MAJOR ARTICLE

Resistance Patterns and Clinical Significance of *Candida* Colonization and Infection in Combat-Related Injured Patients From Iraq and Afghanistan

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Background. Penetrating wounds with environmental contamination are associated with a range of infectious complications, including fungus. This is the first study to examine the epidemiology, resistance patterns, and outcomes of *Candida* infections and colonization in United States military patients injured in Iraq and Afghanistan.

Methods. Clinical information associated with initial unique and serial *Candida* isolates collected from patients (June 2009–October 2013) through the Trauma Infectious Disease Outcomes Study (TIDOS) was evaluated. Susceptibilities were performed using Sensititre YeastOne (YO-9) plates and interpreted by Clinical Laboratory and Standards Institute (CLSI) and adjusted-European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

Results. The analysis included 127 patients with 131 unique *Candida* isolates, of which 102 were *Candida albicans* and 29 non-*albicans Candida* spp. Overall, 99% of patients were male with a median age of 23 and an injury severity score of 22. Injuries were primarily due to blasts (77%) and sustained among personnel serving in Afghanistan (89%). There was a median of 7 days from injury to *Candida* isolation, and 74 isolates were associated with infection. In the multivariate analysis, non-*albicans Candida* spp were associated with prior antifungal exposure, blood isolates, and wound isolates ($P < .01$). Nonsusceptibility by CLSI and EUCAST criteria was associated with non-*albicans Candida* spp ($P < .05$). Patients with *Candida* isolation had a 7.1% mortality rate, compared with 1.4% from the overall TIDOS population.

Conclusions. *Candida* isolation from patients with penetrating war injuries may identify a population at higher risk for death. Prospective studies are needed to determine whether targeted antifungals and surgical management will affect this mortality rate.

Keywords. antifungal resistance; *Candida*; combat-related trauma.

Introduction

Natural disasters and manmade bombings have been associated with both multidrug-resistant (MDR) bacterial infections and invasive mold wound infections (MWIs) [1–7]. The risk factors and role of *Candida* spp colonization and infection in these patients with penetrating trauma (eg, open wound contaminated by environmental and/or organic debris) is unclear. Prior studies have shown high rates of candidemia after a bomb explosion in a market [8] and *Candida* wound infections

Received 10 October 2014; accepted 12 November 2014.

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Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofu109

complicating cluster bomb injuries [9]. Among trauma patients with penetrating injuries related to natural disasters, rates of wound infections with *Candida* may be as high as 20% [1].

In recent years, there has also been a move towards harmonizing Clinical Laboratory and Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) antifungal susceptibility testing (AST) [10]; however, differences in procedures and interpretative breakpoints remain [11–13]. Commercial AST is increasingly used in clinical laboratories, and performance has been variably studied against the new CLSI and EUCAST breakpoints [14, 15]. There are also increasing concerns regarding changes in *Candida* spp distributions, resistance, and correlation of breakpoints with clinical outcomes.

In this study, we sought to identify clinical characteristics and outcomes associated with *Candida* spp colonization and infection in deployment-related injured personnel in Iraq and Afghanistan. We also examined *Candida* spp distributions and resistance patterns according to CLSI and EUCAST breakpoints.

METHODS

Study Population and Definitions

The Trauma Infectious Disease Outcomes Study (TIDOS) was implemented on June 1, 2009. All patients with isolation of *Candida* spp from initiation of TIDOS to October 26, 2013 were included in the analysis. The TIDOS eligibility criteria have previously been described and include active duty personnel or Department of Defense beneficiaries ≥18 years who are injured during deployment requiring evacuation to Landstuhl Regional Medical Center (LRMC) in Germany and ultimately transferring to a participating clinical site in the United States [16]. Trauma history, clinical characteristics on admission, course, and outcomes were obtained retrospectively from the TIDOS database. The study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences in Bethesda, Maryland.

Infectious disease events were classified, as previously described, by a combination of clinical findings, laboratory tests, clinical diagnosis, and/or initiation of directed antimicrobial therapy for ≥5 days [16]. Cultures were performed at the discretion of clinical providers. Isolates associated with infections were collected as part of clinical infection work-ups, whereas isolates were considered to be colonizers if they were specimens obtained for purposes other than infection work-up (eg, surveillance). Susceptibility testing was performed by each hospital's microbiology laboratory and interpreted by CLSI criteria. The TIDOS database was queried for information on concomitant bacterial and MWI within the cohort. Infections, presence of MDR bacteria, and MWI are defined systematically within the TIDOS database [3, 5, 16, 17].

Combat-related injuries were characterized using both Injury Severity Score (ISS) [18] and Abbreviated Injury Scale 2005-Military (AIS) [19, 20]. Wound AIS was defined as the AIS score for the area of a wound or intra-abdominal culture.

Candida Species Isolate Analysis

As part of TIDOS, after identification and susceptibility testing per standard procedures at clinical sites, bacterial and yeast isolates are archived for future study at −80°C. We used all initial unique and serial (≥7 days between same species) *Candida* isolates. Archived isolates were passaged twice on sabouraud dextrose agar before further testing. The BD Phoenix Automated Microbiology System (BD Diagnostics, Sparks, MD) was used to confirm or determine *Candida* species as necessary. Sensititre YeastOne (YO-9) (TREK Diagnostic Systems, Cleveland, OH) plates were used for broth microdilution susceptibility testing [14, 21–23]. Antifungal agents analyzed included anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole, and amphotericin B.

For CLSI interpretations, AST for *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis* were interpreted according to M27-S4 breakpoints [11]. *Candida* spp breakpoints not specifically addressed in M27-S4 were determined according to M27-S3 breakpoints [24]. Amphotericin susceptibility was determined by M27-A3 breakpoints [25]. The EUCAST breakpoints were determined by EUCAST Antifungal Agents Breakpoint Tables for Interpretation of Minimal Inhibitory Concentrations (MIC), version 6.1 [26].

Although EUCAST methods to determine MIC results are intended to yield results that are concordant with CLSI procedures, [12] differences in methodology between AST procedures yield lower breakpoints for anidulafungin and micafungin by EUCAST compared with CLSI [27]. Sensititre YeastOne may also yield higher MICs for select antifungals than the EUCAST method [28], so our MIC results were normalized against the modal MIC and ranges provided by EUCAST rationale documents [27]. The modal MIC and MIC ranges obtained by the Sensititre YeastOne were consistent with those obtained by EUCAST methods for all antifungals tested except anidulafungin and posaconazole [29], which were adjusted as appropriate with breakpoints established 2 dilutions above the respective modal MICs. These modifications yielded the adjusted-EUCAST interpretations (AEIs).

Statistical Analysis

Univariate analysis included χ^2 and Fisher's exact test for categorical variables, as appropriate, and Mann-Whitney *U* for continuous variables. A *P* value of <.05 was used as a significant cutoff. Multivariate analysis was completed with logistic regression of pertinent significant risk factors from univariate analysis. Antifungal susceptibility by CLSI and EUCAST were not

included in multivariate analysis because they were correlated with non-*albicans Candida* species. Statistical analyses were performed using SPSS software (IBM SPSS Statistics Version 19, Chicago, IL).

RESULTS

Overall Demographics and Injury Patterns

During this time period, 5694 trauma patient evacuations occurred through LRMC, and 2567 patients were transferred to TIDOS-participating clinical facilities in the United States. Of these patients, 127 (5%) had *Candida* species for study inclusion. The median age of the patients was 23 years and 99% were male (Table 1). The majority of injuries were due to blasts (71% related to improvised explosive devices) and predominantly were sustained in Afghanistan (89%). The median ISS at LRMC was 22, indicating high injury severity. Eight patients had burn injuries (median total body surface area 18%), and 3 patients had associated inhalational injuries. There were 26 patients with a diagnosis of MDR infection and 25 with MWI.

Table 1. Demographic Characteristics and Injury Circumstances, Number (%) of Military Trauma Patients (*N* = 127) with *Candida* spp Infections and Colonization

Characteristic	Patients
Male gender	126 (99)
Age, median (min-max)	23 (19–45)
Mechanism of injury	
IED blast	90 (71)
Gunshot wound	22 (17)
Non-IED blast	8 (6)
Other	7 (6)
Burn injury	8 (6)
Total body surface area %, median (min-max)	18 (1–45)
Inhalational injury	3 (2)
ISS at LRMC, median (min-max)	22 (8–66)
Wound AIS, median (min-max)[a]	5 (2–5)
Location of initial hospitalization	
Southern Afghanistan	73 (57)
Eastern Afghanistan	41 (32)
Iraq	7 (6)
Other	6 (5)
Facility at initial presentation	
Mobile medical unit within combat zone	26 (20)
Hospital within combat zone	100 (79)
LRMC	1 (1)
Mortality during initial hospitalization	9 (7)

Abbreviations: AIS, abbreviated injury scale; IED, improvised explosive device; ISS, injury severity score; LRMC, Landstuhl Regional Medical Center.

[a] The number of patients with available wound AIS is 35.

Clinical Findings and Outcomes by *Candida* Isolates

Of the 127 patients, there were 131 unique *Candida* isolates. One hundred two unique isolates were *C albicans* and 29 were non-*albicans Candida* spp (Table 2). Seventy-four isolates were associated with infection and 57 were colonizers. The majority of both *C albicans* and non-*albicans Candida* spp were collected from personnel injured in Afghanistan (90% and 86%, respectively). Furthermore, the majority of isolates were collected at institutions within the United States (55%) compared with 45% at LRMC.

Five candidemic patients had prior antifungal exposure with a median of 3 days preceding isolation of *Candida* spp. Thirty-one percent of non-*albicans Candida* isolates and 16% of *C albicans* isolates were from patients who also had MWI. Median time from injury to *Candida* isolation was 6 days for *C albicans* and 14 days for non-*albicans Candida*. Pulmonary and blood isolates were isolated a median of 3 and 6 days after injury, respectively, whereas the remaining sources were a median 8–12 days after injury. All isolate sources had a median ISS of 21–24, except intra-abdominal isolates, which had a median ISS of 34.

The 29 non-*albicans Candida* isolates included 10 *C tropicalis*, 7 *C glabrata*, 6 *C parapsilosis,* 2 *Candida dubliniensis*, 2 *Candida lusitaniae*, and 1 each of *Candida kefyr* and *Candida pelliculosa.* Isolation of non-*albicans Candida* spp was associated with prior antifungal exposure, blood isolates, and wound isolates in the multivariate analysis ($P < .01$). Only 7 patients had recurrent *Candida* of the same species cultured ≥7 days after initial isolation (3 serial wound isolates, 1 blood then wound, 1 serial intra-abdominal, 1 wound then blood, and 1 intra-abdominal then wound). Regarding serial isolates, 6 patients had *C albicans* and 1 patient had *C parapsilosis*. All patients with serial isolates had antifungal exposure between cultures with a median duration of 8 days for echinocandins, 11 for fluconazole, 5 for voriconazole, and 3 for amphotericin. The patient with recurrent *C parapsilosis* had 10 days of echinocandin between isolates and was also the only patient to have *Candida* spp isolated from a wound prior to blood. There was no increased resistance in second isolates.

All-cause mortality during initial hospitalization was 7.1% in patients with *Candida* isolation compared with 1.4% in the overall TIDOS population. There were 10 unique *Candida* isolates associated with the 9 deaths (Table 3). Review of clinical records for these patients did not identify *Candida* as a cause of death by autopsy or death certificate. Prior combination antifungal exposure was noted in 7% of *Candida* isolates associated with survival compared with 30% of *Candida* isolates associated with all-cause mortality ($P < .05$). In addition, 50% and 16% of isolates associated with death and survival, respectively, were from patients with gunshot wounds ($P < .05$). Overall, mortality was not significantly associated with MWI, MDR, *Candida* infection, and either CLSI or AEI nonsusceptibility.

Table 2. Clinical Characteristics, Number (%) of Military Trauma Patients With *Candida albicans* Versus Non-*albicans Candida* Isolates

Characteristic	*C albicans* (*n* = 102)	*Candida* Non-*albicans* (*n* = 29)	Univariate Analysis[a] *P* Value	Multivariate Analysis[a] *P* Value
Age, median (min-max)	23 (19–45)	26 (19–42)	.05	NA
Mechanism of injury			.25	NA
IED blast	71 (70)	21 (72)		
Gunshot wound	19 (19)	5 (17)		
Non-IED blast	8 (8)	0		
Other	4 (4)	3 (10)		
ISS, median (min-max)	22 (8–50)	24 (10–66)	.40	NA
Wound AIS, median (min-max)[b]	4 (2–5)	5 (2–5)	.42	NA
Location of initial hospitalization			.40	NA
Southern Afghanistan	54 (53)	19 (66)		NA
Eastern Afghanistan	38 (37)	6 (21)		NA
Iraq	6 (6)	2 (7)		NA
Other	4 (3)	2 (7)		NA
Mold wound infection	16 (16)	9 (31)	.06	.38
Days from injury to culture, median (min-max)	6 (1–66)	14 (2–127)	<.01	.49
Facility where cultures were collected			<.01	.92
Landstuhl Regional Medical Center	54 (53)	5 (17)		
United States clinical site	48 (47)	24 (83)		
Source of isolate				
Blood[c]	9 (9)	8 (28)	<.01	<.01
Wound	20 (20)	17 (59)	<.01	<.01
Respiratory	59 (58)	4 (14)	<.01	.33
Intra-abdominal	5 (5)	0	.59	NA
Other	9 (9)	0	.21	NA
Clinically diagnosed infection[d]	52 (51)	22 (76)	<.05	.10
Blood	9 (17)	7 (32)		
Wound	18 (35)	14 (64)		
Respiratory	16 (31)	1 (5)		
Intra-abdominal	4 (8)	0		
Other	5 (10)	0		
Prior antifungal exposure	9 (9)	14 (48)	<.01	<.01
Any nonsusceptibility by CLSI	2 (2)	4 (14)	<.05	NA
Any nonsusceptibility by AEI	0	18 (62)	<.01	NA
Mortality during initial hospitalization	7 (7)	3 (10)	.69	NA

Abbreviations: AEI, adjusted-European Committee on Antimicrobial Susceptibility Testing; AIS, abbreviated injury scale; CLSI, Clinical and Laboratory Standards Institute; IED, improvised explosive device; ISS, injury severity score; max, maximum; min, minimum; NA, not applicable.

[a] The univariate and multivariate analyses compare the data of the *C albicans* isolates to the *C* non-*albicans*.

[b] The number of isolates related to wound AIS was 19 and 16 for *C albicans* and *C* non-*albicans*, respectively.

[c] Fifteen of 16 blood isolates were identified as blood stream infections. One isolate identified from a catheter tip culture.

[d] Infectious disease events were classified by a combination of clinical findings, laboratory tests, clinical diagnosis, and/or initiation of directed antimicrobial therapy for ≥5 days [16]. *Candida* spp were isolated during the infection event.

Susceptibility of *Candida* Isolates

Two percent of *C albicans* and 14% of non-*albicans Candida* isolates were noted to be nonsusceptible to at least 1 antifungal by CLSI criteria, whereas 0% and 62%, respectively, were nonsusceptible to 1 or more antifungals by AEI criteria. *Candida albicans* isolates were universally susceptible to micafungin, anidulafungin, voriconazole, fluconazole, and amphotericin by both interpretations (Table 4). Moreover, all *C albicans* isolates were susceptible to caspofungin and itraconazole by CLSI and posaconazole by AEI.

Nonsusceptibility by CLSI was associated with non-*albicans Candida* spp ($P < .05$). By AEI breakpoints, 14% of isolates were nonsusceptible (Table 5). In the multivariate analysis, only non-*albicans Candida* spp remained significantly associated with

Table 3. Clinical Characteristics, Number (%) Among Patients With *Candida* Isolation by Outcome

Characteristic	Survival ($n = 121$)	Death ($n = 10$)	*P* Value
Age, median (min-max)	23 (19–45)	23 (21–41)	.84
Mechanism of injury			.04
IED blast	88 (73)	4 (40)	.06
Gunshot wound	19 (16)	5 (50)	.02
Non-IED blast	8 (7)	0	1.00
Other	6 (5)	1 (10)	.43
ISS, median (min-max)	22 (8–51)	26 (14–66)	.11
Wound AIS, median (min-max)[a]	5 (2–5)	5 (4–5)	.87
Site of wound isolates			.004
Leg	12 (34)	1 (50)	
Arm	4 (11)	0	
Abdomen	2 (6)	0	
Chest/back	0	1 (50)	
Pelvis/groin	9 (26)	0	
Face/head	4 (11)	0	
Other	4 (11)	0	
Location of initial hospitalization			.72
Southern Afghanistan	67 (55)	6 (60)	
Eastern Afghanistan	40 (33)	4 (40)	
Iraq	8 (7)	0	
Other	6 (5)	0	
Mold wound infection	23 (19)	2 (20)	1.00
MDR bacterial infection	24 (20)	3 (30)	.43
Non-*albicans* species	27 (22)	2 (20)	.69
Source of isolate			.16
Blood	14 (12)	3 (30)	
Wound	35 (29)	2 (20)	
Respiratory	60 (49)	3 (30)	
Intra-abdominal	5 (4)	0	
Other	7 (6)	2 (20)	
Clinical evidence of infection	69 (57)	5 (50)	1.00
Prior antifungal exposure	20 (16)	3 (30)	.38
Prior combination antifungal exposure	8 (7)	3 (30)	.04
Any nonsusceptibility by CLSI	5 (4)	1 (10)	.39
Any nonsusceptibility by AEI	16 (13)	2 (20)	.41

Abbreviations: AEI, adjusted-European Committee on Antimicrobial Susceptibility Testing; AIS, abbreviated injury scale; CLSI, Clinical and Laboratory Standards Institute; IED, improvised explosive device; ISS, injury severity score; MDR, multidrug-resistant; max, maximum; min, minimum.

[a] The number of isolates related to wound AIS was 35 and 3 for the survival and mortality categories, respectively.

AEI nonsusceptibility (Table 6). Nonsusceptibility by CLSI, AEI, and discordance between CLSI and AEI interpretations were not associated with death or *Candida* infection.

Of the 6 isolates noted to have nonsusceptibility by CLSI breakpoints, none were associated with mortality or serial isolation. In addition, none of the resistant isolates by CLSI were treated with antifungal monotherapy to which they were not susceptible. Only 2 isolates not associated with infection were treated with echinocandin monotherapy for more than 1 day. These were also the only echinocandin nonsusceptible isolates associated with death.

DISCUSSION

Natural disasters and manmade bombing injuries can be complicated by MDR bacterial infections and MWI. Previous studies have primarily focused on mold infections related to necrotizing cutaneous mucormycosis after the Joplin tornado [6] and MWI complicating combat-related injuries in military personnel from Afghanistan [3–5]. These studies did not specifically address *Candida* spp, despite their frequent isolation from these complicated wounds [3]. Our data reveal that *Candida* isolation is common in combat-related injured personnel and may be reflective of a population at higher risk for death. Although *C albicans* isolation was not associated with resistance in this population, non-*albicans Candida* spp were associated with decreased susceptibility and may be linked with sources more commonly related to infection, such as blood and wounds.

Studies have shown that 5% of deployment-related infections involve *Candida* spp [30], as well as representing the second-most common organism in positive blood cultures in a population of veterans of the recent conflicts [31]. A civilian population of mostly blunt trauma with complicated postoperative courses had rates of *Candida* colonization and infection of 36.6% and 6.1%, respectively [32]. The applicability to complex, penetrating war wounds sustained via blasts is uncertain. Microbiological studies after penetrating injuries during natural disasters commonly isolate *Candida* spp, which in some cases account for almost one fifth of positive cultures [1, 2, 33]. These infections may be underrecognized because they can appear clinically similar to bacterial infections [34].

In our patient cohort, the most frequent source of *Candida* isolation associated with infection was wounds, likely due to the predominance of blast injuries (77%). Wounds were also frequently associated with non-*albicans Candida* spp. Although *Candida* spp are typically considered to be from nosocomial or colonizing sources, there is some question of traumatic inoculation in penetrating injuries. In the MWI cohort, traumatic inoculation of multiple mold species occurred, and 9% of patients also had *C albicans* isolated from wound cultures [3]. A prospective study of 350 patients with blast and fragment injuries after a cluster munitions explosion had a 13.2% rate of *Candida* and mold infections despite weekly fluconazole prophylaxis. The timing of *Candida* isolation was not included, but 10% of patients had late cultures [9]. Another series of patients sustaining non-gastrointestinal injuries after a bomb blast in a crowded marketplace had a 30% rate of candidemia ~12 days after injury

Table 4. *Candida albicans* First Isolates MIC_{50}, MIC_{90}, and CLSI Versus AEI Susceptibility Interpretations to Commonly Used Antifungals (*N* = 102)

					CLSI Interpretation		AEI Interpretation	
Antifungal	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Minimum	Maximum	%Susceptible	%Resistant	%Susceptible	%Resistant
Anidulafungin	0.03	0.06	≤0.015	0.12	100	0	100	0
Micafungin	≤0.008	0.015	≤0.008	0.015	100	0	100	0
Caspofungin	0.06	0.06	0.015	0.12	100	0	NE	NE
Posaconazole	0.015	0.03	≤0.008	0.06	NE	NE	100	0
Voriconazole	≤0.008	0.015	≤0.008	0.06	100	0	100	0
Itraconazole	0.06	0.12	≤0.015	0.12	100	0	NE	NE
Fluconazole	0.05	1	0.25	2	100	0	100	0
5-Flucytosine	0.12	1	<0.06	>64	98	2	NE	NE
Amphotericin	1	1	0.05	1	100	0	100	0

Abbreviations: AEI, adjusted-European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; MIC_{50}, minimum inhibitory concentration to inhibit growth of 50% of organisms; MIC_{90}, minimum inhibitory concentration to inhibit growth of 90% of organisms; NE, not established.

without evidence of preceding *Candida* mucosal colonization. Although there was a higher percentage of total-body surface burn area involvement in candidemic compared with noncandidemic patients, a multivariate analysis found that inhalational injury was the best predictor for candidemia [8]. Other studies have shown that although only fungal wound infection (including both yeasts and molds) is associated with increased mortality in burn patients, fungal wound colonization precedes infection in 40% of cases [35]. Moreover, the number of sites colonized with *Candida* spp has a direct correlation with subsequent risk for candidemia [36]. In contrast, few patients in this series had *Candida*-associated burn injuries, and no patients with *Candida* spp isolated from pulmonary sources had the associated inhalational injury.

In our analysis, we found a 20% rate of MWI and MDR bacterial infections among patients with *Candida*. With environmental contamination of wounds, subsequent infections are often polymicrobial and affected by the conditions of contamination [1–3]. Superinfected wounds after the 2004 tsunami, Marmara earthquake, and Joplin tornado had high rates of MDR bacteria [1, 2, 7]. Whether these were associated with environmental sources or emergency healthcare settings is unclear [1]. After a tornado in Lubbock, Texas, wound infections had 4.6 species per wound in hospitalized patients. Fungal cultures from these patients yielded 3 unspeciated yeasts, 1 *Rhodotorula*, and 8 molds [33]. In the Joplin tornado cohort with *Apophysomyces trapeziformis*-necrotizing wound infections, both pediatric patients and more than half of incident wounds in adults also had *Candida* spp isolated [6, 7]. Although there are data to support environmental sources of wound infection isolates, delayed recovery and resistance patterns of some later isolates also point to the possibility of low initial inoculum, or, more likely, nosocomial sources. More recent studies have reflected the ongoing evolution of microbiology related to both wound infections and colonization as patients are evacuated through echelons of care, emphasizing the importance of infection control, judicious antimicrobial use, and continued microbiological reevaluation with changing clinical status in this severely injured population [37].

Wounded military personnel with *Candida* infection and colonization had a median ISS of 22 and a mortality rate of 7.1%. This result is similar to the median ISS of 20 and crude mortality of 7.8% seen with combat-related MWI [3, 4] and significantly higher than the 1.4% mortality within the overall TIDOS cohort during our study period and the mean ISS of 7.8 from 2003 to 2009 [30]. Although there was no clinical evidence of mortality due to *Candida* infection within this population, the high ISS and mortality rate reflect the severity of injuries associated with *Candida* colonization and infection. This increased injury severity could also have led to more frequent culture obtainment and, thus, increased *Candida* recovery.

Although some studies have shown no difference in mortality in the presence of *Candida* colonization and infection in trauma patients [32], a meta-analysis showed decreased overall and attributable mortality to *Candida* infections with azole prophylaxis in intensive care unit trauma patients [38]. Nonetheless, these studies had heterogeneous populations with high rates of confounding risk factors and primarily blunt-trauma injuries. With prior antifungal exposure associated with non-*albicans Candida* isolation and its decreased antifungal susceptibility, and no evidence of mortality from *Candida* or serial isolation, it is difficult to recommend antifungal prophylaxis in those suffering penetrating trauma. The high rate of MDR bacterial and MWI coexistence in patients with *Candida* wound involvement may favor a strong role for surgical debridement [1–3, 6, 7, 33].

There is concern regarding increasing resistance within *Candida* spp. Overall, the MIC distribution of our first isolates

Table 5. In Vitro Susceptibilities as Determined by Sensititre YeastOne Antifungal Plate by CLSI, and AEI Interpretations for Most Frequently Isolated *Candida* Non-*albicans* Species

		Minimum Inhibitory Concentration (μg/mL)													CLSI Interpretation (%)				AEI Interpretation (%)			
Species	Antifungal	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	64	128	S	SDD	I	R	S	SDD	I	R
C tropicalis (n = 10)	Anidulafungin	NA		3		6			1				NA	NA	90		0	10	90			10
	Micafungin		1	8				1					NA	NA	90		0	10	NE	NE	NE	NE
	Caspofungin			4	5			1					NA	NA	90		0	10	NE	NE	NE	NE
	Posaconazole				1	5	3	1					NA	NA	NE	NE	NE	NE	60			40
	Voriconazole				5	4	1						NA	NA	90	10		0	90			10
	Itraconazole	NA				2	6	2					NA	NA	20	80		0	NE	NE	NE	NE
	Fluconazole	NA	NA	NA	NA				5	3	1	1			80	10		10	80		10	10
	Amphotericin	NA	NA	NA	NA				10				NA	NA	100			0	100			0
C glabrata (n = 7)	Anidulafungin	NA	1[†]	4	1	1							NA	NA	100		0	0	100			0
	Micafungin		7										NA	NA	100		0	0	100			0
	Caspofungin			1	5	1							NA	NA	100		0	0	NE	NE	NE	NE
	Posaconazole								5			2*	NA	NA	NE	NE	NE	NE	NE	NE	NE	NE
	Voriconazole					3	2			2			NA	NA	NE	NE	NE	NE	NE	NE	NE	NE
	Itraconazole	NA						5			2		NA	NA	0	71		29	NE	NE	NE	NE
	Fluconazole	NA	NA	NA	NA							5	1	1		71		29	0		71	29
	Amphotericin	NA	NA	NA	NA				7				NA	NA	100			0	100			0
C parapsilosis (n = 6)	Anidulafungin	NA								6			NA	NA	100		0	0	0		100	0
	Micafungin								4	2			NA	NA	100		0	0	0		100	0
	Caspofungin							6					NA	NA	100		0	0	NE	NE	NE	NE
	Posaconazole		2	4									NA	NA	NE	NE	NE	NE	100			0
	Voriconazole	4	2										NA	NA	100	0		0	100			0
	Itraconazole	NA		1	2	3							NA	NA	100	0		0	NE	NE	NE	NE
	Fluconazole	NA	NA	NA	NA			1	5						100	0		0	100		0	0
	Amphotericin	NA	NA	NA	NA				6				NA	NA	100			0	100			0

Abbreviations: AEI, adjusted-European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; I, intermediate; MIC, minimum inhibitory concentration; NA, not applicable; NE, not established; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

*MIC >8 μg/mL.

[†] MIC ≤0.015 μg/mL.

Table 6. Clinical Characteristics, Number (%) of CLSI Versus AEI Susceptible and Resistant Isolates

Characteristic	CLSI Interpretation		AEI Interpretation	
	Nonsusceptible (*n* = 6)	Susceptible (*n* = 125)	Nonsusceptible (*n* = 18)	Susceptible (*n* = 113)
Age, median (min-max)	23 (19–32)	23 (19–45)	26 (19–42)	23 (19–45)
Mechanism of Injury				
IED blast	5 (83)	87 (70)	13 (72)	79 (70)
Gunshot wound	1 (17)	23 (18)	4 (22)	20 (18)
Non-IED blast	0	8 (6)	0	8 (7)
Other	0	7 (6)	1 (6)	6 (5)
ISS, median (min-max)	22 (8–66)	29 (14–50)	19.5 (10–50)	24 (8–66)
Wound, AIS median (min-max)[a]	4 (2–5)	5 (2–5)	4.5 (2–5)	5 (2–5)
Wound site	2 (33)	35 (28)	12 (67)	25 (22)
Leg	0	13 (37)	6 (50)	7 (28)
Arm	0	4 (11)	1 (8)	3 (12)
Abdomen	1 (50)	1 (3)	2 (16)	0
Chest/back	0	1 (3)	1 (8)	0
Pelvis/groin	0	9 (26)	1 (8)	8 (32)
Face/head	1 (50)	3 (9)	1 (8)	3 (12)
Other	0	4 (11)	0	4 (16)
Location of initial hospitalization				
Southern Afghanistan	4 (67)	69 (55)	12 (67)	61 (54)
Eastern Afghanistan	1 (17)	43 (34)	4 (22)	40 (35)
Iraq	0	8 (6)	1 (6)	7 (6)
Other	1 (17)	5 (4)	1 (6)	5 (4)
Mold wound infection	1 (17)	24 (19)	7 (39)	18 (16)[††]
Non-*albicans* species	4 (67)[†]	25 (20)[†]	18 (100)	11 (10)[††]*
Source of isolate				
Blood	2 (33)	15 (12)	5 (28)	12 (11)[††]
Wound	2 (33)	35 (28)	12 (67)	25 (22)[††]
Respiratory	2 (33)	61 (49)	1 (6)	62 (55)[††]
Intra-abdominal	0	5 (4)	0	5 (4)
Other	0	9 (7)	0	9 (8)
Clinically diagnosed infection	4 (67)	70 (56)	14 (78)	60 (53)
Prior antifungal exposure	2 (33)	17 (14)	8 (44)	15 (13)[††]
Death	1 (17)	8 (6)	2 (11)	8 (7)
Days from culture to death, median (min-max)[b]	10 (10–10)	4.5 (0–71)	17 (17–17)	4.5 (0–71)
Days from injury to death, median (min-max)[b]	19 (19–19)	14.5 (1–76)	36 (29–43)	14.5 (1–76)

Abbreviations: AEI, adjusted-European Committee on Antimicrobial Susceptibility Testing; AIS, abbreviated injury scale; CLSI, Clinical and Laboratory Standards Institute; IED, improvised explosive device; ISS, injury severity score; max, maximum; min, minimum; MWI, mold wound infection.

[†] *P* value <.05 in univariate analysis.

[††] *P* value <.01 in univariate analysis.

* *P* value <.05 in multivariate analysis for AEI only (included MWI Y/N, Non-*albicans* Y/N, antifungal before, source of isolate including blood, wound, and respiratory).

[a] The number of isolates related to wound AIS was 2 and 33 for the CLSI nonsusceptible and susceptible, respectively, and 12 and 23 for AEI nonsusceptible and susceptible, respectively.

[b] The number of isolates related to days from culture/injury to death was 1 and 8 for the CLSI interpretations of nonsusceptible and susceptible, respectively, and 2 and 8 for the AEI interpretations.

matches published epidemiological cutoff values [10]. *Candida albicans* isolates were highly susceptible by both CLSI and AEI criteria. We did not find that antifungal exposure was associated with *C albicans* resistance. This may be related to our lack of resistant *C albicans* isolates, few serial isolates with prolonged, targeted antifungals, or increased non-*albicans Candida* spp recovered after antifungal exposure. Antifungal exposure was associated with nonsusceptibility within non-*albicans Candida* spp.

Although prior studies have shown a direct relationship between infection-related mortality and rising antifungal MICs [10, 15, 39, 40], we did not find an association between antifungal nonsusceptibility and serial isolation, infection, or death. In our population, very few patients with infection were treated with antifungal monotherapy to which the isolate was nonsusceptible. These patients did not fare worse; however, there are multiple limitations of this study that could affect our ability to detect a difference. These include the small number of nonsusceptible isolates [39] and application of established criteria defining pneumonia [16] used in the TIDOS project, which do not require histopathology to more specifically attribute *Candida* isolates from respiratory specimens to actual infection. This predefined criteria led to identifying 17 of 63 pulmonary isolates as associated with infection. In general, these findings support continued correlation of AST with clinical outcomes.

Overall, our data from combat-related injured military personnel support prior clinical and microbiologic studies of fungal traumatic inoculations. In this population, *Candida* isolation is common and may be reflective of a population at higher risk for infections with MDR bacteria, MWI, and death. There was little resistance in *Candida* isolates. Nonetheless, non-*albicans Candida* spp were more likely to be isolated from clinically significant sites such as blood and wounds. These isolates were associated with prior antifungal therapy and, of most immediate clinical concern, decreased susceptibility to antifungals. Without greater numbers of resistant *Candida* isolates associated with infection, the significance of targeted antifungal therapy and/or surgical interventions remains unclear. Further studies focusing on isolates associated with infection, including more resistant isolates, are needed to determine the clinical significance and appropriate management of these infections.

Acknowledgments

We are indebted to the Infectious Disease Clinical Research Program TIDOS study team of clinical coordinators, microbiology technicians, data managers, clinical site managers, and administrative support personnel for their tireless hours to ensure the success of this project.

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Financial support. This work (the Trauma Infectious Disease Outcomes Study; IDCRP-024) was supported by the Infectious Disease Clinical Research Program, a Department of Defense program executed through the Uniformed Services University of the Health Sciences. This project was been funded by the National Institute of Allergy and Infectious Diseases, National Institute of Health (Inter-Agency Agreement Y1-AI-5072) and the Department of the Navy under the Wounded, Ill, and Injured Program (HU001-10-1-0014).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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