnel. The *Enterococcus* genus contains both commensal and pathogenic bacteria found in many combat wounds. We describe the patient population susceptible to *Enterococcus* infection, the characteristics of *Enterococcus* spp. isolated from combat-related wounds, and the microbiological profile of *Enterococcus*-positive wounds.

Methods: Patient and culture data were obtained from the Trauma Infectious Disease Outcomes Study. Subjects were divided into a case group with enterococcal extremity wound infections and a comparator group with wound infections caused by other micro-organisms.

Results: Case and comparator subjects had similar patterns of injury and infection. Case subjects had higher Injury Severity Scores (33 vs. 30; $p < 0.001$), longer hospitalization at U.S. facilities (55 vs. 40 days; $p = 0.004$), and required more large-volume blood transfusions (>20 units) within 24 h post-injury (53% vs. 30%; $p < 0.001$). Approximately 60% of case subjects had three or more infections, and 91% had one or more polymicrobial infections, compared with 43% and 50%, respectively, in the comparator group. The thigh was the most common site of *Enterococcus* spp. isolation, contributing 50% of isolates. *Enterococcus faecium* was the predominant species isolated from case-group infections overall (66%), as well as in polymicrobial infections (74%). Frequent co-colonizing microbes in polymicrobial wound infections with *Enterococcus* were other ESKAPE pathogens (64%) (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* [and *Escherichia coli*], *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) and fungi (35%).

Conclusions: The specific pathogenicity of *Enterococcus* relative to other pathogens in polymicrobial wounds is unknown. Identifying strain-specific outcomes and investigating the interactions of *Enterococcus* strains with other wound pathogens could provide additional tools and strategies for infection mitigation in combat-related wounds.

Keywords: combat-trauma-related infections; *Enterococcus faecium*; *Enterococcus faecalis*; ESKAPE pathogens

ADVANCES IN PROTECTIVE EQUIPMENT and trauma care since World War II have contributed to the lowest overall case-fatality and died-of-wounds rates in U.S. military history during the wars in Iraq and Afghanistan [1]. Survivors of battle wounds tend to have severe orthopedic injuries, exerting a massive cost in terms of both combat-veteran disability and military healthcare-system resource usage [2,3]. Complex injuries and their consequent life-saving interventions, such as high-volume blood transfusions

and amputations, frequently result in additional complications, including wound infections [4,5]. Extremity wound infections, particularly osteomyelitis, are associated with more treatment complications requiring repeated or delayed amputations, re-hospitalization, and reduced return-to-duty rates [6–8].

Although war wounds typically are colonized by commensal gram-positive and saprophytic gram-negative bacteria soon after injury, bacteria associated with wound infections generally

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are not among the colonizing organisms [9–12]. Another source of antibiotic-resistant pathogenic bacteria is the hospital, where nosocomial opportunistic pathogens have become established on abiotic surfaces, and virulent organisms disperse within and among patients [12–14]. Civilian and military hospitals alike are plagued by bacteria collectively called the ESKAPE pathogens, which have been identified as the most common and threatening pathogens. These bacteria are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* (and *Escherichia coli*), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. [15]. In addition to their pathogenicity, the increasing incidence of antibiotic resistance in these bacteria makes it difficult to resolve some infections. Many of the ESKAPE pathogens are extended-spectrum, beta-lactamase-producing gram-negative bacteria, which are well-recognized for causing multidrug-resistant nosocomial infections. Only two of the ESKAPE pathogens are gram-positive, and both are commonly found in healthy humans: the ubiquitous *S. aureus* and the enteric commensal species *E. faecium*.

Enterococcus faecium is a facultative anaerobic lactic acid bacterium that, like other species in the genus, forms small white or yellow colonies on blood agar incubated at body temperature, with or without hemolysis. *Enterococcus faecium* and *Enterococcus faecalis* are the most common enterococcal species causing infections, particularly healthcare-associated infections [16–21], but *E. faecium* surpasses *E. faecalis* in antibiotic resistance [22,23]. In recent years, a notable increase in vancomycin resistance paired with the high innate antibiotic resistance in *E. faecium* has resulted in restricted treatment options for infected patients [24]. Although there are data examining *Enterococcus* spp. with regard to healthcare-associated infections, few published studies are available evaluating the role of *Enterococcus* in civilian wound infections. One analysis from India on civilian trauma wound infections found that *E. faecium* was the most common species of *Enterococcus* in this type of wound, with 8% of *Enterococcus* isolates identified as vancomycin resistant. In addition, 9% of the infections were polymicrobial [25].

FIG. 1. Flowchart of inclusion criteria for subjects and infections. Subjects are a subset of the Department of Defense and Veterans Affairs Trauma Infectious Disease Outcomes Study (TIDOS) population with combat-related extremity injuries and extremity wound infections (skin or soft-tissue infection or osteomyelitis). The cohort was divided into a case group of subjects with at least one *Enterococcus* culture associated with a wound infection and a comparator group of subjects with wound cultures positive for any other microorganism. The total number of infections and the number of wound infections were determined for the case and comparator group.

Although *Enterococcus* spp. often are associated with polymicrobial bloodstream and urinary tract infections [16,17], the role that enterococci play in combat trauma infections has not been investigated extensively.

In 2009, the Department of Defense and Veterans Affairs Trauma Infectious Disease Outcomes Study (TIDOS), a multicenter, observational project, began enrolling participants to investigate infectious disease outcomes in combat-injured U.S. military personnel [4]. The overarching purpose of TIDOS is to improve infectious disease outcomes in military members with traumatic injuries [4,26]. Our study utilized TIDOS data to perform a retrospective analysis to describe the injury patterns, infections, and medical treatment of military personnel with wound infections positive for *Enterococcus* spp. at initial diagnosis. It also describes the variety of species and resistance patterns identified among *Enterococcus* isolates recovered from the wound infections in these patients.

Patients and Methods

Study design

The TIDOS project includes military patients with deployment-related traumatic injuries sustained between June 2009 and December 2014 and admitted to Walter Reed National Military Medical Center (formerly Walter Reed Army Medical Center and National Naval Medical Center) and San Antonio Military Medical Center (formerly Brooke Army Medical Center) after medical evacuation from Iraq and Afghanistan through Landstuhl Regional Medical Center in Germany [4]. The study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences (Bethesda, MD).

The subject dataset for this analysis was determined by querying the entire TIDOS population for patients with at least one skin or soft-tissue infection (SSTI) or osteomyelitis diagnosis meeting the standardized National Healthcare Safety Network-infection diagnostic criteria [27] at an extremity anatomic site and with at least one positive microbiologic culture from the infection site within three days before or after the date of infection diagnosis. Demographics, injury characteristics, and treatment data were obtained from the Department of Defense Trauma Registry [28]. Infection data were collected via the TIDOS supplemental infectious disease module [4]. Body sites classified as extremities were the foot, ankle, lower leg, knee, thigh, groin, gluteal muscles, pelvis, shoulder, upper arm, elbow, forearm, wrist, and hand. The subjects were divided into groups with at least one culture positive for *Enterococcus* (case group) and a comparator group with at least one positive culture, but no *Enterococcus* isolates.

An infection dataset was created by querying all clinical infectious disease events, including, but not limited to, wound infections associated with the subjects in the case and comparator groups. If the a priori criteria were not met, an infection still was included in the analysis if there was a clinical diagnosis associated with directed anti-microbial treatment for five or more days for SSTIs and ≥ 21 d for osteomyelitis. Infections were excluded if an alternative diagnosis was indicated in the medical records and anti-microbial treatment was discontinued. As an individual subject could have more than one infection, the multiple infections in the dataset would belong to the same patient and were counted

individually on a per-infection level. Wound infections were counted by combining the clinical diagnoses of SSTI and osteomyelitis. Polymicrobial wound infections were defined as an SSTI or osteomyelitis diagnosis associated with positive cultures for more than one organism (within three days of infection diagnosis).

The *Enterococcus* isolate dataset was selected by querying all bacterial isolates from the TIDOS Specimen Repository identified clinically as a species of *Enterococcus* and cultured from a wound or bone specimen associated with an SSTI or osteomyelitis within three days before or after infection diagnosis. A wound specimen was defined as tissue, swab, abscess, or drainage material. Anti-microbial susceptibility testing of *Enterococcus* spp. isolates was performed in the clinical microbiology laboratories using either the BD Phoenix automated microbiology system (Becton, Dickinson and Company, Sparks, MD) or VITEK® 2 (bioMérieux, Durham, NC) per manufacturer guidelines and interpreted in accordance with laboratory protocols and the criteria of the Clinical Laboratory

TABLE 1. DEMOGRAPHICS, INJURY CHARACTERISTICS, AND DEATHS OF U.S. SERVICE MEMBERS WITH ENTEROCOCCAL AND OTHER WOUND INFECTIONS

Characteristic (%)	Case subjects (N=155)	Comparator subjects (N=237)	p
Mean age (IQR)	24 (22–27)	23 (21–27)	0.076
Male	153 (98.7)	235 (99.2)	0.650
Rank			0.030
Civilian	0	1 (0.4)	
Enlisted	137 (88.4)	223 (94.1)	
Warrant	0	1 (0.4)	
Officer	18 (11.6)	12 (5.1)	
Branch			0.945
Marine	61 (39.4)	90 (38.0)	
Army	89 (57.4)	137 (57.8)	
Navy	3 (1.9)	7 (3.0)	
Air Force	2 (1.3)	2 (0.8)	
Blast injury mechanism	147 (94.8)	221 (93.2)	0.521
Median Injury Severity Score (IQR)	33 (29–45)	30 (22–38)	<0.001
Injury site			0.777
Upper extremity	2 (1.3)	4 (1.7)	
Lower extremity	23 (14.8)	41 (17.3)	
Both	130 (83.9)	192 (81.0)	
Amputations ^a			0.373
Single			
AK only	10 (6.5)	5 (2.1)	
BK only	18 (11.6)	26 (11.0)	
UE only	2 (1.3)	3 (1.3)	
Multiple	102 (65.8)	115 (48.5)	
Lower extremity amputation plus GU injury	84 (54.2)	97 (40.9)	0.013
Died inpatient (%)	4 (2.6)	6 (2.5)	1.000

^aTwo case subjects had surgical amputations after admission to U.S. hospitals. The remainder of the amputations occurred in the combat theater or at Landstuhl (Germany) and are considered traumatic.

AK=above knee; BK=below knee; GU=genitourinary, IQR=interquartile range; UE=upper extremity.

and Standards Institute [29]. Culture data for the isolates were used to compare proportions of species, antibiotic susceptibilities, anatomic site of culture, and source hospital.

The microbial composition of the polymicrobial wound infections containing *Enterococcus* was determined. The number of unique infections associated with each co-infecting organism was counted.

Statistical analysis

Differences between the case and comparator group related to demographics, injury characteristics, infections, and treatment were evaluated. Categorical data were assessed using the Fisher exact test, the Fisher-Freeman-Halton test, or the χ^2 test, as appropriate. Differences in continuous data were analyzed statistically using the Wilcoxon Mann-Whitney test.

Results

Study population

There were 155 subjects with enterococcal wound infections (SSTI and osteomyelitis) in the case group and 237 comparator-group subjects with wound infections attributable only to non-enterococcal microbes (Fig. 1; Table 1). The demographics of both groups were similar: Subjects were almost all male and predominately enlisted, although there was a small difference in rank distribution between the two groups ($p=0.030$) caused by a slight increase in officers in the case group relative to the comparator group. There was no significant difference in the proportion of subjects from dif-

ferent branches of the service, with both groups containing primarily Marines Corps and Army personnel.

Injury, infection, and treatment

There were no significant differences in the injury pattern for the case and comparator subjects (Table 1). Blast injuries were predominant in both groups (95% and 93% for the case and comparator groups, respectively) with a similar proportion of subjects sustaining injuries to the upper and lower extremities (84% and 81%, respectively). Subjects in the case group had a significantly higher median Injury Severity Score (ISS) than those in the comparator group (33 vs. 30, respectively; $p<0.001$). Furthermore, the case subjects had a higher rate of intensive care unit (ICU) admission ($p<0.001$), received more units of packed red blood cells plus whole blood within 24 h of injury ($p<0.001$), and required a greater number of visits to the operating room in the two weeks after injury ($p<0.001$; Table 2). Lastly, the duration of hospitalization was longer (median 55 vs. 49 days) for the case subjects than for the comparator subjects ($p=0.004$).

Infection patterns were similar in the case and comparator subjects; however, case subjects had a higher incidence of multiple and polymicrobial wound infections. Subjects with three or more in-patient infections represented 60% of the case group, but only 43% of the comparator group ($p<0.001$; Table 2). The proportion of subjects with at least one polymicrobial wound infection was significantly higher in the case group (91% vs. 50%; $p<0.001$). In addition to wound infections, bloodstream infections and pneumonia contributed more than 5% of the total infections in both groups

TABLE 2. MEDICAL EVACUATION, TREATMENT, AND INFECTIONS OF U.S. SERVICE MEMBERS WITH COMBAT-RELATED EXTREMITY WOUND INFECTIONS

Characteristic	Case subjects (N = 155)	Comparator subjects (N = 237)	p
Median evacuation timeline (d) (IQR)			
Injury to CSH admission	2 (2–2)	2 (1–2)	0.128
CSH admission to LRMC admission	2 (2–3)	2 (2–3)	0.355
LRMC admission to discharge	4 (3–5)	3 (3–4)	0.038
U.S. hospital admission to discharge	52 (39–87)	47 (34–66)	0.006
Median operating room visits ^a (IQR)	5 (5–7)	5 (4–6)	<0.001
Intensive care unit admission			<0.001
None	18 (11.6)	44 (18.6)	
LRMC only	10 (6.5)	41 (17.3)	
U.S. hospital with/without LRMC	127 (81.9)	152 (64.1)	
Blood transfused (units) ^b			<0.001
None	0	2 (0.8)	
1–9	20 (12.9)	65 (27.4)	
10–20	47 (30.3)	78 (32.9)	
>20	82 (52.9)	70 (29.5)	
Missing	6 (3.9)	22 (9.3)	
Use of vasopressors at LRMC	20 (12.9)	20 (8.4)	0.153
Median hospitalization (d) (IQR)	55 (41, 90)	49 (37, 68)	0.004
Infections (%)			<0.001
≤2	62 (40.0)	136 (57.4)	
≥3	93 (60.0)	101 (42.6)	
Polymicrobial wound infection (≥1) (%)	141 (91.0)	118 (49.8)	<0.001

^aWithin 24 h of injury.

^bPacked red blood cells plus whole blood within two weeks of injury.

CSH=combat support hospital; IQR=interquartile range; LRMC=Landstuhl Regional Medical Center.

TABLE 3. DISTRIBUTION OF INFECTIONS AMONG U.S. SERVICE MEMBERS WITH COMBAT-RELATED EXTREMITY WOUND INFECTIONS AND POSITIVE MICROBIOLOGY CULTURES^a

Infection type (%)	Case subjects (N=155)	Comparator subjects (N=237)
Total infections	618	648
Wound infection		
Skin and soft-tissue	359 (58.1)	375 (57.9)
Osteomyelitis	49 (7.9)	75 (11.6)
Blood stream	70 (11.3)	75 (11.6)
Pneumonia	44 (7.1)	47 (7.3)
Urinary tract	34 (5.5)	26 (4.0)
Sepsis	24 (3.9)	21 (3.2)
Intra-abdominal	13 (2.1)	9 (1.4)
Central nervous system	1 (0.2)	7 (1.1)
Other ^b	24 (3.9)	13 (2.0)

^aData are presented on a per-infection level.

^bOther infections: *Clostridium difficile* (presumptive and confirmed), eye infection, sinusitis, otitis interna, non-pneumonia lung abscess, tracheobronchitis, and undifferentiated.

(Table 3). As the infection-level data were not independent (i.e., multiple infections from the same patient were counted as individual infections, along with infections from other patients), no statistical analysis was performed on these data.

Microbial isolates

The most common species of *Enterococcus* identified was *E. faecium* (66%), followed by *E. faecalis* (13%) (Table 4). There was one isolate identified as *E. gallinarum*, which was

collapsed into the group of unspciated *Enterococcus* (n=53; 21%) for clarity (Table 4). Vancomycin resistance was not common among *Enterococcus* isolates, but did occur in a higher proportion among uncommon or unspciated *Enterococcus* spp. (15%) than *E. faecium* (1%) or *E. faecalis* (0) (Table 4). Resistance to other classes of antibiotics was higher, with 52% of *Enterococcus* isolates resistant to at least one antibiotic in the beta-lactam class (e.g., penicillin derivatives, cephalosporins, and carbapenems) and 17% resistant to one or more antibiotics in the aminoglycoside class (e.g., gentamicin). The anatomic source of the largest number of isolates was the thigh, followed by the lower leg, pelvis, forearm, and upper arm. The majority of the *Enterococcus* isolates were cultured from polymicrobial wound infections (Table 4).

Polymicrobial wound infections

A total of 211 polymicrobial *Enterococcus* wound infections were identified in the case group, and *E. faecium* was the most common enterococcal species cultured (74%; Table 5). ESKAPE pathogens (other than *Enterococcus* spp.) were co-cultured from 64% of the polymicrobial wound infections, with *E. coli*, *E. cloacae*, *P. aeruginosa*, and *A. baumannii* being the most frequent (Table 5). *Bacillus* spp. (20%) and *Aspergillus* spp. (17%) were the most common non-ESKAPE pathogens co-cultured from polymicrobial *Enterococcus* infections. Mold and yeast growth was identified in 35% of the polymicrobial infections.

Discussion

Wound infection is a major cause of morbidity and death and a primary concern of the U.S. Military Health System as it pursues better outcomes for personnel injured in combat.

TABLE 4. CHARACTERISTICS OF *ENTEROCOCCUS* ISOLATES CULTURED FROM COMBAT-RELATED EXTREMITY WOUNDS WITHIN ± 3 D OF INFECTION DIAGNOSIS

Characteristic (%)	<i>E. faecium</i> (N=163)	<i>E. faecalis</i> (N=31)	<i>Other Enterococcus spp.</i> ^a (N=54)	Total isolates (N=248)
Species proportion	65.7%	12.5%	21.8%	
Antibiotic resistance				
Vancomycin	1 (0.6)	0	8 (14.8)	9 (3.6)
Beta-lactam (class)	106 (65.0)	1 (3.2)	22 (40.7)	129 (52.0)
Aminoglycoside (class)	24 (14.7)	5 (16.1)	13 (24.1)	42 (16.9)
Hospital-level source				
LRMC	74 (45.4)	11 (35.5)	12 (22.2)	97 (39.1)
U.S. hospitals	89 (54.6)	20 (64.5)	42 (77.8)	151 (60.9)
Anatomic source				
Thigh	82 (50.3)	18 (58.1)	25 (46.3)	125 (50.4)
Lower leg	26 (16.0)	4 (12.9)	12 (22.2)	42 (16.9)
Pelvis	14 (8.6)	0	4 (7.4)	18 (7.3)
Forearm	6 (3.7)	2 (6.5)	3 (5.6)	11 (4.4)
Upper arm	6 (3.7)	1 (3.2)	2 (3.7)	9 (3.6)
Specimen source				
Wound	160 (98.2)	30 (96.8)	53 (98.1)	243 (98.0)
Bone	3 (1.8)	1 (3.2)	1 (1.9)	5 (2.0)
From a polymicrobial infection	142 (87.1)	27 (87.1)	51 (94.4)	220 (88.7)

^aIncludes unspciated *Enterococcus* isolates and one isolate identified as *E. gallinarum*.

LRMC=Landstuhl Regional Medical Center.

TABLE 5. PREVALENCE OF PATHOGENS
IN *ENTEROCOCCUS*-POSITIVE POLYMICROBIAL
COMBAT-RELATED EXTREMITY WOUND INFECTIONS

Species	Infections (%) (N=211) ^a
<i>Enterococcus</i>	211 (100)
<i>E. faecium</i>	156 (73.9)
<i>E. faecalis</i>	36 (17.1)
Other <i>Enterococcus</i> spp. ^b	37 (17.5)
ESKAPE pathogens (non- <i>Enterococcus</i> bacteria)	136 (64.5)
<i>Escherichia coli</i>	60 (28.4)
<i>Enterobacter cloacae</i>	49 (23.2)
<i>Pseudomonas aeruginosa</i>	34 (16.1)
<i>Acinetobacter calcoaceticus</i> <i>baumannii</i> complex	30 (14.2)
Non-cloacae <i>Enterobacter</i> spp. (including <i>E. aerogenes</i>)	6 (2.8)
<i>Klebsiella pneumoniae</i>	4 (1.9)
<i>Staphylococcus aureus</i>	3 (1.4)
Other aerobic gram-negative bacteria	57 (27.0)
<i>Stenotrophomonas maltophilia</i>	13 (6.2)
<i>Aeromonas</i> spp.	12 (5.7)
<i>Pseudomonas</i> spp. (excluding <i>P. aeruginosa</i>)	12 (5.7)
<i>Achromobacter</i> spp.	6 (2.8)
<i>Morganella</i> spp.	6 (2.8)
<i>Serratia marcescens</i>	5 (2.4)
<i>Burkholderia</i> spp.	4 (1.9)
Other <i>Serratia</i> spp.	4 (1.9)
<i>Proteus</i> spp.	3 (1.4)
Other ^c	6 (2.8)
Other aerobic gram-positive bacteria	53 (25.1)
<i>Bacillus</i> spp.	42 (19.9)
<i>Staphylococcus</i> spp. (coagulase-negative)	11 (5.2)
<i>Streptococcus</i> spp.	8 (3.8)
Other ^d	2 (0.9)
Anaerobic bacteria	31 (14.7)
<i>Clostridium</i> spp.	16 (7.6)
<i>Bacteroides</i> spp. (excluding <i>B. fragilis</i>)	6 (2.8)
<i>Prevotella</i> spp.	6 (2.8)
<i>Bacteroides fragilis</i>	4 (1.9)
Other ^e	8 (3.8)
Fungi (includes yeasts and molds)	74 (35.1)
<i>Aspergillus</i> spp.	36 (17.1)
<i>Mucor</i> spp.	14 (6.6)
<i>Penicillium</i> spp.	14 (6.6)
<i>Fusarium</i> spp.	11 (5.2)
<i>Alternaria</i> spp.	6 (2.8)
<i>Candida albicans</i>	5 (2.4)
Other <i>Candida</i> spp.	4 (1.9)
<i>Zygomycetes</i> spp.	4 (1.9)
<i>Scopulariopsis</i> spp.	3 (1.4)
<i>Ulocladium</i> spp.	3 (1.4)
Other ^f	26 (12.3)

^aOrganism categories are the total number of infections with those organisms. Species under the categories are counted individually, so may sum to more than the total for each category. Infections are polymicrobial; they may include multiple microorganisms within each category.

^bIncludes unspecified *Enterococcus* isolates and one isolate identified as *E. gallinarum*.

^cIncludes *Acinetobacter radioresistans*, *Acinetobacter ursingii*, *Chryseobacterium* spp., *Citrobacter* spp., and *Ralstonia* spp.

^dIncludes *Lactobacillus* spp. and *Vagococcus* spp.

^eIncludes *Bifidobacterium* spp., *Eubacterium* spp., *Finexgoldia magna*, *Fusobacterium* spp., *Peptostreptococcus* spp., and *Propionibacterium* spp.

^fIncludes *Absidia* spp., *Arthrographis* spp., *Bipolaris* spp., *Chaetomium* spp., *Geotrichum* spp., *Gliocladium* spp., *Malbranchea* spp., *Mycelia sterilia*, *Pithomyces* spp., *Rhizopus* spp., *Scytalidium* spp., *Trichosporon* spp., and unidentified mold, yeast, and fungus.

Whereas other recent studies of trauma wounds in military personnel generally have focused on the role of antibiotic-resistant gram-negative pathogens or invasive fungal infections [30–34], this study is the first targeted investigation of the incidence of *Enterococcus* in combat wounds. The findings indicate that patients with enterococcal wound infections had patterns of injury similar to those of patients with other wound infections but had elevated ISSs, required a greater volume of blood transfused after injury, and had a longer duration of hospitalization in the United States. Individuals with enterococcal wound infections also had more inpatient infections on average than those in the comparator group and a much higher incidence of polymicrobial wound infection.

Treatment of combat-related trauma is challenging because of the high ISS and is often complicated by the development of infections [4,5,35,36]. Because of the high prevalence of extremity trauma among combat casualties from the wars in Iraq and Afghanistan [37–39], we limited our study to subjects with combat-related extremity injuries. This pattern consists mainly of penetrating soft-tissue injuries (53%), with a large number of fractures also reported (26%) [40]. Furthermore, traumatic amputations are common as a result of explosive blasts, which were the predominant injury mechanism in Afghanistan [41–44]. Both the case and comparator groups in this study included a high proportion of blast-injured subjects with severe and complex injuries to both the upper and lower extremities. Although our study does not break out injuries based on pattern, the incidence of SSTIs and osteomyelitis is consistent with previously reported proportions of infections associated with soft-tissue injuries and fracture [14,36,45].

Despite a lack of targeted studies of the microorganism, *Enterococcus* has been a frequently observed gram-positive genus in wounds sustained during the wars in Iraq and Afghanistan [5,11,12]. One prospective study investigating the contribution of early wound-colonizing organisms to later infecting pathogens in open fractures detected *Enterococcus* in extremity wounds. *Enterococcus* spp. were identified in 8 of 14 wounds and in 6 of 10 patients who required amputation of at least one extremity; however, initial and early wound sampling was not predictive of future infecting pathogens [11]. Combining their findings with those reported here, *Enterococcus* appears to be a common early colonizer in severe wounds that may be capable of surviving alongside other pathogens, resulting in infectious complications. Nevertheless, the role of *Enterococcus* as an opportunistic pathogen or an incidental colonizer in the early stages of wound colonization remains unknown.

According to our characterization, *Enterococcus* is an organism of as much interest to the trauma infection community as other wound pathogens. There are a number of reasons *Enterococcus* deserves more attention. Primarily, there is the presence of commensal *Enterococcus* in the healthy skin and gut microbiome, the incidence of *Enterococcus* in polymicrobial wound infections, and the potential additive or antagonistic impacts of co-colonization of *Enterococcus* and other pathogenic organisms.

The high incidence of polymicrobial wound infections in subjects with enterococcal infections is perhaps the most important finding of this study. Not only is the diagnosis of polymicrobial infections challenging because of the limitations of traditional culture methods, such infections can be difficult to treat, as micro-organisms interact to create biofilms that form physical barriers, making even sensitive

pathogens resistant to antibiotics [46,47]. Animal models of polymicrobial infections have shown amplified proliferation of bacterial cells, impaired wound healing, and greater virulence when two organisms co-infect a wound [48,49]. A retrospective case-control study found that the odds of infection recurrence in war wounds were 70 times greater for patients with polymicrobial infection [50]. The findings of our study emphasize the prevalence of polymicrobial wound infections in combat casualties, particularly in patients with a history of *Enterococcus* colonization and severe extremity injury. This contrasts with studies in the civilian trauma sector, which found much lower rates of polymicrobial wound infection with *Enterococcus* [25]. Further research is warranted to investigate the microbial communities of acute traumatic wounds of both military and civilian populations and to determine potential synergies between pathogenic micro-organisms in trauma wounds.

Determining the impact of *Enterococcus* spp. in polymicrobial wound infections is similarly difficult. There are a number of studies indicating that a mixed infection including *Enterococcus* could be detrimental to wound healing. In *in vivo* models, *Enterococcus* exacerbates infections by the gram-negative pathogens *E. coli* and *P. aeruginosa* [51–53]. In cases of co-infections with *E. coli*, a mechanism has been identified whereby *E. faecalis* constitutively produces a soluble, diffusible metabolite that triggers siderophore expression and increases proliferation of the *E. coli* even under iron-limited conditions, such as those present in a wound [53]. These studies posit that *Enterococcus* strains act as enablers, colonizing a wound early and facilitating infection by other, more virulent organisms. Yet the findings of other analyses have suggested that having *Enterococcus* in a wound might promote better infection outcomes. Specifically, as members of the lactic acid bacterial family, enterococcal species produce a variety of anti-microbial peptides (bacteriocins) with a broad range of activity against other pathogens [54–56]. Strains producing one or more of these peptides have protective, probiotic effects on pathogen control in livestock [57,58]. Moreover, in one study of combat-related wound infections, detection of enteric bacteria (e.g., *Enterococcus*) in a wound had a significant negative correlation with treatment failure [59]. It may be the case that the impact of *Enterococcus* in a wound is dependent on both the strain of the *Enterococcus* and the other organisms present.

In conclusion, the results of our study indicate that for the military trauma population, bacteria of the genus *Enterococcus* infect patients with severe injuries who require ICU admission and large-volume blood transfusions. A significantly longer duration of hospitalization also was observed with the *Enterococcus* case subjects. In addition, subjects with enterococcal infections have a higher incidence of multiple infections, and the majority develop at least one polymicrobial infection. Our study did include a large number of unspeciated enterococci because of inconsistencies among the laboratories regarding their speciation capability; these isolates are now undergoing further examination. Future research will include assessing various strains and lineages of enterococcal isolates with differential patient and infection outcomes, screening isolates for antimicrobial peptide genes and expression, and testing for antagonistic or synergistic interactions of *Enterococcus* with other pathogens (including antibiotic-resistant gram-negative pathogens) in *in-vitro* and *in-vivo* models. The role of *En-*

terococcus in combat-related extremity wounds deserves further investigation, particularly in the case of polymicrobial infections. A better understanding of phenotypic differences in enterococcal strains and bacterial interactions between *Enterococcus* and other pathogens could provide additional tools for infection mitigation in combat-related wound injuries.

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