



## Microbiology of combat-related extremity wounds: Trauma Infectious Disease Outcomes Study☆



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### ABSTRACT

We present extremity wound microbiology data from 250 combat casualties (2009–2012). Confirmed extremity wound infections (EWIs) were based on clinical and laboratory findings. Suspected EWIs had isolation of organisms from wound cultures with associated signs/symptoms not meeting clinical diagnostic criteria. Colonized wounds had organisms isolated without any infection suspicion. A total of 335 confirmed EWIs (131 monomicrobial and 204 polymicrobial) were assessed. Gram-negative bacteria were predominant (57% and 86% of monomicrobial and polymicrobial infections, respectively). In polymicrobial infections, 61% grew only bacteria, while 30% isolated bacteria and mold. Multidrug resistance was observed in 32% of isolates from first monomicrobial EWIs  $\pm$  3 days of diagnosis, while it was 44% of isolates from polymicrobial EWIs. Approximately 96% and 52% of the suspected and colonized wounds, respectively, shared  $\geq$ 1 organism in common with the confirmed EWI on the same patient. Understanding of combat-related EWIs can lead to improvements in combat casualty care.

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## 1. Introduction

Extremity wounds are prevalent among combat casualties and often lead to infectious complications, resulting in significant morbidity and mortality (Brown et al., 2010; Murray, 2008; Murray et al., 2011; Tribble et al., 2011). During the wars in Iraq and Afghanistan, multidrug-resistant organisms (MDROs) were frequently associated with wound colonization and trauma-related infections (Hospenthal et al., 2011; Keen et al., 2010; Murray et al., 2006, 2011; Weintrob et al., 2013), presenting further challenges for clinicians and potentially impacting patient outcomes.

Although microbiology of extremity wounds sustained during combat has been examined (Calhoun et al., 2008; Murray et al., 2006; Petersen et al., 2007; Wallum et al., 2015; Yun et al., 2006), prior studies frequently involved a small population, only considered initial cultures, and did not include information on infections. Understanding the complicated wound microbiome is an essential element of improving trauma

patient care. Therefore, we describe the microbiology of combat-related extremity wounds and wound infections, over a three-year period.

## 2. Methods

### 2.1. Study population and design

Data were collected through the Department of Defense (DoD) – Department of Veterans Affairs Trauma Infectious Disease Outcomes Study (TIDOS) (Tribble et al., 2011), an observational, longitudinal, multicenter study of short- and long-term infectious complications following deployment-related trauma. Patients were eligible for inclusion if they sustained a combat-related extremity injury in Iraq or Afghanistan (June 1, 2009–May 31, 2012) requiring medical evacuation to Landstuhl Regional Medical Center (LRMC) in Germany before transfer to a participating US hospital. The analysis was restricted to patients with organisms collected from wound cultures and retained in the TIDOS microbiological repository. Analysis of confirmed infections was restricted to patients with infections directly matched to a wound site. Participating US hospitals were Walter Reed Army Medical Center and National Naval Medical Center in the National Capital Region (Walter Reed National Military Medical Center after September 2011) and

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Brooke Army Medical Center in San Antonio, Texas. The study was approved by the Institutional Review Board of the Uniformed Services University of the Health Sciences (IRB #351767).

## 2.2. Study definitions

An extremity wound infection (EWI) was defined as ‘confirmed’ if the diagnosis was based on a combination of clinical (e.g., localized signs/symptoms from direct observations) and laboratory findings (e.g., microbiology and diagnostic imaging) and classified in accordance with standardized definitions of skin and soft-tissue infections (SSTI) and osteomyelitis from the National Healthcare Safety Network (Centers for Disease Control and Prevention, 2018). In absence of meeting a priori definition, infections were included if there was a clinical diagnosis (based on physician’s direct observations or clinical judgment) associated with directed antimicrobial treatment continued for  $\geq 5$  days for SSTIs and  $\geq 21$  days for osteomyelitis (infections with shorter antibiotic durations included if surgical cure performed). Infections were excluded from analysis if there was a record of an alternate diagnosis along with discontinuation of antimicrobial treatment. A ‘suspected’ infection was defined as isolation of organisms from wound cultures with associated signs and symptoms; however, it did not meet clinical diagnostic criteria of a wound infection. A third group, ‘colonization’ was defined as having organisms isolated from wounds without either a confirmed or suspected clinical infection.

Multidrug resistance was determined based on standardized definitions (Division on Healthcare Quality Promotion, 2018). Organisms resistant to  $\geq 3$  classes of antibiotics (aminoglycosides, beta-lactams, carbapenems, and fluoroquinolones) or if expressed extended-spectrum beta-lactamases or carbapenemases were classified as multidrug-resistant. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* were considered multidrug-resistant.

Patients in this analysis frequently sustained multiple wounds. Therefore, a patient with a confirmed EWI may have had cultures collected from additional wounds, which grew organisms. These additional wounds were classified as either suspected infections or colonization based on the presence or absence of signs and symptoms of a wound infection as described above.

## 2.3. Data sources and statistical analysis

Information on injury patterns, severity of injury, and intensive care unit admission was obtained from the DoD Trauma Registry (Eastridge et al., 2006). Data on inpatient EWIs and microbiological findings were collected from the supplemental TIDOS infectious disease module (Tribble et al., 2011). Characteristics between patients with confirmed monomicrobial and polymicrobial EWIs were compared using Fisher’s exact test. Statistical analysis was performed with SAS® version 9.3 (SAS, Cary, NC).  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Study population

Between 2009 and 2012, 1409 combat casualties with extremity wounds were admitted to LRMC and transferred to a participating US hospital (Fig. 1). A total of 323 (23% of 1409) patients had a confirmed EWI; however only 250 (77% of 323) patients met criteria of having organisms collected from wound cultures and retained in the TIDOS microbiological repository (60 patients without clinical workups and 13 patients without retained organisms excluded; Tables 1 and 2). In addition, 150 (57% of 263) patients with confirmed EWIs had organisms isolated from another wound with a suspected infection, while 42 (16%) patients had organisms recovered from separate colonized wounds.

Twenty-four patients without confirmed EWIs had organisms recovered from extremity wounds with suspected infections (Table 3), while 18 patients without confirmed EWIs had organisms isolated from cultures of colonized wounds (Table 4).

### 3.2. Confirmed extremity wound infections

Among 250 patients with confirmed EWIs, 89 (36%) had only monomicrobial infections and 161 (64%) had polymicrobial infections for a total of 335 unique infections. Blast was the mechanism of injury for 241 (96%) patients, including all 191 patients with traumatic amputations.

A higher proportion of patients with monomicrobial infections had only one limb with an infected wound and associated microbiology data compared to polymicrobial patients with infections ( $P = 0.008$ ; Table 1). Patients with polymicrobial infections had higher injury severity ( $P = 0.001$ ), more traumatic amputations ( $P = 0.001$ ), and a greater number were admitted to the intensive care unit at LRMC ( $P = 0.046$ ) and US hospitals ( $P = 0.027$ ).

#### 3.2.1. Monomicrobial infections

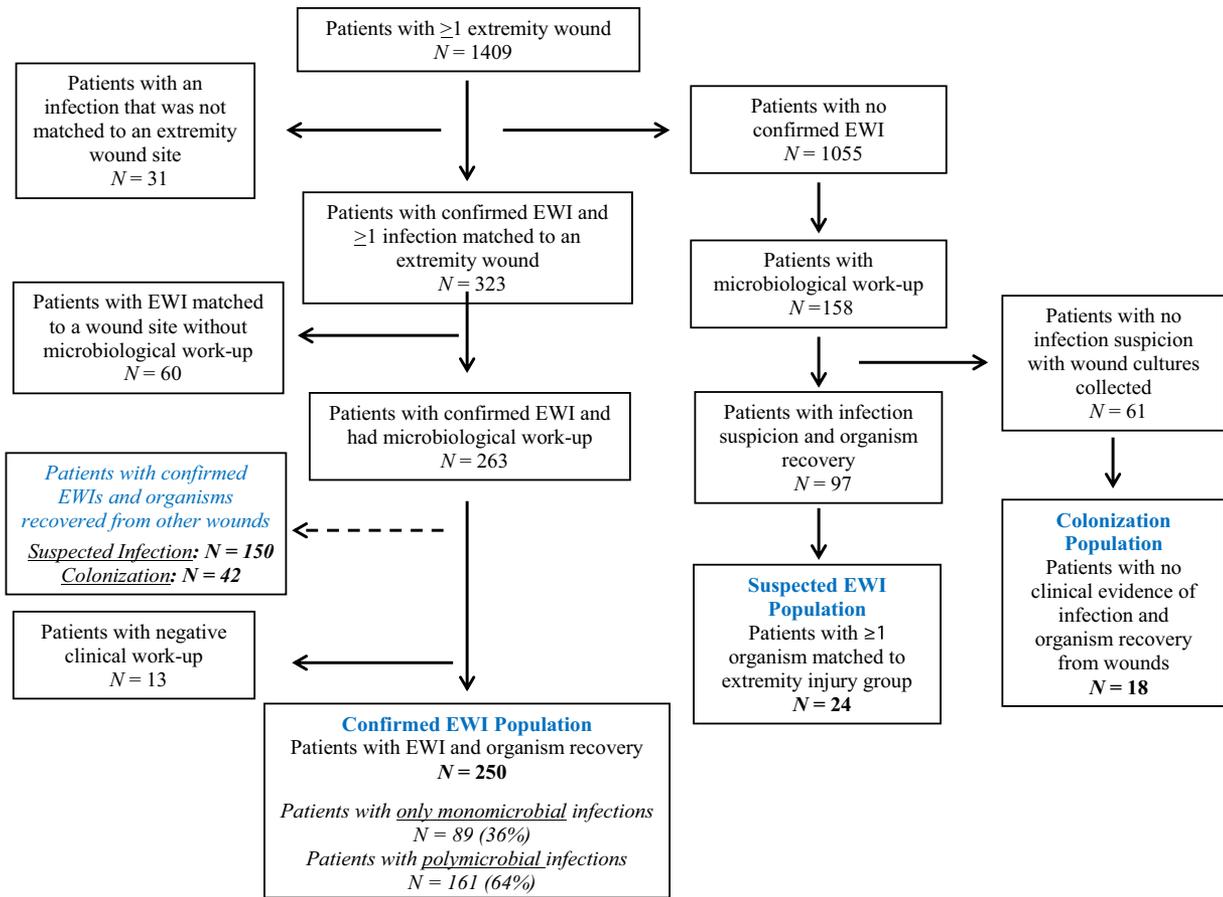
A total of 131 unique monomicrobial infections were identified. The median time from injury to first infection diagnosis was 11 days (Table 2). Recovered isolates were primarily Gram-negative bacteria (57%) with *Acinetobacter* spp. and *Pseudomonas* spp. contributing the largest proportion (28% and 27%, respectively). In addition, 23% of isolates were Gram-positive with *Enterococcus* spp. accounting for the majority (47%), followed by coagulase-negative staphylococci (CNS; 37%).

Seven patients had a monomicrobial infection on two separate injured limbs (second infection diagnosed within 3 days of first infection), of which four patients had organisms in common between the first and second infections (two with *Acinetobacter* spp., one *Enterococcus* spp., and one *Bacteriodes* spp.). Among three patients with a third limb diagnosed with a monomicrobial infection (timing of third infection unspecified), one patient had an *Acinetobacter* spp. in common with the other infections. A single patient had monomicrobial infections in all four limbs (diagnosed same day), involving the same *Acinetobacter* spp. Lastly, six patients had a second infected limb diagnosed  $> 7$  days after initial infection with one patient having an organism in common with the initial EWI.

Among 90 patients with a monomicrobial infection as their first infection, clinical cultures collected after initial diagnosis were examined to assess organism persistence. A total of 105 cultures with organism growth (63 cultures with Gram-negative, 27 Gram-positive, 4 anaerobes, and 11 molds/yeast) were obtained within 3 days of initial infection diagnosis. Among these cultures, 34 (32%) grew MDROs, of which 32 were Gram-negative (51% of 63) and 2 were Gram-positive (7% of 27). Thirty cultures were collected 4–11 days after infection diagnosis and 14 (47%) grew the same organism (7 with Gram-negatives, 5 Gram-positives, 1 anaerobe, and 1 mold), while 6 (20%) identified a different organism (10 cultures negative). Of the 20 cultures with growth, 9 (45%) isolated MDROs (all Gram-negative). Nine cultures were collected 12–19 days post-infection diagnosis with four (44%) being concordant with the initial culture. Concordant cultures grew 3 Gram-negatives and 1 Gram-positive. One culture (11%) grew an organism that was not identified in the initial culture, while four cultures were negative. Of the five cultures that grew organisms during this period, three (60%) isolated MDROs (all Gram-negative).

#### 3.2.2. Polymicrobial infections

A total of 204 unique polymicrobial infections were identified with a median duration of 7 days post-injury to first infection diagnosis (Table 2). Bacteria was the only etiologic agent in 124 infections (61%), followed by bacteria plus mold (30%), bacteria plus yeast (5%), bacteria plus mold and yeast (2%), and mold alone (1%). Similar to monomicrobial infections, Gram-negative bacteria predominated with isolates recovered



**Fig. 1.** Flow diagram of extremity wound infection study population. Confirmed extremity wound infections (EWIs) were based on a combination of clinical and laboratory findings and classified in accordance with standardized definitions from the National Healthcare Safety Network. Patients with confirmed EWIs also had organisms recovered from other wounds (not confirmed to be EWI) with the results being a suspected EWI or colonization. Suspected infections were defined as wound cultures with positive growth, along with signs or symptoms of a wound infection, but did not meet clinical diagnostic criteria. Colonization was defined as organism recovery from wounds without any sign or symptom of infection.

from 86% of infections. *E. coli*, *Enterobacter* spp., and *Pseudomonas* spp. accounted for the majority of isolates (37%, 33%, and 30%, respectively). Gram-positive bacteria were isolated in 72% of polymicrobial infections and *Enterococcus* spp. were most frequent (74%).

Forty patients had  $\geq 2$  injured limbs diagnosed with polymicrobial infections within 3 days of each other, of which 35 (88%) had organisms in common. Specifically, 15 patients had a polymicrobial infection on a second limb that recovered only bacterial organisms with  $\geq 1$  organism in common with the initial infection. Nineteen patients had their second infection involving a combination of bacteria, mold, and/or yeast with  $\geq 1$  concordant organism between the first and second infections. Lastly, one patient with two infected limbs that grew only fungal species had  $\geq 1$  concordant mold. Five patients had a third limb (timing unspecified) with a polymicrobial infection that included  $\geq 1$  organism in common with other infections. Seven patients had a polymicrobial EWI diagnosed in a second limb  $> 7$  days after initial EWI diagnosis with two patients having  $\geq 1$  concordant organism with the initial infection.

Among 157 patients with a polymicrobial infection as their first infection diagnosis, 197 cultures with organism growth were collected within 3 days of diagnosis. *Enterococcus* spp. was the most frequent organism (108 cultures; 55%), while *E. coli* was the most common Gram-negative organism (64 cultures; 32%). Multidrug resistance was identified in 87 cultures (44%), of which 68 and 19 cultures grew multidrug-resistant Gram-negative and Gram-positive organisms, respectively. During the first week after infection diagnosis (4–11 days post-diagnosis), 81 subsequent cultures were collected and 65 (80%) grew a combination of organisms concordant with initial cultures, as well as those that were not previously recovered (16 cultures negative).

Organisms that were concordant were predominantly *E. coli*, *Enterococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., and molds. Approximately half of cultures with growth (33 cultures) identified MDROs (28 with Gram-negative growth, 3 with Gram-positive, and 2 with *Mycobacterium* spp.). On days 12–19 post-infection diagnosis, 25 cultures were collected and 15 (60%) identified organisms concordant with the initial infection along with new organisms (10 cultures negative). Concordant organisms were primarily *Enterococcus* spp., *Acinetobacter* spp., and *Pseudomonas* spp. Of the 15 cultures that grew organisms during this period, 5 (33%) isolated MDROs (all Gram-negative).

### 3.2.3. Suspected infections among patients with confirmed EWIs

Among 263 patients with confirmed EWIs and clinical workups, 150 had organisms recovered from 182 other wounds with suspected infections, resulting in 68 patients with a suspected monomicrobial infection and 114 with a suspected polymicrobial infection (32 patients had both monomicrobial and polymicrobial infections). A total of 142 subjects had organisms recovered from both confirmed EWIs and separate wounds with suspected infections, of which 136 (96%) had  $\geq 1$  organism in common at genus-level. Seventy-one (50%) subjects had the same genus-level microbiological profile between confirmed EWIs and other wounds with suspected infections (26 subjects had only one organism recovered).

A total of 89 monomicrobial cultures were collected from patients with suspected monomicrobial infections from other wounds, which primarily identified Gram-negative bacteria (40 cultures; 45%) with *Acinetobacter* spp. contributing the largest proportion (14 cultures; 35%). Among Gram-positive growth (27 cultures; 30%), *Enterococcus*

**Table 1**  
Characteristics of wounded military personnel with confirmed extremity wound infections, No. (%).<sup>a</sup>

	Patients with only monomicrobial infections (N = 89)	Patients with polymicrobial infections (N = 161)	P-value
Operational Theater			0.293
Iraq	5 (5.6)	4 (2.5)	
Afghanistan	83 (93.3)	149 (92.5)	
Missing	1 (1.1)	8 (5.0)	
Limbs Injured			0.260
1	11 (12.4)	11 (6.8)	
2	23 (25.8)	32 (19.9)	
3	31 (34.8)	67 (41.6)	
4	24 (27.0)	51 (31.7)	
Injured Limbs with Infections and Recovered Isolates			0.008
1	73 (82.0)	105 (65.2)	
2	13 (14.6)	47 (29.2)	
3	2 (2.2)	9 (5.6)	
4	1 (1.1)	0	
Body Region of Injury			0.173
Lower extremity only	69 (77.5)	132 (82.0)	
Lower and upper extremity	7 (7.9)	17 (10.6)	
Upper extremity only	13 (14.6)	12 (7.5)	
Injury Severity Score <sup>b</sup>			0.001
0–9 (mild)	2 (2.2)	3 (1.9)	
10–15 (moderate)	4 (4.5)	9 (5.6)	
16–24 (severe)	27 (30.3)	17 (10.6)	
≥25 (critical)	56 (62.9)	132 (82.0)	
Injury Pattern			0.001
Amputations	57 (64.0)	134 (83.2)	
Open fractures	25 (28.1)	25 (15.5)	
Other wounds	7 (7.9)	2 (1.2)	
Admission to LRMICU	68 (76.4)	139 (86.3)	0.046
Admission to US hospital ICU	55 (61.8)	121 (75.2)	0.027

ICU = intensive care unit; LRMICU = Landstuhl Regional Medical Center.

<sup>a</sup> An extremity wound infection was defined as confirmed if the diagnosis was based on a combination of clinical and laboratory findings and classified in accordance with standardized definitions of skin and soft-tissue infections and osteomyelitis from the National Healthcare Safety Network (Centers for Disease Control and Prevention, 2018). In absence of meeting a priori definition, an infection was included if there was a clinical diagnosis associated with directed antimicrobial treatment.

<sup>b</sup> The Injury Severity Score is an overall measure calculated for each patient based on the top three maximum Abbreviated Injury Scale anatomical region values (Linn, 1995).

spp. and CNS were recovered in 9 cultures (each 33%). Less cultures grew mold and yeast (16 cultures; 18%) and anaerobes (6 cultures; 7%). Isolates were identified a median of 11 days post-injury for Gram-negative organisms, 11 days for mold and yeast, 15 days for Gram-positives, and 33 days for anaerobes.

Regarding suspected polymicrobial infections among patients with confirmed EWIs, 114 cultures were collected, of which 63 (55%) cultures only recovered bacteria, while 32 (28%) were a combination of bacteria and mold, 9 (8%) had bacteria with both yeast and mold, 5 (4%) had bacteria plus yeast, and 5 (4%) only had mold. Among Gram-negative bacteria, *E. coli*, *Pseudomonas* spp., and *Enterobacter* spp. were predominant, while *Enterococcus* spp. was the largest contributor of Gram-positive bacteria. The majority of molds were *Aspergillus* spp., mold from the order Mucorales, and other molds, while it was primarily *Candida* spp. for yeasts. Lastly, *Clostridium* spp. and *Bacteroides* spp. accounted for the majority of anaerobes. Cultures with only bacterial growth were identified a median of 11 days post-injury, while it was 3.5 days for bacteria plus mold, 5 days for mold only, 5 days for bacteria plus mold and yeast, and 16 days for bacteria plus yeast.

### 3.2.4. Colonization among patients with confirmed EWIs

Forty-two patients with confirmed EWIs had organisms recovered from non-infected wounds (colonization), of which 27 and 21 had monomicrobial and polymicrobial colonized wounds, respectively

(6 patients had >1 colonized wound). A total of 31 subjects had organisms recovered from both confirmed EWIs and separate colonized wounds, of which 16 (52%) had ≥1 organism in common at genus-level. Only one subject had the same microbiological profile between the confirmed EWI and colonized wound.

A total of 30 cultures were collected from wounds with monomicrobial colonization and mold and yeast were the largest contributor with 19 cultures (63%), followed by Gram-positive bacteria (5 cultures, 17%), Gram-negative bacteria (3 cultures, 10%), and anaerobes (3 cultures, 10%). Among cultures with mold and yeast, *Aspergillus* spp., mold from the order Mucorales, and other molds were predominant. Gram-positive bacteria included *Enterococcus* spp., *Bacillus* spp., and CNS, while Gram-negative bacteria were *Enterobacter* spp. and *Morganella* spp. Anaerobes recovered from cultures were *Bacteroides* spp. and *Propionibacterium* spp. Isolates were collected a median of 4 days post-injury for Gram-negative bacteria and 3 days for Gram-positive bacteria, anaerobes, mold, and yeast.

Twenty-one cultures collected from colonized wounds were polymicrobial, of which the majority were a combination of bacteria and mold (12 cultures, 57%) followed by only mold (6 cultures, 29%) and only bacteria (3 cultures, 14%). Mold and *Candida* spp. accounted for the largest proportion (18 cultures, 86%) with *Aspergillus* spp. and mold other than from the order Mucorales being the predominant isolates. *Bacillus* spp. and *Enterococcus* spp. were largely recovered from cultures that grew Gram-positive bacteria, while *Enterobacter* spp. was the greatest contributor of Gram-negative bacteria. Only one culture grew an anaerobe (*Clostridium* spp.). Isolates from polymicrobial cultures were identified a median of 2 to 3 days post-injury.

## 4. Discussion

Extremity wounds are the most frequent injury pattern associated with combat-related trauma and often result in infectious complications. Our study found wound infections are predominantly the result of Gram-negative bacteria (57% and 86% of monomicrobial and polymicrobial infections) with *E. coli*, *Enterobacter* spp., *Pseudomonas* spp., and *Acinetobacter* spp. frequently isolated. Gram-positive bacteria were recovered from 72% of polymicrobial confirmed EWIs with *Enterococcus* spp. contributing the majority. While mold/yeast and anaerobes were recovered at a lower proportion (40% and 17% of polymicrobial confirmed EWIs, respectively), they cannot be discounted and warrant consideration. Over half of wounds with infections are polymicrobial with 38% growing a combination of bacteria plus mold and/or yeast.

Although they are less frequent, infections involving mold, Gram-positive bacteria, and anaerobes have been observed among combat casualties. In particular, 6.8% of combat casualties injured between 2009 and 2011 developed an invasive fungal wound infection (IFI). Nonetheless, presence of mold from wound cultures alone is not sufficient to diagnose IFIs. For a wound with mold culture growth to be classified as a possible IFI requires recurrent wound necrosis following ≥2 surgical debridements. Wounds meeting these criteria should also have tissue specimens sent for histopathological analysis to diagnose IFI with a higher degree of certainty (Weintrob et al., 2015). Without the hallmark of recurrent wound necrosis, wounds that grow mold can be considered colonized and do not require antifungal therapy (Rodriguez et al., 2014).

Obligate anaerobes and *Candida* spp. isolates recovered from combat casualties have also been examined. In an analysis of anaerobes (primarily *Bacteroides* spp.) collected from wound cultures from 59 combat casualties, 85% of isolates were recovered from infected wounds. Although 6.8% of patients with anaerobe recovery died, the pathogenic role of anaerobes is uncertain and their occurrence may be a marker of disease severity (White et al., 2016). *Candida* spp. were also isolated from wounds with confirmed EWIs in our analysis, as well as suspected infections and colonization. In a prior study, 131 *Candida* spp. isolates

**Table 2**  
Confirmed monomicrobial and polymicrobial extremity wound infections<sup>a</sup>.

	Monomicrobial infections <sup>b</sup>		Polymicrobial infections <sup>c</sup>	
	Number of cultures (%)	Median days from injury to first infection (min-max)	Number of cultures (%)	Median days from injury to first infection (min-max)
<b>Total unique infections</b>	<b>131</b>	<b>11 (1–136)</b>	<b>204</b>	<b>7 (1–120)</b>
<b>Gram-negative bacteria</b>	<b>75 (57.3)</b>	<b>14 (4–47)</b>	<b>175 (85.8)</b>	<b>7 (1–120)</b>
<i>Acinetobacter</i> spp.	21 (28.0)	8 (5–28)	36 (20.6)	13 (2–44)
<i>Enterobacter</i> spp.	12 (16.0)	17.5 (7–34)	57 (32.6)	4 (1–42)
<i>Escherichia coli</i>	9 (12.0)	14 (5–47)	64 (36.6)	8 (1–33)
<i>Klebsiella</i> spp.	1 (1.3)	10 (10–10)	6 (3.4)	13.5 (4–27)
<i>Pseudomonas</i> spp.	20 (26.7)	14.5 (4–33)	52 (29.7)	7 (2–120)
Other Gram-negatives	12 (16.0)	24.5 (5–36)	59 (33.7)	6 (1–42)
<b>Gram-positive bacteria</b>	<b>30 (22.9)</b>	<b>7.5 (1–136)</b>	<b>146 (71.6)</b>	<b>6 (1–120)</b>
Coagulase-negative staphylococci	11 (36.7)	17 (4–69)	17 (11.6)	4 (1–31)
<i>Enterococcus</i> spp.	14 (46.7)	6 (1–18)	108 (74.0)	6 (2–44)
<i>Staphylococcus aureus</i>	2 (6.7)	76 (16–136)	7 (4.8)	11 (2–120)
Other Gram-positives	3 (10.0)	9 (5–27)	55 (37.7)	3 (1–22)
<b>Mold or yeast</b>	<b>20 (15.3)</b>	<b>10.5 (3–65)</b>	<b>80 (39.2)</b>	<b>5 (1–43)</b>
<i>Aspergillus</i> spp.	4 (20.0)	8 (7–30)	26 (32.5)	4.5 (1–19)
<i>Candida</i> spp.	3 (15.0)	11 (11–65)	15 (18.8)	12 (2–43)
Order Mucorales	4 (20.0)	8 (5–17)	19 (23.8)	5 (2–15)
Other mold	9 (45.0)	11 (3–31)	46 (57.5)	4.5 (1–21)
Other yeast	0	NA	2 (2.5)	5.5 (4–7)
<b>Anaerobes</b>	<b>6 (4.6)</b>	<b>5.5 (5–10)</b>	<b>35 (17.2)</b>	<b>4 (2–32)</b>
<i>Bacteroides</i> spp.	2 (33.3)	5 (5–5)	14 (40.0)	4.5 (3–32)
<i>Clostridium</i> spp.	2 (33.3)	5.5 (5–6)	16 (45.7)	3 (2–11)
Other anaerobes	2 (33.3)	9 (8–10)	13 (37.1)	6 (2–18)

<sup>a</sup> An extremity wound infection was defined as confirmed if the diagnosis was based on a combination of clinical and laboratory findings and classified in accordance with standardized definitions of skin and soft-tissue infections and osteomyelitis from the National Healthcare Safety Network (Centers for Disease Control and Prevention, 2018). In absence of meeting a priori definition, an infection was included if there was a clinical diagnosis associated with directed antimicrobial treatment. Data are presented from first recorded confirmed extremity wound infection.

<sup>b</sup> Blast trauma patients contributed 126 monomicrobial infections (96% of 131). All *Enterococcus* spp., *S. aureus*, *E. coli*, *Klebsiella* spp., anaerobes, mold, and *Candida* spp. isolates recovered from monomicrobial infections were from blast wounds.

<sup>c</sup> Polymicrobial infections have more than one organism identified so the values sum to greater than the total number of infections. Blast trauma patients contributed 200 polymicrobial infections (98% of 204). All coagulase-negative staphylococci, *E. coli*, *Klebsiella* spp., *Bacteroides* spp., mold, and yeast isolates were collected from patients with blast trauma.

recovered from combat casualties were evaluated and included 37 (28%) isolates collected from wound cultures, of which 86% were associated with infections. As there was no significant association between

*Candida* isolation and mortality, it is presumably that *Candida* spp. isolation resulted from frequent culture collection due to high injury severity and was not an indication of pathogenicity (Blyth et al., 2014).

**Table 3**  
Suspected monomicrobial and polymicrobial extremity wound infections<sup>a,b</sup>.

	Suspected wound infection cultures, No.	
	Only monomicrobial <sup>c</sup>	Polymicrobial <sup>d</sup>
<b>Total positive cultures</b>	<b>22</b>	<b>8</b>
<b>Gram-negative bacteria</b>		
<i>Achromobacter</i> spp.	0	1
<i>Acinetobacter</i> spp.	3	4
<i>Alicigenes denitrificans</i>	1	0
<i>Chryseobacterium</i> spp.	2	1
<i>Enterobacter</i> spp.	1	1
<i>Pseudomonas</i> spp.	2	2
<b>Gram-positive bacteria</b>		
<i>Bacillus</i> spp.	0	1
Coagulase-negative staphylococci	10	0
<i>Enterococcus</i> spp.	0	1
<b>Mold</b>		
<i>Aspergillus</i> spp.	1	1
Order mucorales	0	1
Other mold	2	5

<sup>a</sup> A suspected infection was defined as isolation of organisms from wound cultures with associated signs and symptoms; however, it did not meet clinical diagnostic criteria of a wound infection.

<sup>b</sup> Total is 24 patients with suspected extremity wound infections (18 with monomicrobial cultures and 8 with polymicrobial cultures; 2 patients had more than one suspected EWI). Twenty-three (96%) patients injured were via a blast (17 with monomicrobial wounds and 8 with polymicrobial wounds). Only *Alcaligenes denitrificans* and one *Pseudomonas* spp. isolate were recovered from non-blast wounds.

<sup>c</sup> Median time from injury to culture was 3 days for patients who grew mold, 15 days for Gram-positive bacteria, and 17 days for Gram-negative bacteria.

<sup>d</sup> Polymicrobial cultures have more than one organism identified so the values sum to greater than the total number of cultures. Median time from injury to culture was 27 days for patients who grew bacteria alone, 6 days for bacteria plus mold, and 3 days for mold alone.

**Table 4**  
Cultures from non-infected extremity wounds with colonization<sup>a,b</sup>.

	Colonized wound cultures, No.	
	Only monomicrobial <sup>c</sup>	Polymicrobial <sup>d</sup>
<b>Total positive cultures</b>	<b>11</b>	<b>8</b>
<b>Gram-negative bacteria</b>		
<i>Acinetobacter</i> spp.	1	3
<i>Enterobacter</i> spp.	0	2
<i>Escherichia coli</i>	1	2
<i>Pseudomonas</i> spp.	1	0
<i>Serratia</i> spp.	0	1
<b>Gram-positive bacteria</b>		
<i>Bacillus</i> spp.	0	1
Coagulase-negative staphylococci	2	1
<i>Enterococcus</i> spp.	1	2
<i>Peptostreptococcus</i> spp.	0	3
<b>Mold or yeast</b>		
<i>Aspergillus</i> spp.	2	1
<i>Candida</i> spp.	1	0
Other mold	2	5
Other yeast	0	1

<sup>a</sup> Colonization was defined as having organisms isolated from wounds without either a confirmed or suspected clinical infection.

<sup>b</sup> Total is 18 patients with colonized wounds (11 with monomicrobial cultures and 8 with polymicrobial cultures; one patient had more than one colonized wound). Fourteen (78%) patients injured were via a blast (7 with monomicrobial wounds and 7 with polymicrobial wounds). All mold and yeast, as well as all Gram-positive bacteria from polymicrobial wounds, were recovered from blast wounds.

<sup>c</sup> Median time from injury to culture was 9 days for patients who grew Gram-negative bacteria, 5 days for Gram-positive bacteria, and 3 days for mold and *Candida* spp.

<sup>d</sup> Polymicrobial cultures have more than one organism identified so the values sum to greater than the total number of cultures. Median time from injury to culture was 6 days for bacteria only, 4.5 days for mold only, 3 days for bacteria plus mold, and 46 days for bacteria plus yeast.

The large proportion of polymicrobial wounds in our study (64% of patients with confirmed EWIs) is consistent with prior reports of combat extremity trauma (Petersen et al., 2007; Wallum et al., 2015). Specifically, an assessment of 14 mangled lower extremities that underwent amputation found that wounds were polymicrobial and frequently grew both Gram-negatives and Gram-positives with a smaller proportion identifying mold and/or yeast. The Gram-negative bacteria were generally *Pseudomonas* spp., while *Enterococcus* spp., represented the majority of Gram-positive isolates (Wallum et al., 2015). An additional study found that *Enterococcus* spp. were the highest bacterial contributor to polymicrobial SSTI and fungal-infected wounds from blast trauma (Warkentien et al., 2015). Polymicrobial infections was also frequently observed in an analysis of patients with enterococcal wound infections (Heitkamp et al., 2018). Although *E. coli* and *Enterobacter* spp. were the primary Gram-negative organisms identified from polymicrobial confirmed EWIs in our study, *Pseudomonas* spp. still contributed a high proportion. Moreover, over 70% of cultures collected from polymicrobial wounds grew Gram-positive bacteria, which were predominantly *Enterococcus* spp.

The microbiological profile of confirmed and suspected EWIs from the same patient were similar (96% with  $\geq 1$  organism in common) with *Acinetobacter* spp., *E. coli*, *Pseudomonas* spp., CNS, and *Enterococcus* spp. as the predominant organisms. While patients in the suspected EWI and colonization populations (N = 24 and 18, respectively) did not have anaerobe growth, anaerobes were isolated from wounds of confirmed EWI patients (both confirmed infections and wounds with suspected infections and colonization). Unlike confirmed and suspected EWIs, cultures collected from colonized wounds were largely monomicrobial (58%) and primarily identified molds; however, bacteria (*Enterococcus* spp. and *Acinetobacter* spp.) were still recovered.

Our findings are comparable with prior investigations of wound microbiology. In an analysis of civilian trauma patients injured in an earthquake in China, wound infection cultures primarily recovered *E. coli*, *Enterococcus* spp., *Acinetobacter baumannii*, and *S. aureus* (Zhang et al., 2012), which was similar to the bacterial distribution from infected wounds in our study. Regarding combat-related wounds, an examination of extremity wounds (predominantly lower extremities) found that *A. baumannii* accounted for 63% of identified isolates followed by *Enterococcus faecium* (12%) and *E. coli* (7%). Among cultures collected during the first week post-injury, 7.7% were polymicrobial compared to 2.9% and 2.0% in the second and third weeks. *A. baumannii* remained the largest bacterial contributor across 3 weeks, while *E. faecium* increased during the second week and decreased in the third. In contrast, *E. coli* decreased in the second week, but rose in the third (Sheppard et al., 2010). Our analysis found a high proportion of *Acinetobacter* spp. recovered from patients with confirmed and suspected EWIs (both monomicrobial and polymicrobial cultures). Furthermore, 22% and 20% of cultures with *E. coli* and *Enterococcus* spp. identified in the initial polymicrobial EWI had the same organisms recovered a week later.

Our study does have limitations that should be considered. Our population consisted of severely injured combat casualties (76% of patients had traumatic amputations); therefore, the wound microbiology data may not be generalizable to all wounded personnel. Furthermore, we do not have a way of definitively determining which organisms isolated in polymicrobial wounds were the true pathogen.

#### 4.1. Conclusions

Gram-negative bacteria are predominant in both monomicrobial and polymicrobial confirmed EWIs, while Gram-positive bacteria also substantially contributed to polymicrobial EWIs. Combat casualties with confirmed EWIs often had additional colonized wounds or wounds with suspected infections. The majority of suspected and colonized wounds shared  $\geq 1$  organism in common with the confirmed EWI on the same subject. Overall, the results of our study confirm the complexity of combat-related wounds with cultures frequently growing

multiple bacteria, as well as anaerobes, mold, and yeast. Describing the microbiome of these combat extremity wounds leads to a better understanding of the complexity of and infection burden, which will result in improved wound management and infection outcomes in future conflicts by applying appropriate wound care and antimicrobial therapies. Furthermore, infection control is an important component of patient care to prevent nosocomial transmission of pathogens. Knowledge of the wound microbiome will lead to improvements in infection control strategies, which may result in less infectious complications and the prevention of nosocomial transmission. Future investigations will focus on outcomes related to specific microbiology, particularly *Enterococcus* spp., and risk factors for wound infection.

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#### Conflicts of Interest

None.

#### Disclaimer

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