2016 INTERNATIONAL STATE-OF-THE-SCIENCE MEETING
MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES
LITERATURE REVIEW
Executive Summary

To inform the 2016 International State-of-the-Science Meeting, the United States Department of Defense Blast Injury Research Program Coordinating Office requested a review of recent research literature directed at minimizing the impact of wound infections following blast-related injuries. This literature review addresses specific research questions about: 1) predictive risk factors of wound infection following blast-related injuries; 2) identification of candidate biomarkers to advance wound infection diagnosis capabilities; and 3) emerging prevention and treatment strategies, including vaccines, in an era of antimicrobial resistance.

Wound infection following blast-related injuries continues to be a significant source of morbidity and mortality in the modern era of military healthcare. Approximately a quarter of combat wounds become infected, having significant impact on patient outcomes and healthcare costs. Several studies report increasing rates of nosocomial infections as patients experience prolonged hospitalization and progress through higher echelons of care. Additionally, combat wound infections due to drug-resistant or multidrug-resistant organisms have increased in military personnel that served in Iraq and Afghanistan.

Risk factors associated with combat wound infection include injury characteristics, such as mechanism of injury, severity of injury, and region of injury. Environmental characteristics and healthcare-associated exposures, such as blood transfusions, medical implants, and delayed antibiotic treatment, also contribute to increasing risk of infection. Improved approaches to diagnose and detect infection would promote better prediction of infection, earlier diagnosis, earlier treatment application, individually-tailored treatments, and improved understanding of the epidemiology of wound infection.

While clinical practice guidelines are in place to guide detection and diagnosis of wound infection, and provide recommendations for post-injury antimicrobials and antifungals, debridement and irrigation, surgical wound management, and facility infection control measures for implementation from prehospital field care to regional Level IV hospitals; limited information is available about specific diagnostic capabilities across military treatment facilities. Development of novel objective biomarkers would enable faster and more precise wound infection diagnosis capabilities. National and international researchers from government, private, and non-profit organizations are seeking to develop novel infection biomarker approaches, including proteins and enzymes, proteomic analysis, metabolomics, next-generation sequencing, biofilm detection, electrochemical sensors, intelligent wound dressings, and digital microscopy.

In addition, these organizations are collaborating to develop new prevention and treatment approaches as alternatives to antimicrobials, including vaccines, passive immunological therapy, phage therapy, antimicrobial peptides, photodynamic therapy, quorum sensing, nanoparticles, iron chelators, lectin inhibitors, FimH inhibitors, lactoferrin, hypothiocyanite, bioengineered tissue, bacterial gene transfer, probiotics, and plant compounds.
Challenges posed by the provision of healthcare in austere environments, increasing nosocomial transmission, and the emergence of drug-resistant infection present capability gaps in the mission to minimize wound infection following blast-related injury. To bridge these gaps, experts have identified various research needs in three areas. First, basic science studies designed to achieve a better understanding of physiological processes including the pathophysiology of infection and the host immune response to infection, the association between biofilms and infection, and the mechanism of action for existing antibiotics and immunoprotection. Secondly, studies focused on the military healthcare system including continued epidemiological assessment of bacterial and fungal infection, assessment of the availability and use of diagnostic techniques for wound infection, and assessment of the delivery of antimicrobials following injury and subsequent infection rates. Third, studies advancing the development of novel products or methods enabling new diagnosis, prevention, and treatment approaches including biomarkers including biofilm detection methods, new vaccine candidates, and improved animal models that more accurately reflect clinical wound infection.

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as official Department of the Army position, policy, or decision.
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**Purpose**

The mission of the United States (US) Department of Defense (DoD) Blast Injury Research Program Coordinating Office (PCO) is to assist in fulfilling the DoD Executive Agent responsibilities and functions related to medical research to prevent, mitigate, and treat blast injuries in accordance with DoD Directive 6025.21E. The Blast Injury Research PCO coordinates and manages relevant DoD medical research efforts and programs, including identifying blast injury knowledge gaps, shaping medical research programs to fill identified gaps, facilitating collaboration among diverse communities within and outside the DoD, and disseminating blast injury research information.

To achieve these objectives, the Blast Injury Research PCO convenes an annual International State-of-the-Science (SoS) Meeting to assist in identifying knowledge gaps pertaining to key blast injury issues. These annual SoS meetings are highly focused to help determine what is known and unknown about particular blast injury topics. The topic of the 2016 International SoS Meeting is Minimizing the Impact of Wound Infections Following Blast-Related Injuries.

To inform the 2016 SoS Meeting, the Blast Injury Research PCO requested a literature review about minimizing the impact of wound infections following blast-related injuries. The literature review focuses on evidence from clinical and laboratory research aimed at developing prediction, identification, prevention, and treatment strategies for minimizing the impact of wound infections following blast-related injuries. The literature review seeks to address the following research questions:

1. What risk factors contribute to acquisition or persistence of wound infections following blast-related injuries?
2. What are current and potential biomarker approaches for identification of wound infections following blast-related injuries?
3. What are emerging prevention and treatment strategies for wound infections following blast-related injuries?
   a. What candidate vaccine approaches have the potential to immunize against wound infections following blast-related injuries?
   b. What treatment strategies have the potential to counteract the impact of drug-resistant pathogens in wound infections following blast-related injuries?
Methodology

This literature review searched PubMed, the Defense Technical Information Center (DTIC), Google, and Google Scholar using search terms (see Appendix 1) to identify English language clinical and basic science articles published in the last 10 years (between 2006 and 2016, inclusive). The DTIC documents selected were limited to those assigned for public distribution (Distribution A). Identified articles published prior to 2006 were included in the literature review only if they were determined to be critical to addressing the research questions or understanding the topic.

Search terms were generated in collaboration with the Blast Injury Research PCO and the 2016 SoS Meeting Planning Committee. In addition to the search terms listed in Appendix 1, ad hoc searches on key principal investigators or specific topics were performed. Publications identified in the bibliographies of reviewed articles were also considered for this literature review. Table 1 lists the search inclusion and exclusion criteria for this literature review.

Table 1. Literature Search Inclusion and Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. English language articles only</td>
<td>1. Articles not directly addressing research questions</td>
</tr>
<tr>
<td>2. Articles published between 2006 and 2016 (inclusive)*</td>
<td>2. DTIC documents not approved for public release</td>
</tr>
<tr>
<td>3. Clinical and animal model studies</td>
<td></td>
</tr>
<tr>
<td>4. DTIC documents assigned Distribution A:</td>
<td></td>
</tr>
<tr>
<td>Approved for public release: distribution unlimited</td>
<td></td>
</tr>
</tbody>
</table>

* Older publications were included when potentially critical to addressing the research questions or understanding the topic.

Articles meeting the inclusion criteria were further reviewed to determine whether they directly informed the research questions and merited inclusion in the literature review. Articles were reviewed for the following elements:

- Study design
- Study population
- Outcome measures
- Results and statistics
- Conclusions, study limitations, and recommendations

Following this strategy, the literature search yielded 557 references that met the parameters of the search terms and inclusion/exclusion criteria (Table 1). This literature review report includes a total of 345 references that directly informed the research questions and merited inclusion.
Epidemiology

The infectious organisms encountered in the management of war wound infection has changed from World War I to modern conflicts due to advances in treatment such as surgical wound debridement and the use of antibiotics (Aronson, Sanders, & Moran, 2006). Despite advances in treatment, wound infection remains a significant source of morbidity and mortality for US military Service Members who survive combat-related injuries (Eardley, Brown, Bonner, Green, & Clasper, 2011), including those sustained during Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) (Blyth, Yun, Tribble, & Murray, 2015; Hospenthal & Murray, 2011; Murray, 2008a, 2008b). For example, infection is the most common cause of death in military and civilian burn patients (Gomez et al., 2009; Murray, Loo, et al., 2008). Invasive fungal infections (IFIs) have also recently been associated with a mortality rate of 7.8 percent in combat trauma patients (Weintrob et al., 2015).

Military healthcare experts have initiated large-scale studies utilizing medical records from the Department of Defense Trauma Registry (DoDTR), formerly the Joint Theater Trauma Registry (JTTR), to investigate infection-related complications (Murray et al., 2009; Murray, Wilkins, et al., 2011), including the incidence of post-hospitalization infections (Tribble, Conger, et al., 2011). Understanding the epidemiology of wound infection is critical to reduce the incidence and impact of infections following blast-related injuries.

Wound Infection Rates

Studies of war- or blast-related wound infection report infection rates that vary between pathogen, injury type, study population, infection outcome measure, and geographic region. A recent review of 21 studies (Table 2) conducted in Middle East conflict zones reported war-related infection rates from 4.9 percent to 78 percent (Sahli, Bizri, & Abu-Sittah, 2016). These studies included non-blast wounds, non-US military populations, and civilian populations in Iraq, Syria, Israel, and Lebanon. The most commonly reported infection-causing pathogens from these studies were Pseudomonas aeruginosa, Acinetobacter baumannii-calcoaceticus complex (ABC), and Staphylococcus aureus.
Table 2. Reports Describing the Microbiology of War Wound Infections in the Middle East

<table>
<thead>
<tr>
<th>Military/Civilian</th>
<th>Study sample</th>
<th>Site</th>
<th>Infection rate</th>
<th>Outcome</th>
<th>Most common organism</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iraq</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>British military</td>
<td>48</td>
<td>Open femur fractures</td>
<td>8%</td>
<td>4% underwent amputation</td>
<td>S. aureus</td>
<td>Bennett, Sargeant, Myatt, &amp; Penn-Barwell, 2015</td>
</tr>
<tr>
<td>US military</td>
<td>300</td>
<td>Lower extremity amputations</td>
<td>27%</td>
<td>53% underwent reoperation</td>
<td>not stated</td>
<td>Tintle et al., 2014</td>
</tr>
<tr>
<td>British military</td>
<td>182</td>
<td>Chest</td>
<td>43%</td>
<td>4.9% overall mortality</td>
<td>not stated</td>
<td>Senanayake, Poon, Graham, &amp; Midwinter, 2014</td>
</tr>
<tr>
<td>US military</td>
<td>192</td>
<td>Diaphyseal tibia fractures</td>
<td>27%</td>
<td>22% underwent amputation</td>
<td>ABC (surveillance) S. aureus (infected)</td>
<td>Burns et al., 2012</td>
</tr>
<tr>
<td>US military</td>
<td>16,742</td>
<td>Variable</td>
<td>5.5%</td>
<td>0.6% overall mortality</td>
<td>Gram negatives</td>
<td>Murray, Wilkins, et al., 2011</td>
</tr>
<tr>
<td>Civilian</td>
<td>137</td>
<td>Chronic osteomyelitis</td>
<td>78%</td>
<td>not stated</td>
<td>S. aureus</td>
<td>Murphy et al., 2011</td>
</tr>
<tr>
<td>Military and civilian</td>
<td>211</td>
<td>Variable</td>
<td>26.5%</td>
<td>3.57% mortality among infected</td>
<td>ABC</td>
<td>Petersen et al., 2007</td>
</tr>
<tr>
<td>US military</td>
<td>49</td>
<td>Variable</td>
<td>49%</td>
<td>not stated</td>
<td>Coagulase-negative staphylococci</td>
<td>Murray et al., 2006</td>
</tr>
<tr>
<td>Syria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civilian</td>
<td>100</td>
<td>Variable</td>
<td>12%</td>
<td>2% overall mortality</td>
<td>not stated</td>
<td>Biswas et al., 2016</td>
</tr>
<tr>
<td>Military and civilian</td>
<td>66</td>
<td>Cranial trauma</td>
<td>10.6%</td>
<td>4.5% overall mortality</td>
<td>not stated</td>
<td>Barhoum et al., 2015</td>
</tr>
<tr>
<td>Military and civilian</td>
<td>345</td>
<td>Variable</td>
<td>18%</td>
<td>not stated</td>
<td>P. aeruginosa</td>
<td>Teicher et al., 2014</td>
</tr>
<tr>
<td>Israel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civilian</td>
<td>21</td>
<td>Variable</td>
<td>30%</td>
<td>43% mortality rate</td>
<td>Candida</td>
<td>Wolf et al., 2000</td>
</tr>
<tr>
<td>Military and civilian</td>
<td>142</td>
<td>Chest trauma</td>
<td>4.9%</td>
<td>not stated</td>
<td>not stated</td>
<td>Romanoff, 1975</td>
</tr>
<tr>
<td>Military</td>
<td>624</td>
<td>Variable</td>
<td>12.5%</td>
<td>6 cases of bacterial sepsis</td>
<td>P. aeruginosa</td>
<td>Klein, Berger, &amp; Yekuti, 1975</td>
</tr>
<tr>
<td>Lebanon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Military and civilian</td>
<td>350</td>
<td>Total body cluster munitions</td>
<td>19.4%</td>
<td>not stated</td>
<td>P. aeruginosa</td>
<td>Fares, El-Zaatari, Fares, Bedrosian, &amp; Yared, 2013</td>
</tr>
<tr>
<td>Military and civilian</td>
<td>1021</td>
<td>Head and neck injuries</td>
<td>12%</td>
<td>not stated</td>
<td>S. aureus</td>
<td>Zaytoun, Shikhani, &amp; Salman, 1986</td>
</tr>
</tbody>
</table>

*ABC, Acinetobacter baumannii-calcoaceticus complex*

Adapted from Sahli 2016
Studies of wound infection in US military populations also report a range of wound infection rates across multiple variables. Military healthcare experts have generally estimated that about 25 percent of combat wounds subsequently become infected (Hospenthal & Murray, 2011). A recent comparison of combat-related wound infection during Vietnam and OEF/OIF (Blyth et al., 2015) reviewed multiple studies documenting infection rates in US military populations (Table 3). Several recent studies of wound infection during OEF/OIF draw from the DoDTR/JTTR, which was developed to study and improve outcomes after battlefield injury (Eastridge et al., 2009, 2010; Eastridge, Jenkins, Flaherty, Schiller, & Holcomb, 2006). An analysis of over 16,000 deployment-related DoDTR/JTTR injury records found that 5.5 percent of patients had one or more infections (Murray, Wilkins, et al., 2011). The authors noted that this figure was likely an underrepresentation of the actual infection rate, given the limitations of the DoDTR/JTTR.
### Table 3. Studies of Aerobic Bacteria Isolated From Wound Cultures Before or Shortly After Initial Debridement of War Wounds

<table>
<thead>
<tr>
<th>Wound Culture Results Before Surgical Intervention</th>
<th>Wound Culture Results on Follow-up Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OIF</td>
</tr>
<tr>
<td>Reference</td>
<td>Murray et al., 2006</td>
</tr>
<tr>
<td>No. Patients</td>
<td>49</td>
</tr>
<tr>
<td>No. wounds evaluated</td>
<td>61</td>
</tr>
<tr>
<td>No. bacteria isolated</td>
<td>37</td>
</tr>
<tr>
<td>Timing of culture</td>
<td>Within ED</td>
</tr>
<tr>
<td>Gram positive, %</td>
<td>93</td>
</tr>
<tr>
<td>S. aureus, %</td>
<td>11</td>
</tr>
<tr>
<td>Coagulase-negative Staphylococcus species, %</td>
<td>86</td>
</tr>
<tr>
<td>Enterococcus species, %</td>
<td>0</td>
</tr>
<tr>
<td>Gram negative, %</td>
<td>7</td>
</tr>
<tr>
<td>P. aeruginosa, %</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacter baumannii complex, %</td>
<td>0</td>
</tr>
<tr>
<td>E. coli, %</td>
<td>33</td>
</tr>
<tr>
<td>Proteus species, %</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacter species, %</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella species, %</td>
<td>0</td>
</tr>
<tr>
<td>Serratia marcescens, %</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cultures from mangled lower extremity injuries only.

** Cultures from open tibial fractures only.

‡ Cultures from biopsy of open wounds being treated with vacuum-assisted wound closure devices.

ED: emergency department; Level V, tertiary care hospital within the continental United States

Adapted from Blyth 2015
Another analysis of DoDTR/JTTR medical records of 192 trauma patients across three Level V military treatment facilities (MTFs; Table 4) found an overall incidence of infection of 26.6 percent (Tribble, Conger, et al., 2011); within this cohort, 14.8 percent of ward patients and 50 percent of intensive care unit (ICU) patients had infections. Furthermore, in a study of severe open tibia fractures treated at the San Antonio Military Medical Center, 27 percent of wound cultures were positive for infection (Burns et al., 2012). In addition, the rate of culture-positive wound infection was 46 percent for combat-related calcaneal fractures treated at Walter Reed National Military Medical Center between March 2003 and August 2010 (Dickens et al., 2013).

Table 4. Levels of Care for Injured Military Personnel

<table>
<thead>
<tr>
<th>Role 1/Level I</th>
<th>Role 2/Level II</th>
<th>Role 3/Level III</th>
<th>Role 4/Level IV</th>
<th>Role 5/Level V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Buddy/Self care or Battalion aid station</td>
<td>Forward resuscitative surgical system</td>
<td>Deployed inpatient hospital</td>
<td>Fixed hospital outside theater of operations</td>
</tr>
<tr>
<td>Purpose</td>
<td>Immediate first aid delivered at the scene</td>
<td>Resuscitation and stabilization, inpatient care for &lt; 72 hours</td>
<td>Restoration of functional health, stabilization for evacuation, full inpatient care</td>
<td>Definitive medical and surgical care outside the combat zone, ability to return Service Members to duty</td>
</tr>
<tr>
<td>Capabilities</td>
<td>Initial treatment of nuclear, biological, and chemical injuries; treatment of toxic industrial material exposure; primary disease prevention; and combat stress control measures</td>
<td>Basic primary care, laboratory, surgery, x-ray, optometry, dental, stress control, and mental health capabilities</td>
<td>Preoperative diagnostics, intensive surgical and critical care, and microbiology laboratory</td>
<td>Full tertiary care hospital and intensive rehabilitation</td>
</tr>
</tbody>
</table>

Adapted from: Petersen et al. (2007) and https://ke.army.mil/bordeninstitute/other_pub/ews/Chp2LevelsOfCare.pdf

As reviewed by Blyth et al. (2015), the bacteriology of war wound infections changes as patients move through echelons of care. Cultures of the wounds of combat-related trauma patients admitted to combat support facilities (North Atlantic Treaty Organization [NATO] Role 3 or Level III) found that most infected wounds were contaminated by low-virulence Gram positive skin commensals and environmental Gram negative bacteria (Murray et al., 2006; Wallum et al., 2015; White et al., 2016). Several studies report increasing rates of nosocomial-associated and drug-resistant Gram negative bacterial infections as patients experience prolonged hospitalization and progress through higher echelons of care (Burns et al., 2012; Johnson, Burns, Hayda, Hospenthal, & Murray, 2007; Kaspar et al., 2009; Keen et al., 2010; Mende et al., 2014; Mody et al., 2009; Murray, Hospenthal, Kotwal, & Butler, 2011; Petersen et al., 2007; Sheppard et al., 2010; Wallum et al., 2015; Weintrob et al., 2010, 2013). Common etiologic agents identified in these studies include *A. baumannii*, *Klebsiella* spp., *P. aeruginosa*, and *Escherichia coli*. In spite of increasing Gram negative bacterial contamination in the
hospital care system, infection-related persistent wound complications are more commonly associated with *S. aureus* and other Gram positive organisms (Burns et al., 2012; Johnson et al., 2007; Murray, Hospenthal, et al., 2011; Yun, Branstetter, & Murray, 2008).

Throughout OEF/OIF, the US military healthcare system has encountered a steady increase of infections due to drug-resistant or multidrug-resistant organisms (MDRO) in military personnel (Calhoun, Murray, & Manring, 2008; Hospenthal, Crouch, et al., 2011; Murray, 2008a; Scott et al., 2007; Vento et al., 2013). In a recent study of 2,079 OEF/OIF combat trauma patients admitted to two (Level V) US hospitals, 14 percent had positive cultures for drug-resistant Gram negative bacteria (Gilbert et al., 2016). The most common drug-resistant Gram negative bacteria detected in this cohort was *E. coli* (74 percent), followed by *A. baumannii* complex (15 percent), *K. pneumonias* (10 percent), *Enterobacter cloacae* (1 percent), and *Citrobacter* spp. (<1 percent) (Gilbert et al., 2016). Surveillance cultures collected between June 2009 and May 2012 detected multidrug-resistant Gram negative bacteria in 6.6 percent of patients at Landstuhl Regional Medical Center (Level V) and 12.4 percent of patients admitted to three Level V US MTFs (Weintrob et al., 2013).

Studies have observed a higher prevalence of multidrug-resistant *E. coli*, *A. baumannii*, and *P. aeruginosa* from Service Members deployed overseas, compared to non-deployed Service Members or civilians (Davis, Moran, McAllister, & Gray, 2005; Gilbert et al., 2016; Vento et al., 2013).

Trauma-related IFIs appear to be less common than bacterial infections following combat injuries (Tribble et al., 2015; Tribble & Rodriguez, 2014). Analysis of DoDTR/JTTR data for 1,133 US military personnel injured in Afghanistan between June 2009 and August 2011 found an overall IFI rate of 6.8 percent (Weintrob et al., 2015). Within this cohort, the IFI rate was 0.2 percent for ward admissions and 11.7 percent for ICU admissions. An earlier study of 2,413 patients evacuated from Afghanistan to Landstuhl Regional Medical Center (Level IV) between June 2009 and December 2010 identified IFI rates up to 3.5 percent following combat injury (Warkentien et al., 2012). Traumatic wound IFIs are associated with significant adverse clinical outcomes in military populations (Blyth et al., 2014; Lewandowski, Purcell, Fleming, & Gordon, 2013; Murray, Loo, et al., 2008; Warkentien et al., 2012, 2015), and studies of trauma-related IFIs in civilians report mortality rates as high as 38 percent (Fanfair et al., 2012; Tribble et al., 2015).
Risk Factors

Identifying risk factors associated with the development or persistence of wound infection following blast injury is necessary to inform development of diagnosis, prevention, and treatment approaches. Several studies have identified prominent risk factors for infection from preinjury through the continuum of care.

Injury Characteristics and Circumstances

Different injury characteristics confer varying risks of infection. Numerous studies have investigated patterns of injury and clinical characteristics of combat and blast injuries to identify infection-related risk factors.

Mechanism of Injury

Blast injury is a primary risk factor for combat injury-related infection. In studies assessing US military personnel deployed during OEF/OIF, blast injury was significantly associated with the development of infections, including both bacterial (Murray, Wilkins, et al., 2011; Petersen et al., 2007) and fungal (Murray et al., 2009; Rodriguez, Weintrob, Shah, et al., 2014). In one study, blast was the mechanism of injury for 100 percent of the IFI cases and controls, with 92 percent of these personnel injured while on foot patrol (Lewandowski et al., 2016). The complex penetrating and burn injuries that often result from blast exposure can provide an efficient vector for deep implantation of environmental organisms into soft tissue (Lewandowski et al., 2016).

Injury Severity

Injury severity is a known risk factor for wound infection following trauma. Blast exposure often results in polytrauma that requires debridement of a large surface area. As the surface area of a wound increases, the likelihood of contamination from pathogenic environmental organisms increases, in addition to the likelihood of deeply implanted debris or devitalized tissue present during debridement (Hajdu, Obradovic, Presterl, & Vécsei, 2009; Petersen et al., 2007).

The presence of three or more injury locations or limb loss has been associated with significant increases in combat injury-related infections (Petersen et al., 2007). Several studies have reported an association between a higher Injury Severity Score (ISS) and the development of infections (Dickens et al., 2013; Murray et al., 2009; Murray, Wilkins, et al., 2011; Penn-Barwell et al., 2016; Petersen et al., 2007). Notably, the ISS is a composite score that reflects additional risk factors, such as the requirement for massive blood transfusions or life-sustaining invasive devices, which are risk factors discussed below.

The severity of combat-related open fractures and soft tissue injuries, as measured by the Gustilo-Anderson classification system, has also been associated with increased risk of infection, amputation, and prolonged time to union of fractures (Burns et al., 2012; Lack et al., 2015; Weber, Dulai, Bergman, Buckley, & Beaupre, 2014; Westgeest et al., 2016). Specifically, type IIIIC fractures sustained during combat were significantly
more likely to develop deep infections than type II A fractures (Burns et al., 2012). Higher Gustilo-Anderson scores and deep infection are associated with delayed healing and nonunion of open long bone fractures (Westgeest et al., 2016).

**Body Region**

Although the extensive nature of blast injuries increases the overall risk of exposure to microorganisms and subsequent development of infections, recent studies in military populations suggest that injuries to specific body regions pose greater risk of infection.

Extremity injuries and musculoskeletal trauma resulting from blast exposure have been associated with soft tissue infections and eventual amputation (Belmont et al., 2013; Casey, Demers, Deben, Nelles, & Weiss, 2015). Injuries to the lower extremities, especially to the tibia, are associated with an increased risk of infection development (Brown, Murray, & Clasper, 2010; Rodriguez, Weintrob, Dunne, et al., 2014). Open tibia fractures sustained in recent conflicts are usually the result of penetrating trauma from blast mechanisms and gunshot wounds (Burns et al., 2012). In a study of US military personnel injured during OEF/OIF, those who sustained a calcaneal fracture, ipsilateral talar fracture, or forefoot fracture, and who had a more severe Gustilo and Anderson fracture type had a significantly higher risk of developing a wound infection (Dickens et al., 2013). Penn-Barwell et al. (2013) report that the infection rate of British military personnel with a severe open tibial fracture sustained between 2006 and 2010 was 23 percent. In addition, Service Members with bilateral lower-extremity injuries exhibit both local and systemic cytokine responses that are associated with an increased risk of infection, wound dehiscence, and heterotopic ossification (Lisboa et al., 2013). Similarly, the severe immunosuppression resulting from polytrauma and the administration of massive blood products following blast-related hemipelvectomy has been shown to be associated with a significantly increased risk of both bacterial infections and IFI (D’Alleyrand et al., 2015).

In addition, a study of combat-injured military personnel reported that, on a per extremity wound basis, patients with IFI had a significantly higher median number of operative procedures, risk of proximal amputation revisions, and an higher number of days to initial wound closure than controls without IFI (Lewandowski et al., 2016). Similarly, another study reported that patients with IFI had a significantly higher number of lower extremity amputations, a greater proportion of above-the-knee amputations, pelvis and/or hip injuries, genitalia and/or groin injuries, and an increase in the requirement for colostomy than controls (Rodriguez, Weintrob, Shah, et al., 2014). Notably, among patients with a combat-related vascular injury during the Syrian conflict, those that underwent amputation had a higher rate of infection compared to patients with salvaged limbs (Şişli, Kavala, Mavi, Sariosmanoğlu, & Oto, 2016).

Thoracic and abdominal cavity injuries have also been associated with an increased risk of infection in both military personnel (Conger et al., 2008; Petersen et al., 2007) and civilian populations (Fares et al., 2013). The primary risk factor for infection following thoracic trauma is retained hemothorax (Conger et al., 2008; Martin, Dunne, Cho, & Solomkin, 2011). Following blast injury, Service Members who developed IFI commonly
had lower extremity amputations with perineal or pelvic injury and received massive blood transfusions (Warkentien et al., 2012). Petersen et al. (2007) suggest that abdominal injuries are more likely to become infected than extremity injuries because damage to the viscera may result in the leakage of and exposure to bowel contents and subsequent sepsis.

**Dismounted Patrol**

Geographic constraints on the ground have necessitated more frequent dismounted patrols in southern Afghanistan, placing Service Members who incur blast injuries at particularly high risk for IFIs (Tribble & Rodriguez, 2014). Multivariate analyses have reported that military personnel who are on dismounted patrol when injured by blasts are at the greatest risk of infection (Lloyd, Weintrob, Rodriguez, et al., 2014; Rodriguez, Weintrob, Shah, et al., 2014; Warkentien et al., 2012). In general, Service Members on foot patrol are at increased risk of directly encountering ground-emplaced improvised explosive devices (IEDs), thereby increasing the overall risk of blast exposure, penetrating trauma, and multiple extremity amputation (Evriviades et al., 2011; Fleming, Waterman, Dunne, D’Alleyrand, & Andersen, 2012; Lewandowski et al., 2016; Radowksy, Strawn, Sherwood, Braden, & Liston, 2011). Studies report that Service Members sustaining a dismounted complex blast injuries are highly susceptible to both bacterial and invasive fungal infections, which can present concurrently following injury (Cannon et al., 2016; Ficke et al., 2012).

**Healthcare-Associated Characteristics**

Nosocomial transmission during the care of patients within the military healthcare system, particularly at field hospitals, has been reported as an important risk factor for infection with MDROs (Kaspar et al., 2009; Scott et al., 2007). A recent study of patients from conflict areas within the Middle East reported that the hospital environment is a major source of MDROs, which are often associated with infections of blast-induced injuries (Sahli et al., 2016). The temporary nature of field hospitals complicates the maintenance of infection control and environmental cleaning practices, and the frequent influx of military and civilian casualties increases the risk of environmental contamination with these microorganisms (Scott et al., 2007). Prolonged hospital stays have been also identified as a risk factor for infection (Moultrie, Hawker, & Cole, 2011). This finding is likely related to an increased risk of nosocomial transmission, but may also reflect severity of injury. The number of operating room visits and days spent in the ICU have been reported to be significant risk factors for developing IFIs (Lloyd, Weintrob, Rodriguez, et al., 2014). Procedures and devices typically associated with admission to the ICU (e.g., ventilators, catheters, central lines) have been linked to increased risk of infections (Petersen et al., 2007).

**En Route Care**

Evacuation times have decreased with each successive conflict. During the Vietnam War, casualties were evacuated and admitted to treatment facilities within 1–4 hours of injury, whereas during OEF/OIF, the average time from injury to admission decreased to
45 minutes (Langan, Eckert, & Martin, 2014). Medical evacuation can be delayed by combat and environmental conditions, which may significantly affect risk of infection. Petersen et al. (2007) evaluated trauma casualties evacuated from theatre to a US Navy hospital ship and reported that external fixation and a delay to ship transport greater than 3 days were independently associated with increased risk of infection. Studies in civilian populations indicate that increased time between injury and trauma center admission is associated with wound infection (Pollak et al., 2010), while increased time between trauma center admission and initiation of irrigation and debridement is not (Srour et al., 2015). Prolonged out-of-hospital time following injury may be associated with higher risk of infection (Pollak et al., 2010).

**Massive Blood Transfusions**

Despite aggressive resuscitation protocols and damage control procedures that include blood transfusions, blast injury patients are often hypotensive, hypothermic, and anemic, thereby increasing the risk for wound infection (Casey et al., 2015). Patients who develop infections after injury have had a significantly higher mean number of massive blood transfusions within the first 24 hours following injury than patients who do not develop infections (Evriviades et al., 2011; Lewandowski et al., 2016; Murray, Wilkins, et al., 2011; Rodriguez, Weintrob, Shah, et al., 2014). There is some variation in the literature as to how many units of blood are considered a massive transfusion; one study assessed patients who received at least eight units (Evriviades et al., 2011), and other studies assessed patients who received at least 20 units (Lewandowski et al., 2016; Lloyd, Weintrob, Rodriguez, et al., 2014; Rodriguez, Weintrob, Shah, et al., 2014). The need for such massive transfusions may reflect the severity of injury and may not be considered an independent risk factor for infection (Murray, Wilkins, et al., 2011). However, in one study, the number of patients with IFI receiving massive transfusions within 24 hours after injury was significantly greater than the number of non-infected controls, even when the groups had comparable injury severities (Lewandowski et al., 2016). The same study reported that IFI cases had a significantly larger proportion of patients with a shock index greater than 1.5 (Lewandowski et al., 2016), and other studies reported that IFI patients were significantly more hypotensive and acidic upon admission to a medical facility (Lloyd, Weintrob, Rodriguez, et al., 2014; Rodriguez, Weintrob, Shah, et al., 2014). Taken together, these data suggest that patients at increased risk for massive transfusion are also at increased risk of infection. Massive blood transfusions may result in temporary immunosuppression via the modulation of chemokines and cytokines, which can subsequently increase the risk of infection (Dunne et al., 2009; Evriviades et al., 2011; Hajdu et al., 2009; Radowsky et al., 2011; Rodriguez, Weintrob, Shah, et al., 2014; Tribble & Rodriguez, 2014; Warkentien et al., 2012). In addition, iron overload that may result from a massive blood transfusion may increase risk of IFI development. Since Mucorales are known to use iron as a nutrition source, the increased serum iron availability immediately after injury increases the risk of mucormycosis (Lloyd, Weintrob, Rodriguez, et al., 2014; Rodriguez, Weintrob, Dunne, et al., 2014; Tribble & Rodriguez, 2014; Warkentien et al., 2012).
Invasive Devices

Medical devices that are necessary for the resuscitation of patients may also pose an increased risk for infection (Carpenter, Hartzell, Forsberg, Babel, & Ganesan, 2008; Evriviades et al., 2011). Ventilator dependency has been reported to be a significant risk factor for infection in Service Members injured during OEF/OIF (Moultrie et al., 2011; Murray, Wilkins, et al., 2011). Ventilators themselves can be contaminated in combat environments and ventilator dependency is associated with immune dysfunction, thereby increasing the risk of developing an infection (Radowsky et al., 2011). The use of invasive devices or procedures (e.g., placement of a central line or nasogastric tube, use of total parenteral nutrition) was significantly higher in injured Service Members who developed trauma-related infections than in injured Service Members without infections (Petersen et al., 2007). It is unclear whether these devices serve as portals of infection or are surrogate markers of severe injuries that require critical care support and are consequently more likely to result in an infection (Petersen et al., 2007).

Fracture Fixation Strategies

In studies of US military Service Members injured in Iraq and Afghanistan, the presence of an orthopedic device was a significant risk factor for recurrent osteomyelitis (Yun et al., 2008); however, external fixation and intramedullary nail fixation were not significantly associated with infection in British military casualties (Brown et al., 2010). In a study of US military Service Members, an initial diagnosis of osteomyelitis was more commonly associated with external fixation, and recurrent osteomyelitis was more frequently associated with internal fixation (Murray, Obremskey, et al., 2011; Yun et al., 2008). Type IIIC tibia fractures are the most prevalent type of fracture seen in OEF/OIF combat-injured populations, and these fractures are associated with increased rates of infection (Burns et al., 2012; Dickens et al., 2013). Optimal fixation strategies for combat-related open tibia fractures at specific levels of care has been debated by experts (Murray, Obremskey, et al., 2011). Internal fixation is not recommended in combat environments, while external fixation has been widely used with few complications (Murray, Obremskey, et al., 2011). Combat-injured patients with type IIIC open tibia fractures treated with external fixation had an overall deep infection rate of 8 percent (Keeling, Gwinn, Tintle, Andersen, & McGuigan, 2008), while another study reported that patients treated with intramedullary nailing had an overall infection rate of 14.3 percent (Murray, Obremskey, et al., 2011). Similarly, studies in civilian populations report positive outcomes following external fixation, though one study reported a 43 percent pin sepsis rate and a 38 percent incidence of malalignment greater than 5 degrees (Murray, Hsu, et al., 2008).

Delayed Antibiotic Therapy and Wound Coverage

Current guidelines for tactical combat casualty care recommend initiation of antimicrobials as soon as possible (Murray, Obremskey, et al., 2011) and wound closure at approximately 5 days following injury if there is no evidence of infection (Murray, Obremskey, et al., 2011). However, these guidelines are based on civilian studies, which generally have not shown significant differences in infection rates based
upon timing of antibiotic therapy or wound coverage (Al-Arabi, Nader, Nader, Hamidian-Jahromi, & Woods, 2007). There is a shortage of studies on post-injury antimicrobial delivery and subsequent infection rates in military populations. A study of type III open tibia fractures in a cohort of patients within a Level 1 trauma facility reported that antibiotic therapy delayed beyond 66 minutes and wound coverage delayed beyond 5 days significantly independently predicted the development of an infection (Lack et al., 2015). This study suggests that the timing of antibiotic prophylaxis and wound coverage may be more important to patient outcomes than previously thought; however, this finding has several limitations. For example, antibiotic administration and wound coverage could be delayed by parallel resuscitative measures. Similarly, severe fractures with extensive soft tissue damage are themselves at higher risk of infection. The delay to coverage may be related to the time required for the wound to be deemed stable for coverage rather than the delay in coverage itself. An additional caveat to the findings of this study is that they are specific to type III fractures and it is unknown how they translate to other injuries, such as those incurred during combat.

Environmental Characteristics

IFIs have emerged as a serious complication of combat trauma-associated infections in Service Members injured during OEF/OIF. Injuries sustained in southern Afghanistan are significantly more likely to be contaminated with mold because of the geographical characteristics of the region (Rodriguez, Weintrob, Shah, et al., 2014; Tribble et al., 2015). The low elevation, warm climate, and large agricultural areas of the southern region of Afghanistan are conducive to a more dense concentration of decaying vegetation and environmental mold, compared to the more arid regions of Afghanistan or Iraq (Rodriguez, Weintrob, Shah, et al., 2014; Warkentien et al., 2012). Military personnel who developed IFIs or that had mold-contaminated wounds are more likely to have sustained injuries in the southern province of Afghanistan within the vicinity of agricultural zones (Rodriguez, Weintrob, Shah, et al., 2014; Tribble et al., 2015). Notably, the molds found in southern Afghanistan are pathogenic species, such as Mucorales and Aspergillus spp. (Tribble et al., 2015).

Other Risk Factors

A variety of other factors have been associated with an increased risk of infection following combat-related injury. Murray et al. (2011) associated Glasgow Coma Scale score with risk of infection based on univariate analysis, but it was not considered a risk factor upon multivariate analysis (Murray, Wilkins, et al., 2011). Burns et al. (2012) evaluated surveillance cultures obtained from injured Service Members within 72 hours of arrival to a medical facility. Patients with positive initial surveillance cultures were significantly more likely to develop deep infections, osteomyelitis, and the need for amputation than patients with negative initial surveillance cultures. Furthermore, the more bacteria identified on surveillance cultures, the higher the likelihood patients had of developing a deep infection and osteomyelitis (Burns et al., 2012).
Diagnosis

Diagnosis of wound infection is critical for optimal wound management. Current diagnostic approaches in the military healthcare system seek to identify wound infection as rapidly and accurately as possible using resources available in potentially austere environments. US and international researchers across academic, private, and government organizations seek to develop novel objective biomarkers to enable faster and more precise identification of infection pathogens. More effective diagnosis capabilities through the use of advanced biomarkers would confer multiple benefits relevant to minimizing wound infection following blast-related injury (Dupuy et al., 2013; Strimbu & Tavel, 2010), including:

- **Prediction of wound infection.** Diagnostic approaches that can be utilized close to the point of injury and predict whether contaminated or already colonized wounds have an increased probability of developing an infection will help direct the use of prophylactic antimicrobial therapies.

- **Early diagnosis of wound infection.** Diagnostic approaches that can rapidly detect and diagnose wound infection enable prompt treatment that can result in decreased morbidity and improved outcomes.

- **Tailored treatments.** Diagnostic approaches that can identify pathogens and determine their antimicrobial susceptibility will enable the use of more focused and effective treatments. Wounded Service Members typically receive broad-spectrum antibiotics that are active against a wide range of bacteria; however, these drugs may be ineffective against certain pathogens and their use also increases the risk of multidrug resistance. Moreover, use of broad-spectrum antibiotics increases risk for adverse events that may be associated with antimicrobial toxicities and side effects (Metzger, Frobel, & Dunne, 2014). Precise wound infection diagnosis would facilitate targeted therapeutic approaches.

- **Enhanced epidemiological understanding of wound infection.** Diagnostic approaches may provide critical epidemiological information regarding the etiological agents of wound infections that can facilitate targeted surveillance efforts (Rota, Trees, MacCannell, & Gerner-Smidt, 2015).

**Current Wound Infection Diagnostic Approaches**

Currently, biomarkers for the identification of wound infections following blast-related injuries can be detected by both clinical and molecular methods. However, diagnosing a wound infection and identifying the etiologic agent remains challenging; therefore, rapid diagnostics and better wound infection biomarkers are needed.

**Clinical Assessment**

Clinical assessment is a common method utilized for the detection of wound infections and is based on an evaluation of clinical signs and symptoms, such as localized pain, redness, increased temperature, purulent discharge, delayed healing, abscess...
formation, fever, dehiscence, edema, and malodor (Cutting & White, 2004; Gardner, Frantz, & Doebbeling, 2001). Despite widespread use by healthcare providers, the validity of clinical sign as an indicator of infection has been called into question recently (Blokhuis-Arkes et al., 2015; Gardner et al., 2001). Evidence suggests that secondary clinical signs (e.g., serous exudate, delayed healing, discoloration of granulation tissue) are more accurate indicators of chronic infection than classical signs (e.g., pain, edema, heat, erythema, purulence) (Gardner et al., 2001). In a study of chronic wounds (venous leg ulcerations), clinical signs were unreliable for the diagnosis of wound infection (Serena, Hanft, & Snyder, 2008). In contrast, in a study of 300 military personnel who had sustained combat-related lower extremity amputations, there was a significant association between certain clinical signs (erythema and/or drainage) and wound infection, but not with others, such as edema and wound infection (Polfer et al., 2014). As with most clinical practice guidelines (CPGs), current Joint Theater Trauma System (JTTS) CPGs recommend the use of a subset of clinical signs in the diagnosis of infection, often in conjunction with additional diagnostic methods; however, supporting evidence for the JTTS CPGs has not been identified.

**Culture-Based Methods**

Culture-based methods are widely used in parallel with other methods of diagnosing wound infection and have the potential to provide a variety of clinically relevant information, including: (1) quantitative measure of wound bioburden, (2) identification of etiologic agent(s), and (3) susceptibility of pathogen to antimicrobials and the identification of multidrug-resistant organisms (Pfaller et al., 2015).

**Wound Bioburden Quantification**

Before the emergence and refinement of modern diagnostic technologies, bacterial quantification was utilized as a diagnostic method. Diagnosis was predicated based on the relationship between the number of bacteria and the time between injury and treatment. The greater the amount of time between injury and treatment, the greater the bacterial colonization, and the greater the risk of wound infection (Robson, Duke, & Krizek, 1973). Recent studies, however, do not support the utility of bacterial quantification in wound management (Kallstrom, 2014). It is now understood that a definitive diagnosis and prediction of wound outcome based solely on bacterial number have limited utility due to what is now known about wound microbial diversity, variable virulence, and the synergy between different bacterial species (Tay, Chong, & Kline, 2016).

**Pathogen Identification**

Culture-based methods have been used routinely to identify common pathogens and multidrug-resistant organisms in acutely infected wounds and to guide the use of antibiotic therapies. These culture-based methods continue to evolve and now include commercially available, automated systems for the identification of microbes (Tribble, Conger, et al., 2011). Swab cultures remain the most commonly used method of microbial culture, and samples for culture may be obtained via tissue biopsy or curettage (Drinka et al., 2012). Regardless of the sampling method, wound cultures are
commonly used to identify pathogens; however, culture-based methods have several limitations. Culture-based methods can only detect viable microorganisms and only a small fraction of microbes can be grown on standard microbiological media (Stewart, 2012). Culture-based methods are biased toward planktonic microorganisms, which grow well on laboratory media, but are not always the cause of an infection (Hodkinson & Grice, 2015a; Rhoads, Wolcott, Sun, & Dowd, 2012). Rhoads, Wolcott, Sun, & Dowd (2012) demonstrated that culture-based methods not only underreport the microbial diversity within a wound, but also fail to identify the most abundant bacteria in more than 50 percent of wounds (Rhoads et al., 2012). In addition, when conventional culture-based methods were compared to peptide nucleic acid-based fluorescence in situ hybridization methods of bacterial identification in chronic wounds suspected of P. aeruginosa infection, there was no correlation between the bacteria detected by these two techniques (Kirketerp-Møller et al., 2008). In a civilian study of 81 acute and chronic wounds, the diagnoses of infection by clinical assessment or culture were not significantly associated (Blokhuis-Arkes et al., 2015). Furthermore, culture-based methods may be inadequate for the detection and identification of pathogens comprising biofilms because they do not accurately reflect biofilm viability and composition (Vyas & Wong, 2016). Notably, culture-based methods can be too slow to be clinically meaningful, as specimen collection and culture can take several days to weeks, depending on the pathogen (Pfaller et al., 2015). For example, the JTTS CPG for fungal infection recommends that fungal cultures be checked frequently for 2 weeks and then once a week for 4 additional weeks before final results are assured (JTTS, 2012).

Pathogen Susceptibility

Susceptibility testing is routine and many tests are commercially available in fully-automated systems (Leber, 2016). These tests may be used in tandem with culture-based diagnostic techniques (Leber, 2016), and their results are widely accepted as clinically relevant for the successful selection of effective antimicrobial therapies. The benefits accrued by susceptibility testing include more timely alterations to the course of antimicrobial treatment, fewer laboratory tests overall, reduced need for invasive procedures, and reduced risk for developing multidrug resistance (Barenfanger, Drake, & Kacich, 1999; Doern, Vautour, Gaudet, & Levy, 1994).

Overall, using multiple diagnostic approaches is critical because of the limitations of each culture-based method when used independently. For example, a study of wounded US military Service Members at Level IV and V facilities concluded that the combination of positive fungal cultures and recurrent necrosis determined via clinical assessment supports a diagnosis of IFI and constitutes a need for antifungal therapy (Rodriguez, Weintrob, Dunne, et al., 2014). Thus, multiple approaches may help to accurately distinguish between fungal colonization and IFI.

Histopathology

Similar to clinical assessment and culture-based methods, histopathological methods are often performed in conjunction with other approaches for the identification of wound
infections. For example, although culture is still required for a definitive diagnosis, fungi may be visualized under a microscope following Periodic acid-Schiff or Grocott’s methenamine silver staining (JTTS, 2012). Diagnosis of Pythium infection via conventional histopathology and culture is difficult because broad aseptate hyphae are morphologically similar to members of the Mucorales order (Farmer et al., 2015). Despite their morphological similarities, the two species are genetically and physiologically distinct, and require distinct treatment strategies (Farmer et al., 2015). In addition, while the assessment of frozen sections has a high specificity for the identification of IFI, its low specificity precludes it from being used as a lone diagnostic (Heaton et al., 2016).

Molecular Techniques

Polymerase chain reaction (PCR) is a molecular technique used to amplify DNA, enabling the detection of microorganism-specific DNA sequences. PCR and other molecular techniques can be used to identify pathogens and offer unparalleled speed, sensitivity, and specificity relative to conventional culture and histopathological techniques (Tatum & Dowd, 2012; Weile & Knabbe, 2009). PCR and methods of sequencing 16S ribosomal RNA (rRNA) genes have been effectively utilized to complement conventional culture techniques, identify novel and emerging pathogens, and define complex microbial communities. In a retrospective study of 168 chronic wounds, PCR-based methods identified a total of 338 bacterial taxa, whereas culture-based techniques identified only 17 taxa (Rhoads et al., 2012). In pleural fluid samples evaluated via clinical assessment and conventional culture, PCR and 16S rRNA sequencing diagnosed infection in 82 percent of samples, whereas conventional cultures diagnosed infection in only 55 percent of samples (Insa et al., 2012). Wolcott, Cox, & Dowd (2010) demonstrated that the combination of PCR and sequencing techniques significantly reduces unnecessary use of expensive, first-line treatments for methicillin-resistant *S. aureus* (MRSA) by accurately identifying MRSA in chronic wounds (Wolcott et al., 2010).

Although PCR-based methods are not included in the current wound management recommendations of the JTTS CPGs, they are being used within the military healthcare system. The extent to which they are available and regularly employed in practice is unknown, however. In a case study, Farmer et al. (2015) report the use of PCR and sequencing-based techniques in conjunction with conventional culture and histopathology in Role IV and V facilities to identify unusual fungal elements of infection in a US military Service Member who had sustained extensive blast-related burns and polytrauma (Farmer et al., 2015).

Diagnosis of Combat Wound Infection

The diagnostic capabilities associated with wound infections vary across the different levels of care within the military healthcare system, and there is limited information available regarding the diagnostic capabilities of each level of the JTTS. At Level I and II military treatment echelons, CPGs specify clinical assessment for diagnosis and treatment of wound infection (Hospenthal et al., 2008). Some Level III facilities have
laboratory support (i.e., limited microbiological capabilities) to enhance diagnostic capacity (Hospenthal et al., 2008). Furthermore, at Level IV and V facilities, CPGs recommend that specimens be collected and evaluated via histopathology and culture from patients who are at risk for developing IFIs (e.g., demonstrate poor wound appearance, tissue necrosis, or tissue compromise) (JTTS, 2012). Although specialized diagnostic assays (e.g., acid-fast bacilli testing for Mycobacteria) are not routine, they can be requested (JTTS, 2012).

In response to an IFI outbreak that began in 2009 among US military Service Members in Afghanistan, the military trauma community developed and implemented a CPG at Landstuhl Regional Medical Center that called for the screening of wounded patients who were at increased risk for developing IFI (JTTS, 2012; Lloyd, Weintrob, Rodriguez, et al., 2014). Although this CPG recommended only conventional culture and histopathological techniques, its implementation significantly reduced the time to diagnosis in Service Members following injury due to explosive blast (Lloyd, Weintrob, Rodriguez, et al., 2014).

**Wound Infection Biomarker Development**

US and international researchers across academic, private, and government organizations are working to develop novel wound infection biomarkers to enhance diagnostic capabilities. These research efforts pursue several approaches, including protein and enzyme analysis, proteomics, metabolomics, next-generation sequencing, microarrays, biofilm detection, electrochemical sensors, intelligent dressings, and multiplexed automated digital microscopy.

**Protein and Enzyme Analysis**

An infected wound is a complex environment, resulting from the innate host microbiome interacting with proteins and enzymes secreted into the wound as part of the host immune response (Grice & Segre, 2012; Masic, Gardner, & Grice, 2014). Expression levels of protein or enzyme compounds within the wound environment are thought to be potential biomarkers for infection and wound status (Forsberg, Potter, Polfer, Safford, & Elster, 2014; Hahm, Glaser, & Elster, 2011; Reinhart, Bauer, Riedemann, & Hartog, 2012; Tegl, Schiffer, Sigl, Heinzel, & Guebitz, 2015; Vlek, Bonten, & Boel, 2012). Two of the most widely-studied protein biomarkers of infection are procalcitonin (PCT) and C-reactive protein (CRP). Serum PCT levels have been used as an early indicator of septic complication and response to antimicrobial therapy in patients with severe burn injuries (Mokline et al., 2015) and have been found to correlate with wound dehiscence following closure of severe open extremity wounds (Forsberg et al., 2008). CRP is produced in response to inflammation and widely acknowledged as a marker of infection (Tegl et al., 2015). In orthopedic cases, elevated levels of CRP were reported to be an effective screening test for the presence of infection (Greidanus et al., 2007). In patients with open fractures, serial serum measurements of CRP have been used to diagnose infections before they were clinically relevant (Douraiswami, Dilip, Harish, & Jagdish, 2012). In a systematic review and meta-analysis of studies that simultaneously investigated PCT and CRP levels as markers for bacterial infection, PCT levels were
reported to be more accurate markers than CRP levels (Simon, Gauvin, Amre, Saint-Louis, & Lacroix, 2004).

Many other proteins and enzymes have been described as potential wound infection biomarkers (Tegl et al., 2015). Neutrophil-derived enzymes, such as myeloperoxidase (MPO), human neutrophil elastase (HNE), cathepsin G (CAT G), lysozyme (LYS), and matrix metalloproteinases (MMPs), appear in the early stages of infection and are being explored as biomarkers to monitor wound status (Hasmann et al., 2013; Andrea Hasmann et al., 2011). There are significantly higher levels of proteases, including MMPs and elastases, in infected wounds than in non-infected wounds, suggesting a potential role as biomarkers of wound infection (Heinzle et al., 2013). Blokhuis-Arkes et al. (2015) recently investigated the diagnostic properties of enzyme analysis (i.e., HNE, MPO, LYS, and CAT G) versus wound swabs and clinical judgement for detecting infection in acute and chronic wounds, and reported that one or a combination of enzymes could be used in various predictive models to positively identify infection. Other promising biomarkers in the management of antibiotic therapy in acute infections include Soluble Triggering Receptor Expressed on Myeloid cells-1, Soluble urokinase-type Plasminogen receptor, proadrenomedullin, and Presepsin (Dupuy et al., 2013).

While biomarker research in civilian populations can potentially be applied to military applications, the unique characteristics of wound infection in the combat environment (Hahm et al., 2011) have prompted researchers to investigate biomarkers in military populations with combat wounds. Proteomic analysis of wound effluent retrieved from US military Service Member patients with combat or traumatic wounds identified 52 candidate protein biomarkers of wound infection (Chromy et al., 2013). A study of 19 patients with severe, combat-related, open extremity wounds found a correlation between dehiscence following wound closure and levels of PCT, interleukin-13, and Regulated on Activation, Normal T Cell Expressed and Secreted chemokine (Forsberg et al., 2008). In a prospective study of 38 traumatic extremity combat wounds from 25 patients evacuated from Iraq and Afghanistan, impaired wounds had significantly elevated levels of serum MMP2 and MMP7, and significantly reduced levels of effluent MMP3 than wounds that healed, suggesting that concentrations of these MMPs could potentially predict wound closure or other outcomes (Utz et al., 2010). In a related study, analysis of effluent and wound bed tissue biopsies in 52 extremity wounds from 33 patients found that the proinflammatory cytokines interleukin-6 and interleukin-8, and macrophage inflammatory protein-1a were significantly elevated in sera of patients whose wounds dehisced (Hawksworth et al., 2009). These data suggest that cytokine and chemokine proteins display a condition of inflammatory dysregulation that leads to poor wound healing and dehiscence.

**Proteomic Analysis**

Matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS) systems have become increasingly common in clinical microbiology laboratories (Patel, 2015). This technique is based on laser-induced ionization and fragmentation of sample proteins, followed by their acceleration in an electric charge and measurement of the time of flight. Microbes are identified by comparing the
fingerprint of unknown microbes to fingerprints of known microbes in a database (Patel, 2015). Two systems have been approved by the U.S. Food and Drug Administration (FDA) for the identification of cultured bacteria: the VITEK® MS (bioMerieux Inc.) and the MALDI Biotyper CA System (Bruker Daltronics Inc.). Moreover, the VITEK® MS has also been approved for the identification of fungi (Patel, 2015).

MALDI-TOF MS systems have been extensively evaluated for their ability to accurately identify various microbes, including bacteria that are aerobic and anaerobic, as well as mycobacteria and fungi (Patel, 2015). In one study, 440 common and unusual Gram negative bacilli were sampled. The MALDI Biotyper CA System correctly identified 93 percent and 82 percent of common and unusual bacteria, respectively, compared to 83 percent and 75 percent identified by conventional biochemical methods (Saffert et al., 2011). Additional studies have consistently demonstrated that MALDI-TOF MS is comparable or superior to conventional biochemical identification of common bacteria and yeast. Although MALDI-TOF MS has been successfully applied to culture-isolated microbes, it has not been extensively used on clinical samples, such as those derived from wound exudate. Its clinical utility has been limited to analyzing microbes from positive blood cultures and urine samples with a relatively short turnaround time (e.g., 20 to 30 minutes in positive blood cultures) (Leli et al., 2013). MALDI-TOF has also been used to identify pathogens in patients with sepsis and to improve the targeted antibiotic treatment of bacteremia (Rodríguez-Sánchez et al., 2014). In case studies of wound infections, MALDI-TOF MS has been used to correctly identify Photobacterium damselae and Vibrio harveyi (Hundenborn, Thürig, Kommerell, Haag, & Nolte, 2013), Sporolactobacillus laevolaticus (Abat, Kerbaj, Dubourg, Garcia, & Rolain, 2015), and Yersinia ruckeri (De Keukeleire et al., 2014). MALDI-TOF has also been used in conjunction with PCR to identify MRSA from burn wounds (Madhava Charyulu, Gnanamani, & Mandal, 2012).

A limitation of using MALDI-TOF MS is that highly active antimicrobial resistance-associated proteins (e.g., beta-lactamases) are expressed at concentrations below the level of detection for current MALDI-TOF MS system (Patel, 2015). Another limitation is the variability in MALDI-TOF MS methodologies caused by differences in sample preparation, matrix solutions, and organic solvents (De Bruyne et al., 2011). In response researchers have developed a procedure for the detection of bacteria using reference organisms, which was reported to have a high overall accuracy for the detection of Fructobacillus and Lactococcus bacteria (De Bruyne et al., 2011).

**Metabolomics**

Metabolomics, which is the study of small molecular metabolites, may be useful for the detection of disease biomarkers, particularly in the context of biofilms (Vyas & Wong, 2016). During biofilm formation, both the pathogen and the host may undergo metabolic changes that could be detectable via metabolomic analysis. Furthermore, nuclear magnetic resonance metabolomics analyses may be useful for the study of biofilm formation and antibiotic resistance (Zhang & Powers, 2012).
Next-Generation Sequencing

Next-generation sequencing (NGS), also known as high-throughput sequencing, is a collection of modern technologies that enable more rapid and inexpensive sequencing of DNA and RNA as compared to traditional methods. NGS techniques are culture-independent and, therefore, eliminate the biases inherently introduced by culture-based methods (Hodkinson & Grice, 2015b), such as the bias toward microorganisms that grow well in standard laboratory conditions, as well as the poor representation of microbial diversity and microbial load (Be et al., 2014; Gardner, Hillis, Heilmann, Segre, & Grice, 2013; Han et al., 2011; Price et al., 2009). Five major NGS platforms have been used to study the wound microbiome: 454 (Roche), Illumina (Solexa), SOLiD (Thermo Fisher Scientific), Ion Torrent (Thermo Fisher Scientific), and PacBio (PACBIO) (Hodkinson & Grice, 2015b). NGS approaches are being used to gain insight into microbial composition, diversity, and dynamics during infection, as well as how these relate factors to wound healing and the risk of complications (Be et al., 2014; Hodkinson & Grice, 2015b; L. B. Price et al., 2009; Tay et al., 2016).

There are few published reports describing the use of NGS to provide relevant diagnostic or clinical information. In a case study of a venous leg ulcer, NGS was used to characterize weekly changes in bacterial load, community structure, and bacterial diversity over a 15-week course of treatment and healing. Bacterial bioburden was more dynamic that previously appreciated and changes in the bacterial load and community structure correlated with wound expansion, antibiotic therapy, and healing (Sprockett, Ammons, & Tuttle, 2015). In other studies, NGS has been used to 1) diagnose a rare case of meningoencephalitis and to facilitate the use of targeted and efficacious antimicrobial therapies (Wilson et al., 2014), 2) identify infectious microbes from septic patients using plasma-circulating DNA (Grumaz et al., 2016), 3) diagnose an unusual and fatal case of progressive encephalitis in an immunocompromised adult (Naccache et al., 2015), and 4) analyze paired noninvasive and invasive group A streptococcal strain isolates from a patient with a skin/soft tissue infection (Flores et al., 2014).

Although NGS continues to become more accessible and more affordable, challenges remain in bringing these technologies into clinical settings as wound infection diagnostic or prognostic tools (Hodkinson & Grice, 2015b). For example, NGS approaches are not free from bias, which can be introduced at many stages (e.g., sample preparation, selection of primer sequences, inherent error profiles of each NGS platform) (Misić et al., 2014). In addition, there is little standardization across different microbiome studies with respect to quality control of sequence data, use of controls, and the types of analyses performed (Clooney et al., 2016; Misić et al., 2014). Management and analysis of the significant volume of data generated by NGS also represents a notable challenge to diagnostic efforts (Hodkinson & Grice, 2015b).

Microarrays

DNA microarrays are a molecular platform by which specific DNA, oligonucleotides, or cDNA are bound to a matrix that is used to detect individual DNA sequences from a sample of fluorescently labeled DNA. Microarrays can provide broad-spectrum detection
and genetic characterization of microbes. Microarrays have been designed with probes for microbial identification and discovery (Palacios et al., 2007; Vijaya Satya, Zavaljevski, Kumar, & Reifman, 2008; Wang et al., 2007). A pan-Microbial Detection Array was designed to detect all known viruses, bacteria, and plasmids; identify them at the family and species level as confirmed by PCR; and detect mixtures of microbes from complex samples (Gardner, Jaing, McLoughlin, & Slezak, 2010). This MDA was renamed the Lawrence Livermore Microbial Detection Array (LLMDA) and used for the microbial analysis of 124 extremity wound samples collected between September 2007 and January 2012 from 44 combat-injured Service Members (Be et al., 2014). This LLMDA analysis indicated that wounds that heal successfully frequently contain microorganisms that differ from those in wounds that fail to heal. For instance, *Acinetobacter* and multiple *Pseudomonas* species were detected in wounds that dehisce (i.e., wound failures) than in healed wounds. In addition, wounds with similar characteristics did not necessarily have similar LLMDA analysis outcomes, indicating that wound healing failure is due to a range of factors beyond simply the presence or absence of specific organisms (Be et al., 2014).

Recent studies have extended the clinical utility of microarray analysis beyond the detection of pathogen DNA. Yan et al. (2015) used microarrays to build statistical prognostic models to predict infection outcomes and inform early triage of burn patients (Yan et al., 2015). Dix et al. (2015) used a whole-genome microarray approach to perform a transcriptome analysis of human whole-blood infected with bacterial or fungal pathogens and identified 38 biomarker genes associated with sepsis (Dix et al., 2015). Thus, microarrays may be used to create predictive models for susceptibility to infection and investigate the host response to infection.

**Biofilm Detection**

Biofilms are produced when microorganisms adhere to surfaces and proliferate, secreting a matrix of microbial and host-derived protein, polysaccharides, and extracellular DNA. Biofilms are associated with 65 percent of nosocomial infections (Sevgi, Toklu, Vecchio, & Hamblin, 2013), 80 percent of bacterial infections (Akers et al., 2014), and are common in chronic wounds (Akers et al., 2014; Bertesteanu et al., 2014; Sevgi et al., 2013). Biofilms provide an optimal environment for microbial growth by enabling pathogens to bypass host immune responses and impeding the penetration of antimicrobials at the site of infection (Akers et al., 2014; Bertesteanu et al., 2014; Percival, McCarty, & Lipsky, 2015; Sevgi et al., 2013). Thus, the production of biofilms puts patients at risk for delayed healing and persistent wound infection, and requires specialized wound management practices (Akers et al., 2014).

The presence of one type of microorganism is sufficient to generate a favorable environment for other organisms to colonize a wound, and two or more non-pathogenic microorganisms may interact synergistically to cause an infection (Bertesteanu et al., 2014). However, determining whether an infection is biofilm-related or due to planktonic microorganisms remains a challenge in clinical practice. A clinical algorithm has been developed to facilitate the recognition of clinical indicators of wound biofilms and subsequent wound management practices (Metcalf, Bowler, & Hurlow, 2014).
Additionally, a gold nanoparticle-based multichannel fluorescence sensor has been used in a bacteria-mammalian cell co-culture wound model to detect and identify the species composition of biofilms based on their overall physicochemical properties (Li et al., 2014).

Research investigating the relationship between biofilms and infection is still in its infancy and currently there are no tools that confirm the presence of a wound biofilm (Hurlow et al., 2015). Additional studies are needed to determine infection risk and to improve the detection of biofilm-associated infections after blast or other combat-related injuries.

**Electrochemical Sensors**

Electrochemical sensors offer a simple and inexpensive alternative to culture-based methods and molecular techniques for the detection of microbes. These sensors can identify the presence of bacteria based on the detection of electrochemical changes, such as those induced by bacterial quorum sensing molecules or host immune responses. As part of the Wound Etiology and Healing Study, Sismaet et al. (2016) developed an inexpensive, disposable electrochemical sensor to detect pyocyanin, which is a unique, redox-active quorum-sensing molecule released by *P. aeruginosa*, in wound exudate from chronic wounds. The assay required less than 1 minute to complete using 7.5 microliters of wound exudate, yielding 71 percent sensitivity and 57 percent specificity for detection of *Pseudomonas*. Ciani et al. (2012) reported an electrochemical biosensor system that detected multiple host antigens simultaneously from mock wound fluid at concentrations relevant for the detection of infection. The test was performed directly on mock wound fluid without an extensive workup and completed in less than an hour. Gou et al. (2014) described a carbon nanotube-based solid-state sensor that can wirelessly measure real-time pH fluctuations over a wide range (pH 2–12) and can assist in the early detection of infection. This device was attached to a passively powered radio-frequency identification tag and was able to successfully transmit pH data through simulated skin. Additionally, array-based gas sensors are being developed to detect infection based on wound odor. These “electronic nose” devices have demonstrated the ability to detect bacteria and volatile organic compounds from cultures obtained from infected wounds (Byun, Persaud, & Pisanelli, 2010; Gardner, Craven, Dow, & Hines, 1998). Yan et al. (2012) are further developing gas sensors by improving algorithms for identifying wound infection based on the wound odor of mice infected with three common bacterial species (Yan et al., 2015). With further development, these electrochemical sensors may be used as rapid point-of-care diagnostic tools.

**Intelligent Dressings**

Large and chronic wounds often require frequent removal and changes of dressings to inspect wound status. Sensors embedded within wound dressings are being developed to function as “intelligent dressings” that can provide insight into the wound healing process and the development of infections (Mehmood, Hariz, Fitridge, & Voelcker, 2014). Noninvasive sensing and wireless technologies incorporated into bandages can
provide information on wound parameters (e.g., temperature, bacterial load, pH, odor, moisture level of dressing) that are indicative of infection and overall wound status (McLister, Phair, Cundell, & Davis, 2014; Mehmood et al., 2014). Wound dressings have been designed to release an inert dye and visually indicate infection with antibiotic-resistant bacteria (Brocklesby, Johns, Jones, Sharp, & Smith, 2013), fluoresce when exposed to pathogenic bacteria (Zhou et al., 2011) or pathogenic wound biofilms (Thet et al., 2015), and to electrochemically and wirelessly detect uric acid concentrations in wound exudate that correlate with bacterial infection (Kassal et al., 2015). Additionally, there is a patent application for a wound management system comprising a wound dressing embedded with temperature, pH, moisture, and cell impedance sensors that transmits data wirelessly and simultaneously delivers regenerative therapy via electromagnetic stimulation (Elder, 2013).

Significant challenges to the development of intelligent dressings include providing clinically informative systems that are robust, disposable, and economically viable. Some wound monitoring devices have already been incorporated into commercial products. WoundSense™ (Ohmedics) is a wound moisture monitoring system that informs clinical decisions regarding whether to change or remove a dressing (Mehmood et al., 2014). However, this dressing only focuses on one parameter and does not itself detect bacterial colonization or infections. Most devices monitor only one wound parameter at a time, and there is a shortage of literature on integrated systems and studies within biological environments (Mehmood et al., 2014).

Multiplexed Automated Digital Microscopy

Multiplexed automated digital microscopy (MADM) begins with live microbial cell extraction from a specimen and cell immobilization in an analyzer cartridge. A computerized microscope then takes time-lapse images of the immobilized cells as they are exposed to various tests, and an image analysis program interprets and reports the results in standard clinical terms (Chantell, 2015). In clinical samples, MADM can identify the presence of bacteria or yeast in 1 hour and complete susceptibility testing in 5 hours (Chantell, 2015). Furthermore, MADM has been used to identify and quantify multiple pathogens, including A. baumannii, P. aeruginosa, extended-spectrum beta-lactamase producing Enterobacteriaceae, and MRSA within 2 hours and characterized multiple major modes of antibiotic resistance within 6 hours (Metzger et al., 2014; Price, Kon, & Metzger, 2014).

Clinical Trials

A number of clinical trials are being conducted to improve the diagnosis and detection of infections. A list of these trials can be found in Table 5. Some of these trials are investigating specific methodologies and techniques to detect or validate known biomarkers, while others are investigating novel biomarkers. Only two of these studies specify the inclusion of military populations, and none of the identified clinical trials specify blast-injured populations. Although the objectives of these trials may enhance current diagnostic approaches, to date, no results have been reported from these studies.
<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Title</th>
<th>Purpose</th>
<th>Status (as of July 2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00287599</td>
<td>Rapid Identification of Key Pathogens in Wound Infection by Molecular Means</td>
<td>Evaluate real-time PCR methods for the rapid identification and quantification of wound pathogens in military and civilian populations</td>
<td>Completed; no study results posted</td>
</tr>
<tr>
<td>NCT01198262</td>
<td>Rapid Test to Detect Staphylococcus Aureus in Blood and Wound Infections</td>
<td>Evaluate the ability of the Cepheid GeneXpert system to detect methicillin-resistant and -susceptible S. aureus in blood cultures and wound swabs from adult and geriatric patients</td>
<td>Completed; no study results posted</td>
</tr>
<tr>
<td>NCT02508272</td>
<td>Transcriptomic Profiling in Severely Injured Patients</td>
<td>Link defined clinical phenotypes with RNA data obtained by high throughput technologies in polytrauma patients with systemic inflammation</td>
<td>Completed; no study results posted</td>
</tr>
<tr>
<td>NCT01379053</td>
<td>Volatile Organic Compounds in Staphylococcus Aureus Patients (MRSAVOC)</td>
<td>Evaluate the ability of the noninvasive zNose® MRSA test to detect the presence of S. aureus in patients with suspected infection or colonization</td>
<td>Completed; no study results posted</td>
</tr>
<tr>
<td>NCT01875692</td>
<td>Can we Better Understand the Development of VAP and Eventually Predict and Prevent it?</td>
<td>Determine biomarkers of VAP in the oropharyngeal juice and tracheal aspirates in adult ICU patients</td>
<td>Completed; no study results posted</td>
</tr>
<tr>
<td>NCT01496014</td>
<td>Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage (Bioburden)</td>
<td>Characterize wound bioburden at the time of definitive wound closure of severe tibia fractures in military and civilian populations</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT02457663</td>
<td>Identification and Validation of Biomarkers for Infections in Burns</td>
<td>Validate previously identified biomarkers and identify novel biomarkers of infections in burn patients using discovery proteomics</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT02323165</td>
<td>A New Method for Detection of Bacteria in the Bloodstream</td>
<td>Evaluate the use of PCR with universal bacterial 16S primers for detection and identification of bacteria in burn patients</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02753608</td>
<td>Early Detection of Ventilator-associated Pneumonia (VAP) (cheqVAP)</td>
<td>Identify biomarkers for VAP in exhaled breath condensates from patients receiving invasive ventilation</td>
<td>Not yet recruiting</td>
</tr>
<tr>
<td>NCT00258869</td>
<td>Observational Study of Sepsis and Pneumonia to Develop Diagnostic Tests</td>
<td>Identify biomarkers in blood samples that predict outcome in patients with sepsis and community acquired pneumonia using advanced bioinformatic, metabolomic, proteomic and mRNA sequencing technologies</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Source: [https://clinicaltrials.gov/ct2/home](https://clinicaltrials.gov/ct2/home)
Prevention and Treatment

The prevention and treatment of wound infection is vital to treating blast-related injuries (Balazs, Blais, Bluman, Andersen, & Potter, 2015). The emergence of drug-resistant pathogens has complicated efforts to prevent and treat wound infection following combat injuries in US (Calhoun et al., 2008; Hospenthal, Crouch, et al., 2011; Murray et al., 2010; Vento et al., 2013) and Allied MTFs (Fletcher, Hutley, Adcock, Martin, & Wilson, 2013; Mérens et al., 2014). Numerous studies have noted the increasing role of nosocomial transmission associated with drug-resistant infections (Hospenthal, Green, et al., 2011; Kaspar et al., 2009; Keen et al., 2010; Petersen et al., 2007; Sheppard et al., 2010), prompting the need for healthcare system-wide infection control measures of prevention and treatment.

Existing CPGs provide an evidence-based framework for the prevention and treatment of combat-related wound infection in the modern era of drug resistance (Hospenthal, Murray, et al., 2011; Hospenthal & Murray, 2011; JTTS, 2012). Medical experts continue to explore ways of improving upon existing infection control practices and medical treatment approaches, including the use of antimicrobials. Global research efforts are also underway to develop new prevention and treatment approaches as alternatives to antimicrobials.

Clinical Practice Guidelines

In 2007, a committee of military and civilian experts convened to develop evidence-based recommendations for the reduction or prevention of combat-related infections, which were published as CPGs the following year (Hospenthal et al., 2008). Development of these CPGs was based on a detailed review of military and civilian literature and a systematic evaluation of: 1) the strength of recommendations and 2) the quality of evidence available to support recommendations based on ratings systems from the Infectious Diseases Society of America and the US Public Health Service. These CPGs provided infection prevention recommendations from point-of-injury through Level III facilities (Hospenthal et al., 2008), as well as from Level IV to Level V facilities by anatomic site or type of injury, including extremity injury (Murray, Hsu, et al., 2008), central nervous system injury (Wortmann, Valadka, & Moores, 2008), thoracic and abdominal cavity injuries (Conger et al., 2008), head and neck injury (Petersen, Hayes, Blice, & Hale, 2008), and burns (D’Avignon, Saffle, Chung, & Cancio, 2008). Evaluations of compliance with the 2008 CPG found increased compliance rate for some injury types in 2009 and 2010 but also identified ongoing need for continued compliance improvements (Lloyd, Weintrob, Hinkle, et al., 2014; Tribble, Lloyd, et al., 2011).

Military and civilian experts reconvened in 2011 to develop an update to the 2008 infection prevention CPGs, which was published later that year (Hospenthal, Murray, et al., 2011). The updated 2011 recommendations were also based on a review of evidence available in military and civilian literature, as well as ratings of strength and the quality of supporting evidence based on the Grades of Recommendation, Assessment,
Development and Evaluation (GRADE) methodology.¹ Evidence-based reviews of literature informing the 2011 CPG recommendations were similarly focused on five injury types including: 1) extremity injuries (Murray, Obremskey, et al., 2011), 2) central nervous system injuries (Forgione, Moores, & Wortmann, 2011), 3) eye, maxillofacial, and neck injuries (Petersen, Colyer, Hayes, Hale, & Bell, 2011), 4) thoracic and abdominal cavity injuries (Martin et al., 2011), and 5) burn injuries (D’Avignon, Chung, Saffle, Renz, & Cancio, 2011). The 2011 infection prevention CPGs provide recommendations for the use of post-injury antimicrobials, debridement and irrigation, and surgical wound management from prehospital field care through Role 4/Level IV care (Table 6). Significant updates to the previous 2008 infection prevention CPGs noted by the authors include guidelines for antimicrobial selection based on pattern of injury, the use of negative pressure wound therapy (NPWT), and the use of oxygen supplementation during aeromedical evacuation (Hospenthal, Murray, et al., 2011).

¹Grades of Recommendation, Assessment, Development and Evaluation (GRADE), www.gradeworkinggroup.org

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## Table 6. Recommendations to Prevent Infections Associated With Combat-Related Injuries

<table>
<thead>
<tr>
<th>Level of Care*</th>
<th>Care Category</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| Role 1/Level 1 (prehospital) | Initial care in the field | • Bandage wounds with sterile dressings (avoid pressure over eye wounds)  
• Stabilize fractures  
• Transfer to surgical support as soon as feasible |
| | Post-injury antimicrobials | • Provide single-dose point-of-injury antimicrobials if evacuation is delayed or expected to be delayed |
| Role 1/Level I  
Role 2/Level II without surgical support (IIa) | Post-injury antimicrobials | • Provide IV antimicrobials as soon as possible (within 3 h)  
• Provide tetanus toxoid and immune globulin as appropriate  
• Enhance gram-negative coverage with aminoglycoside or fluoroquinolone not recommended  
• Addition of penicillin to prevent clostridial gangrene or streptococcal infection is not recommended  
• Redose antimicrobials if large volume blood product resuscitation  
• Use only topical antimicrobials for burns  
• Irrigate wounds to remove gross contamination with normal saline, sterile, or potable water, under low pressure (bulb syringe or gravity flow) without additives  
• Do not attempt to remove retained deep soft tissue fragments if criteria met.†  
  Provide cefazolin 2 g IV × 1 dose  
• Provide IV antimicrobials as soon as possible (within 3 hours)  
• Provide tetanus toxoid and immune globulin as appropriate  
• Enhance gram-negative coverage with aminoglycoside or fluoroquinolone not recommended  
• Addition of penicillin to prevent clostridial gangrene or streptococcal infection is not recommended  
• Redose antimicrobials if large volume blood product resuscitation  
• Use only topical antimicrobials for burns |
| Role 2/Level II with surgical support (IIb)/  
Role 3/Level III | Debridement and irrigation | • Irrigate wounds to remove contamination with normal saline or sterile water, under low pressure (5–10 PSI, e.g., bulb syringe or gravity flow) without additives (use 3 L for each Type I, 6 L for each Type II, and 9 L for each Type III extremity fractures)  
• Do not attempt to remove retained deep soft tissue fragments if criteria are met.†  
  Provide cefazolin 2 g IV × 1 dose  
• Do not obtain cultures unless infection is suspected  
• Surgical evaluation as soon as possible  
• Only dural and facial wounds should undergo primary closure  
• NPWT can be used  
• External fixation (temporary spanning) of femur/tibia fractures  
• External fixation (temporary spanning) or slit immobilization of open humerus/forearm fractures |
| | Surgical wound management | • Use only topical antimicrobials for burns  
• Antimicrobial beads or pouches may be used  
• Provide post-splenectomy immunizations if indicated  
• Irrigate wounds to remove contamination with normal saline or sterile water, under low pressure (5–10 PSI, e.g., bulb syringe or gravity flow) without additives (use 3 L for each Type I, 6 L for each Type II, and 9 L for each Type III extremity fractures)  
• Do not attempt to remove retained deep soft tissue fragments if criteria are met.†  
  Provide cefazolin 2 g IV × 1 dose  
• Do not obtain cultures unless infection is suspected  
• Surgical evaluation as soon as possible  
• Only dural and facial wounds should undergo primary closure  
• NPWT can be used  
• External fixation (temporary spanning) of femur/tibia fractures  
• External fixation (temporary spanning) or slit immobilization of open humerus/forearm fractures |
| | Post-injury antimicrobials | • Complete course of post-injury antimicrobials  
• Antimicrobial beads or pouches may be used  
• Provide post-splenectomy immunizations, if indicated  
• Irrigate wounds to remove contamination with normal saline or sterile water, under low pressure (5–10 PSI, e.g., bulb syringe or gravity flow) without additives (use 3 L for each Type I, 6 L for each Type II, and 9 L for each Type III extremity fractures)  
• Do not attempt to remove retained deep soft tissue fragments if criteria are met.†  
  Provide cefazolin 2 g IV × 1 dose  
• Do not obtain cultures unless infection is suspected  
• Wounds should not be closed until 3–5 d post-injury  
• Only dural and facial wounds should undergo primary closure  
• NPWT can be used  
• External fixation (temporary spanning) of femur/tibia fractures  
• External fixation (temporary spanning) or slit immobilization of open humerus/forearm fractures |

* Role of care, level of care, and echelon of care are considered synonymous with role currently the preferred US military term. Definitions of role/level/echelon of care: Role 1—self-aid, buddy aid, combat lifesaver, and combat medic/corpsman care at the point-of-injury; physician/physician assistant care at battalion aid station (BAS; US Army) or shock trauma platoon (US Marine Corps [USMOC]); no patient holding capacity; Role 2—medical company (includes forward support medical company, main support medical company, and area support medical company in US Army) or expeditionary medical support (EMEDS, US Air Force [USAF]); 72 h patient holding capacity, basic blood transfusion, radiography, and laboratory support. May be supplemented with surgical assets (forward surgical team, US Army; mobile field surgical team, USAF; forward resuscitative surgical system, USMOC); Role 3—combat support hospital (CSH, US Army), Air Force theater hospital (AFTH, USAF), or casualty receiving ships (USN); full inpatient capacity with intensive care units and operating rooms; Role 4—regional hospital (Landstuhl Regional Medical Center, Germany) or USNS hospital ships (USN), typically outside of the combat zone; general and specialized inpatient medical and surgical care; Role 5—care facilities within United States, typically tertiary care medical centers.† Criteria for allowing retained fragments to remain behind: entry/exit wounds <2 cm; no bone, joint, vascular, and body cavity involvement; no high-risk etiology (e.g., mine); no obvious infection; and assessable by X-ray. Adapted from Hospenthal, Murray, et al. (2011)
The 2011 CPGs also recommend infection control and prevention measures at each MTF, including: 1) hand hygiene with compliance monitoring and oversight, 2) patient isolation, 3) patient cohorting, 4) assignment of infection control officers (IOCs), and 4) antimicrobial stewardship. In response to previously identified challenges in infection control within the deployed environment (Hospenthal & Crouch, 2009), an educational course was created to train ICO personnel (Crouch, Murray, & Hospenthal, 2010); additionally, clinical support resources including standard operating procedure templates and teleconsultation services were deployed (Hospenthal et al., 2010). In 2010, the US Army required assignment of trained ICOs at all deployed Role 3 MTFs (Hospenthal, Green, et al., 2011).

Development of the 2011 infection control CPGs led to the identification of research gaps that, if addressed, would advance clinical recommendations for the prevention and treatment of combat-related infection (Table 7).

**Table 7. Research Gaps Relevant to Prevention of Combat Injury-Related Infection**

<table>
<thead>
<tr>
<th>Type of Research</th>
<th>Research Gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic Research</td>
<td>• Better understand the microbiome and biofilm development associated with wounds</td>
</tr>
<tr>
<td></td>
<td>• Better understand the pathophysiology and host immune response</td>
</tr>
<tr>
<td></td>
<td>• Increased epidemiological data, which should include data on invasive fungal infections</td>
</tr>
<tr>
<td>Applied Research</td>
<td>• Elucidation of ideal post-injury antimicrobials and antimicrobial therapy timing, including the shortest effective duration necessary</td>
</tr>
<tr>
<td></td>
<td>• Better understand topical wound therapies and topical decolonization/cleansing interventions</td>
</tr>
<tr>
<td></td>
<td>• Develop novel prevention, diagnostic, and treatment strategies/technologies</td>
</tr>
</tbody>
</table>

Adapted from Hospenthal, Murray, et al., 2011.

In 2011, Landstuhl Regional Medical Center implemented a process improvement CPG designed to reduce the time to diagnosis for IFIs and standardize the use of early antifungal therapies in patients at high risk of IFI (Lloyd, Weintrob, Rodriguez, et al., 2014). An analysis of DoDTR records from June 2009 to August 2011 found that earlier screening for IFIs achieved earlier diagnosis and treatment following implementation of the CPG, with a non-significant reduction in mortality from 11.4 percent to 6.7 percent (Lloyd, Weintrob, Rodriguez, et al., 2014). In 2012, the DoD JTTS published CPGs describing injury characteristics associated with IFI and providing IFI management guidelines (JTTS, 2012; Sheean, Tintle, & Rhee, 2015). In brief, these recommendations included aggressive surgical debridement; specific antifungal therapies, including topical applications consisting of Dakin’s solution (McCullough & Carlson, 2014) in conditions of suspected IFI; and antifungal bead application in culture-verified or strongly suspected circumstances (JTTS, 2012).

The Healthcare Infection Control Practices Advisory Committee (HICPAC), which resides within the Centers for Disease Control and Prevention (CDC), provides advice and guidance to the CDC and the Secretary of the Department of Health and Human
Services (HHS) regarding infection prevention, control, and surveillance strategies in US healthcare settings (CDC, 2016). These infection control guidelines may also inform practices in the military healthcare setting. HICPAC has also published guidelines for the management of MDROs (Siegel, Rhinehart, Jackson, Chiarello, & Healthcare Infection Control Practices Advisory Committee, 2007), environmental infection (Sehulster, Chinn, CDC, & HICPAC, 2003), as well as the appropriate disinfection and sterilization techniques within healthcare facilities (Rutala, Weber, Healthcare, & Infection Control Practices Advisory Committee (HICPAC), 2008). HICPAC is currently updating existing guidelines for the prevention of surgical site infections (Mangram, Horan, Pearson, Silver, & Jarvis, 1999), which is scheduled for release in 2016 (Berríos-Torres, 2016).

Improvement of Current Prevention and Treatment Approaches

In the years that followed the publication of the 2011 CPGs for infection prevention and the 2013 CPGs for IFI management, researchers have been working to advance existing prevention and treatment approaches. The US military launched the Multidrug-resistant Organism Repository and Surveillance Network to study drug-resistant infections in Service Members and improve the management of these infections in US Army hospitals (Lesho et al., 2011). This program instituted a comprehensive analysis and repository of clinical isolates that aims to provide the data necessary to improve clinical decision making (e.g., choosing the correct antibiotic), improve prevention efforts, and reduce medical costs.

Novel Antibiotics

Pathogens are increasingly acquiring resistance to the currently available arsenal of antibiotics, resulting in significant research efforts to identify and develop new drugs that are safe and effective for a wide range of bacterial infections (Brown & Wright, 2016; Taneja & Kaur, 2016). While new bacteriostatic and bacteriocidal antibiotics were successfully discovered from the 1960s to the early 1990s, the process of discovering or developing new antibiotics has become long and costly in the modern era and has not lead to new clinical successes (Brown & Wright, 2016). Challenges facing antibiotic development are highlighted in Table 8 below.
Table 8. Challenges and Solutions in Antibiotic Discovery

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efforts to identify and exploit new antibiotic targets utilizing genomics approaches have not been successful.</td>
<td>Efforts should be directed towards identifying novel targets and approaches in antibiotic discovery.</td>
</tr>
<tr>
<td>The mechanisms by which current antibiotics work is not well enough elucidated.</td>
<td>Additional efforts to understand the mechanism of action of existing antibiotics is needed.</td>
</tr>
<tr>
<td>Conventional methods of drug discovery (e.g., development of synthetic chemicals) are unlike the natural products initially discovered during the golden era.</td>
<td>Additional studies are needed to better understand the mechanisms by which bacteria block the permeability of antibiotics. In addition, more natural products should be revisited and new technologies utilized to assess the suitability of certain compounds as effective antibiotics.</td>
</tr>
<tr>
<td>Minimum inhibitory concentration (MIC) is the measure utilized to determine antibiotic effectiveness. However, MIC may not be an appropriate measure of effectiveness for novel antibiotics that work via different targets and pathways than those of traditional antibiotics.</td>
<td>Additional, more relevant measures of antibiotic effectiveness need to be developed.</td>
</tr>
</tbody>
</table>

Adapted from Brown & Wright, 2016.

Various drug discovery pathways are available for the identification and development of novel antibiotics. For example, assessing already FDA-approved drugs for their effectiveness as antibiotics is a mechanism by which novel uses for these drugs can be determined without the need for complex, lengthy approval processes (Andersson et al., 2016). Furthermore, as current medical practices have often implicated the use of broad spectrum antibiotics that are extremely challenging to develop and can contribute to resistance, a shift is needed towards development of narrow-spectrum treatments that target specific etiologic agents. The shift towards such treatments not only requires advancements in drug discovery, but also an increased understanding of the pathogens that cause disease and the mechanisms by which they can be rapidly and effectively identified (Brown & Wright, 2016).

In the European Union, the Innovative Medicines Initiative has invested more than €660 million to aid in the discovery and development of new antibacterial agents (Kostyanov et al., 2016). This initiative seeks to unite public and private organizations to combat the significant threat of drug-resistant pathogens. In the US, the Generating Antibiotics Incentives Now Act was signed in 2012 to extend the period of time by which specific antibiotics can be sold without competition from generics by 5 years (Brown & Wright, 2016).

In addition to the discovery of novel antibiotics, improving treatment parameters, such as timing or delivery methods, may enhance the management of combat wound-related bacterial infections. Researchers are exploring the use of new combinations of existing antibiotics to improve the treatment of infections, such as for *P. aeruginosa* (Chatterjee et al., 2016). Optimizing the timing of antibiotic administration has been noted as a key factor in the management of wound infection following open fractures (Sheean et al., 2015), with one recent study demonstrating that delay of more than 66 minutes following injury is an independent predictor of subsequent infection (Lack et al., 2015).
Furthermore, in a rat model, antibiotic delivery via a bioabsorbable gel more effectively suppressed *S. aureus* infection compared to commonly-used polymethylmethacrylate beads (Penn-Barwell, Murray, & Wenke, 2014). A novel chitosan and polyethylene glycol (PEG) sponge delivery system is being developed to enable the application of antibiotics in combination with antifungals as an adjunctive therapy (Parker et al., 2015).

**Novel Antifungals**

Recent antifungal recommendations for wound infection include the use of amphotericin B and triazonole (Tribble & Rodriguez, 2014; Warkentien et al., 2012); however, further study is needed to elucidate the pharmacokinetics and wound penetration of these more commonly used antifungals (Akers et al., 2015). Recent success using Dakin’s solution (Barsoumian et al., 2013; McCullough & Carlson, 2014) in conjunction with NPWT has indicated another potential approach to combatting fungal infections (Lewandowski et al., 2013). Furthermore, a recent review by Moriyama et al. (2014) identified clinical trials investigating several potential antifungal agents for *Candida* spp., including two for invasive Candidemia: isavuconazole (Astellas Pharma Inc, 2016) and SCY-078 (Scynexis, Inc., 2016).

**Antimicrobial Textiles and Dressings**

Researchers are working to develop polymers or textiles with antimicrobial or antifungal properties via the utilization of nanotechnology or other techniques (El-Shanshory, Chen, El-Hamshary, Al-Deyab, & Mo, 2015; Hossain et al., 2016; Jain et al., 2014). The antimicrobial properties of silver nanoparticles have been studied for many years for different infection control applications, including the incorporation into textile fibers and wound dressings to inhibit microbial growth and biofilm formation (Marin et al., 2015; Sacco, Travan, Borgogna, Paoletti, & Marsich, 2015; Velázquez-Velázquez et al., 2015), which are particularly useful in military environments (Barillo, Pozza, & Margaret-Brandt, 2014). Wound dressings with antimicrobial properties are commercially available (Guthrie et al., 2014; Velázquez-Velázquez et al., 2015). A DoD Small Business Innovation Research (SBIR) program is seeking to develop antimicrobial textiles for military uniforms and combat medical applications, such as for infective wound dressings, hospital textiles, bedding, and medical devices (SBIR Source, 2016).

**Negative Pressure Wound Therapy**

Researchers continue to evaluate NPWT as an adjuvant treatment for wound infection (Hinck, Franke, & Gatzka, 2010; Murray, Obremskey, et al., 2011). Application of NPWT reduced mortality and *P. aeruginosa* levels in a mouse model of burn wounds (Liu et al., 2014). In a swine model of blast injury, NPWT significantly reduced bacterial loads (Li et al., 2013). However, observations that NPWT can reduce the effectiveness of local antibiotics calls for future work to better understand how adjuvant use of this treatment with antibiotics can best be achieved (Stinner, Hsu, & Wenke, 2012).

**Biofilms**

Some prevention and treatment research efforts are directed at interruption or inhibition of biofilms. Studies assessing the effectiveness of PEG sponges (Parker et al., 2015).
and wound dressings (Franci et al., 2015; Yang, Larose, Della Porta, Schultz, & Gibson, 2016) on bacterial infections have also demonstrated biofilm reduction. Several topical antimicrobial agents have emerged recently for biofilm disruption in burn wound infections (Sevgi et al., 2013). Recently, application of a nanoemulsion developed to increase the solubility of chlorhexidine, a non-antibiotic agent, demonstrated effectiveness against a MRSA biofilm both in vitro and in vivo (Song et al., 2016). Some alternative prevention and treatment approaches (see below) may also be effective against wound biofilms.

**Development of Alternative Prevention and Treatment Approaches**

The emergence of drug- and multi drug-resistance has prompted US and international researchers across academic, private, and government organizations to develop alternative prevention and treatment approaches for bacterial and fungal infections. Experts have noted that while antibiotic stewardship and hospital hygiene should continue to play a role in infection control in light of drug resistance, development of new and alternative treatments is needed (Garcia-Quintanilla, Pulido, & McConnell, 2013). Successful development of alternative prevention or treatment approaches could potentially be applied to minimizing wound infections following blast related injuries in US military Service Members. Recent review articles identified by this literature review provide an overview of ongoing and efforts to develop of alternative prevention and treatment approaches for drug- or multidrug-resistant pathogens.

A major area of research for development of alternative prevention and treatment approaches is immunological approaches against bacterial pathogen (Ahmad, El-Sayed, Haroun, Hussein, & El Ashry, 2012; Chatterjee et al., 2016; Chen, 2015; Sause, Buckley, Strohl, Lynch, & Torres, 2016) and fungal pathogen (Medici & Del Poeta, 2015; Santos & Levitz, 2014) infections. Other alternative approaches include phage therapy, antimicrobial peptides, photodynamic therapy, quorum sensing, nanoparticles, iron chelators, lectin inhibitors, FimH inhibitors, lactoferrin, hypothiocyanite, bioengineered tissue, bacterial gene transfer, probiotics, and plant compounds (Chatterjee et al., 2016; García-Quintanilla, Pulido, López-Rojas, Pachón, & McConnell, 2013; Tillotson & Theriault, 2013).

**Active and Passive Immunological Approaches**

Immunological approaches to preventing or treating infection are classified as either active or passive. Active immunization (i.e., vaccination) is the administration of an antigen that elicits a host immune response, including immunological memory, that confers protection against subsequent exposure to the intended pathogen. Passive immunization is the transfer of antibodies that elicit endogenous antibacterial or antifungal activity in a non-immune host.

While vaccination is a cost-effective and proven method of preventing infection, it requires a period of time for the development of immunological memory responses and consequently is not ideal for application in urgent cases or nosocomial infections (Garcia-Quintanilla et al., 2013). Vaccine candidates are based on either single, purified...
antigens (i.e., monovalent vaccines), or those with multiple antigenic components (i.e., multivalent vaccines), such as from whole cells or bacterial membrane complexes. While each of these approaches confers notable advantages and disadvantages (Table 9) the failure of single antigen approaches in recent years is prompting researchers to emphasize multiple antigen approaches (Giersing, Dastgheyb, Modjarrad, & Moorthy, 2016).

### Table 9. Advantages and Disadvantages of Single and Multiple Antigen Vaccine Strategies

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Monovalent vaccines   | • Well-defined composition  
• Low levels of reactogenic impurities  
• Existence of standardized methods for industrial production                                                                 | • Concerns regarding expression of the antigen in all strains  
• Adaptation to immune pressure via antigen down-regulation is more feasible  
• Purification process can alter the native antigen conformation |
| Multivalent vaccines  | • Higher strain coverage due to targeting of multiple antigens  
• Reduced risk of adaptation due to immune pressure  
• Antigens can be maintained in their native conformations                                                                 | • Difficult to standardize all vaccine components between production lots  
• Presence of impurities that could produce side effects (e.g., lipopolysaccharide) |

Adapted from Garcia-Quintanilla et al. 2013

Approaches to prevention and treatment utilizing passive immunity have the potential to confer immediate protection following administration; however, these approaches are largely unproven in the clinical setting and are likely to be more costly (Garcia-Quintanilla et al., 2013).

Most scientific reviews and primary research studies regarding the development of immune-based approaches to prevention and treatment against bacterial infections are directed towards a specific bacterial species. The following summaries of active and passive immunological development research literature are organized by pathogen.
Acinetobacter baumannii

According to a review by Chen (2015), the development of vaccine candidates for the prevention of A. baumannii infections lags behind that of other nosocomial infections, with no vaccine candidates for this pathogen being evaluated in clinical trials. Researchers have successfully identified multiple antigens that induce humoral immunity and confer protection against subsequent bacterial challenges in animal models; however, a better understanding of the underlying mechanisms of immunoprotection against this pathogen is needed. Given the differences between animal models, additional research is needed to identify the ideal animal model to best mimic human infections and immune responses against A. baumannii infection and vaccination (Chen, 2015).

Examples of recent primary research studies reflect ongoing interest and progress in vaccine development targeting A. baumannii. In a study by Zhang et al. (2016), mice intranasally immunized with outer membrane protein A produced systemic and mucosal antibodies against A. baumannii. In addition, these animals had a significantly higher survival rate than non-immunized animals following challenge with drug-resistant strains of A. baumannii. Huang et al. (2016) recently identified another outer membrane protein, Omp22, as a potential protective antigen by demonstrating a high degree of conservation, as well as increased survival rates in mice treated with Omp22 in a sepsis model. The authors also note other previously reported antigen candidates, including iron-regulated outer membrane proteins, formalin-inactivated whole cells, outer membrane complexes, outer membrane vesicles, biofilm-associated protein, poly-N-acetyl-β-(1–6)-glucosamine, trimeric autotransporter protein, and K1 capsular polysaccharide, as potentially protective antigens against A. baumannii.

Pseudomonas aeruginosa

A wide range of immunological approaches have been investigated for the treatment of drug-resistant P. aeruginosa, which is a major cause of combat-related wound infection; however, none are currently commercially available (Chatterjee et al., 2016). Priebe & Goldberg (2014) describe various immunological treatment approaches and identify two with relative promise: 1) a vaccine candidate based on outer membrane proteins F and I, which has been studied in recent clinical trials (Valneva Austria GmbH, 2016a, 2016b); and 2) the passive transfer of anti-PcrV antibodies that block the Type Three Secretion System of P. aeruginosa, which was shown to mediate significant bacterial clearance from the lungs of infected mice (Baer et al., 2009). Priebe & Goldberg (2014) also describe challenges in vaccine development against P. aeruginosa. For example, current animal models don’t accurately reflect the comorbid conditions that typically characterize P. aeruginosa-infected human patients, such as combat injuries, cystic fibrosis, and an immunosuppressed state. Additionally, vaccine trials are plagued by design challenges, including a minimal understanding of the association between bacterial colonization and infection. The authors suggest that future vaccine development be tailored toward specific patient populations and specific types of infections.
**Staphylococcus aureus**

Vaccine development against *S. aureus* has focused largely on multivalent approaches following the failure of several single antigen vaccine candidates (Giersing et al., 2016). Notably, the vaccine SA4Ag (Anderson et al., 2012) is currently being evaluated in two Phase II clinical trials (Pfizer, 2016a, 2016b). Three passive immunization candidates, MEDI4893 (MedImmune LLC, 2016), 514G3 (XBiotech, Inc., 2016) and KBSA301 (Aridis Pharmaceuticals, Inc., 2016), are currently being evaluated in Phase II clinical trials. The vaccine candidate NDV3 is under Phase I clinical trial evaluation (NovaDigm Therapeutics, Inc., 2016) and may also be protective against *Candida* infection. As reviewed by Giersing et al. (2016), there are additional vaccine candidates in pre-clinical phases of investigation. In this review, the authors also noted that vaccine development has not been included in national and/or international antimicrobial resistance agendas and requires stronger consideration by policy makers. Additionally, efforts to develop *S. aureus* vaccines in low- and middle-income countries have yet to be initiated.

Three challenges have hindered progress in the development of passive immune approaches to combat *S. aureus*: 1) an overreliance on strategies that target a single antigen, 2) dependence on phagocytic activity as an indicator of efficacy, and 3) a lack of animal model-based research that is readily translatable to clinical studies (Sause et al., 2016). Current approaches are focused on combining antibodies to target different antigens, targeting the mechanisms by which bacteria circumvent immune responses, and tailoring approaches to attack *S. aureus* in both extracellular and intracellular environments.

**Klebsiella pneumoniae**

Researchers are pursing active and passive immune therapy approaches against *K. pneumoniae* (Ahmad et al., 2012); however, there are currently no active clinical trials directed toward wound infections. Recent advances in the biological understanding of T helper cells, specifically Th17 cells, are contributing to potential vaccine development efforts against this pathogen (Kumar, Chen, & Kolls, 2013). Researchers are working to identify novel vaccine antigen candidates by screening cDNA-based expression libraries (Hoppe, Bier, & von Nickisch-Rosenegk, 2014) and small fragment genome libraries (Lundberg, Senn, Schüler, Meinke, & Hanner, 2013). *K. pneumoniae*-derived extracellular vesicles, also identified as a potential vaccine candidate, were recently found to confer protection against bacterial infection in vitro (Lee et al., 2015).

**Fungal Pathogens**

Vaccines and passive antibody transfer present potential alternative therapies for the treatment of fungal infection (Datta & Hamad, 2015). Vaccines are in development for several fungal pathogens, including *C. albicans*, *Aspergillus spp*, *Cryptococcus spp*, *Blastomyces spp*, *Paracoccidioides brasiliensis*, and *Sporothrix spp* (Kniemeyer et al., 2016; Medici & Del Poeta, 2015; Nanjappa & Klein, 2014; Santos & Levitz, 2014; Wang et al., 2015). A vaccine candidate for *C. albicans*, NDV-3, which also confers protection against *S. aureus*, has completed a Phase I clinical trial (De Bernardis et al., 2012; NovaDigm Therapeutics, Inc., 2016; Schmidt et al., 2012). Wang et al. (2015) reviewed
approaches that could strengthen immune responses to vaccines against *C. albicans*, including targeting cell wall proteins, developing new or modified adjuvants, delivery of vaccine by dendritic cells, and passive immunization. The development of fungal vaccine candidates is hindered by several challenges (Edwards, 2012; Medici & Del Poeta, 2015), which include: 1) safety concerns regarding the use of live attenuated vaccines; 2) risks associated with vaccine administration to immunocompromised patients, which as noted previously, are a major at-risk population for fungal infection; and 3) the high cost of vaccine development in relation to the relatively small at-risk target population, which may not attract market-based investment incentives. In addition to vaccines, researchers are developing passive antibody transfer therapies and dendritic cell immunotherapies to combat fungal infections (Santos & Levitz, 2014).

**Other Alternative Approaches**

In addition to immunotherapy, additional alternatives to antibiotic or antifungal medication are being pursued as treatments for drug-resistant infections (Chatterjee et al., 2016; García-Quintanilla et al., 2013; Tillotson & Theriault, 2013). These alternatives include phage therapy, antimicrobial peptides, photodynamic therapy, quorum sensing, and nanoparticles, iron chelators, lectin inhibitors, FimH inhibitors, lactoferrin, hypothiocyanite, bioengineered tissue, bacterial gene transfer, probiotics, and plant compounds.

*Phage Therapy*

Phage therapy refers to the application of bacteriophages (i.e., viruses that infect bacteria) to combat infections (Qadir, 2015; Wittebole, De Roock, & Opal, 2014). Phage therapy has been used as an alternative to antibiotics in Eastern Europe and the Soviet Union for over 60 years (Mihu & Martinez, 2011; Pires, Vilas Boas, Sillankorva, & Azeredo, 2015), and is being investigated as a potential treatment for *A. baumannii* infection (García-Quintanilla et al., 2013), *P. aeruginosa* (Chatterjee et al., 2016), *S. aureus* (Kaźmiarczak, Górski, & Dąbrowska, 2014; Magana et al., 2015), and *K. pneumoniae* (Taneja & Kaur, 2016).

A study by Lin et al. (2010) indicated that *A. baumannii*-specific phage rapidly lysed 89 percent of *A. baumannii* strains tested, 97.3 percent of which were multidrug-resistant. Since then, *in vitro* studies have confirmed the efficacy of additional phage against *A. baumannii*; however, it appears that each phage has a limited spectrum of specificity to *A. baumannii* strains (García-Quintanilla et al., 2013). An *in vivo* study investigated the effect of the phage BS46 in *A. baumannii* infection, finding that treatment with BS46 conferred protection to mice inoculated with five times the LD$_{50}$ (i.e., the known lethal dose at which 50 percent of the mice die following inoculation) of this particular *A. baumannii* strain (García-Quintanilla et al., 2013). As the phage investigated have a high specificity for *A. baumannii*, these treatments may preserve the host flora, unlike many conventional antibiotic therapies (García-Quintanilla et al., 2013).

Research of phage therapy as a treatment for *P. aeruginosa* (Chatterjee et al., 2016; Krylov, Shaburova, Krylov, & Pleteneva, 2012; Pires et al., 2015) has led to clinical trials in patients with burns (Pherecydes Pharma, 2016; Rose et al., 2014) and chronic otitis
As reviewed by Chatterjee et al. (2016), lytic phages prevent in vitro biofilm formation by P. aeruginosa. These authors also note that phage therapy used in combination with antimicrobials, such as streptomycin, ceftriaxone, or chlorine, has a synergistic effect that reduces bacterial numbers and biofilm formation. Studies have also indicated that using multiple phages concurrently is more effective against P. aeruginosa than the use of a single phage (Hagens, Habel, & Bläsi, 2006; Torres-Barceló et al., 2014). Phage therapy has demonstrated efficacy against P. aeruginosa infection in several animal studies (Debarbieux et al., 2010; Hall, De Vos, Friman, Pirnay, & Buckling, 2012; Hawkins, Harper, Burch, Anggård, & Soothill, 2010; Heo et al., 2009; Khairnar, Raut, Chandekar, Sanmukh, & Paunikar, 2013; Marza, Soothill, Boydell, & Collyns, 2006; McVay, Velásquez, & Fralick, 2007; Morello et al., 2011; Tiwari, Kim, Rahman, & Kim, 2011; Watanabe et al., 2007).

The advantages of phage therapy over antibiotics includes potentially greater effectiveness against biofilms and the capacity for genetic modification to reduce host inflammatory responses (Chatterjee et al., 2016). Potential limitations of phage therapy include rapid clearance of bacteriophage by the host immune response and the production of anti-bacteriophage antibodies (Mihu & Martinez, 2011). Additionally, bacteria can develop a resistance to bacteriophage and there is also a limited understanding of the safety of phage therapy (Pires et al., 2015). Further studies are needed to advance the clinical application of phage therapy for multidrug-resistant infections (García-Quintanilla et al., 2013; Mihu & Martinez, 2011).

**Antimicrobial Peptides**

Antimicrobial peptides, which are naturally occurring components of the immune system, are being pursued as a potential treatment for wound infection against several Gram negative bacterial species (Otvos & Ostorhazi, 2015; Tillotson & Theriault, 2013). Research investigating the use of synthetic antimicrobial peptides against P. aeruginosa has yielded positive results (Chatterjee et al., 2016; Melvin et al., 2016), including a Phase I clinical trial (Polyphor Ltd., 2016). Antibacterial peptides have been studied in vitro and in animal models of A. baumannii infection (García-Quintanilla et al., 2013). One candidate, A3-APO, has demonstrated efficacy in a blast injury model (Ostorhazi et al., 2010). Limitations to the clinical use of antimicrobial peptides include susceptibility to degradation by endogenous enzymes, toxicity, and high development costs (Peters, Shirliff, & Jabra-Rizk, 2010).

**Photodynamic Therapy**

Photodynamic therapy, which generates antimicrobial reactive oxygen species through the application of light and photoreactive dyes, is a potential treatment for bacterial infections (Magana et al., 2015) and fungal infections (Baltazar et al., 2015), including the prevention of biofilm formation (Biel, 2015). Zhang et al. (2014) demonstrated that a multidrug-resistant A. baumannii strain isolated from a military combat wound patient was susceptible to photodynamic therapy in vitro. In a murine burn wound model of A. baumannii infection, treatment with photodynamic therapy killed bacteria and improved survival without having an impact on wound healing (Dai, Huang, & Hamblin, 2009).
Photodynamic therapy is limited by the fact that it must be applied topically and is non-selective, so it may be damaging to non-infected, healthy host cells (García-Quintanilla et al., 2013).

**Quorum Sensing**

Quorum sensing is a cell communication phenomenon involving the cell-density-dependent release of molecules that coordinate collective cell responses and gene expression in a given cell population (Castillo-Juárez et al., 2015; Tillotson & Theriault, 2013). Quorum sensing controls bacterial virulence factors, which is targeted by quorum sensing inhibitors or quorum quenching approaches (Grandclément, Tannières, Moréra, Dessaux, & Faure, 2016). Research in the area of quorum sensing and quenching is being applied to the development of treatments against *P. aeruginosa* (Chatterjee et al., 2016), *A. baumannii* (Polkade, Mantri, Patwekar, & Jangid, 2016), and *S. aeurus* infections (Khan, Yeh, Cheung, & Otto, 2015; Magana et al., 2015). Because of their potential to inhibit biofilm formation, quorum sensing inhibitors may be useful prophylactics on medical devices, such as catheters and ventilators, to prevent infection (Tillotson & Theriault, 2013).

**Nanoparticles**

The application of nanoparticles is being investigated for use as treatment for a number of diseases, including bacterial infections (Franci et al., 2015; Tillotson & Theriault, 2013; Yah & Simate, 2015). Nanoparticles are small enough to diffuse though the cell wall of bacterial cells and have demonstrated bactericidal effects against pathogens, including *S. aeurus* (Magana et al., 2015) and *P. aeruginosa* (Chatterjee et al., 2016). However, nanoparticle research is currently limited to preclinical studies.

**Iron Chelators**

The application of iron chelators or gallium-based formulations is a potential antibacterial therapeutic approach (Foley & Simeonov, 2012; Kelson, Carnevali, & Truong-Le, 2013; Thompson, Corey, Si, Craft, & Zurawski, 2012). These substances interrupt iron uptake, which is critical for bacterial survival and biofilm formation (Banin, Vasil, & Greenberg, 2005). Iron chelators and gallium nitrate both have shown modest antibacterial effects *in vitro* against *A. baumannii* (Thompson et al., 2012). Mice treated *in vivo* with gallium had less *A. baumannii* compared to untreated animals (de Léséleuc, Harris, KuoLee, & Chen, 2012). Gallium has also been shown to inhibit *A. baumannii* growth in human serum *in vitro* (Antunes, Imperi, Minandri, & Visca, 2012; García-Quintanilla et al., 2013). Similarly, gallium has been shown to be effective for the treatment of *P. aeruginosa* infections *in vivo* (Chatterjee et al., 2016; Kaneko, Thoendel, Olakanmi, Britigan, & Singh, 2007; Rangel-Vega, Bernstein, Mandujano-Tinoco, García-Contreras, & García-Contreras, 2015). In combination with certain antibiotics, gallium treatment prevents *P. aeruginosa* biofilm production (Halwani et al., 2008; Moreau-Marquis, O’Toole, & Stanton, 2009).
Lectin Inhibitors

Lectin inhibitors prevent microbes from attaching to host epithelial cells (Chemani et al., 2009). *In vitro* use of lectin inhibitors has been shown to prevent *P. aeruginosa* biofilm production (Grishin, Krivozubov, Karyagina, & Gintsburg, 2015). In a clinical trial of cystic fibrosis patients, treatment with fructose/galactose inhalation therapy reduced numbers of *P. aeruginosa* in the sputum of these patients (Hauber et al., 2008; Kolomiets et al., 2009; Taneja & Kaur, 2016).

FimH Inhibitors

FimH is an fimbrial adhesin utilized by *E. coli* to adhere to host epithelial cells, allowing the bacteria to effectively colonize and invade the host cells to cause infection (Tchesnokova et al., 2011). Notably, FimH inhibitors derived from mannoses inhibited *in vitro* biofilm formation and prevented/treated a urinary tract infection in a murine infection model (Chen et al., 2009; Tillotson & Theriault, 2013).

Lactoferrin & Hypothiocyanite Therapy

Hypothiocyanite, a bactericidal molecule present in airways, has been shown to control *P. aeruginosa* infection in patients with cystic fibrosis (Georgi et al., 2011). The European Medicines Agency and the US FDA have granted a hypothiocyanite and lactoferrin inhalable treatment orphan drug status (Hurley, Cámara, & Smyth, 2012). Furthermore, this drug combination was able to effectively prevent *P. aeruginosa* biofilm formation and, when used with the antibiotics tobramycin and aztreonam, was able to reduce already established biofilms (Chatterjee et al., 2016; Moreau-Marquis, Coutermarsh, & Stanton, 2015).

Bioengineered Tissue

Bioengineering human skin cells to produce peptides with microbicidal activity may be another novel mechanism of combatting multidrug-resistant pathogens, such as *A. baumannii* (Heilborn et al., 2003; Mihu & Martinez, 2011; Thomas-Virnig et al., 2009). For example, genetically engineered non-tumorigenic, pathogen-free human keratinocyte progenitor cells have been developed to produce human cathelicidin hCAP-18, which is a host defense peptide that has been implicated in wound healing and antimicrobial activity (Thomas-Virnig et al., 2009). Notably, *A. baumannii*-infected burn wounds of mice treated with NIKShCAP-18 cells resulted in a significant reduction in bacterial numbers and improved wound healing, compared to control untreated animals (Heilborn et al., 2003).

Bactericidal Gene Transfer Therapy

Bactericidal gene transfer therapy utilizes the transfer of plasmids containing lethal genes from host cells into targeted bacterial pathogens (Mihu & Martinez, 2011; Shankar et al., 2007). In a murine burn wound infection model of a multidrug-resistant strain of *A. baumannii*, animals treated with this therapy had reduced wound colonization when compared to untreated control animals (Shankar et al., 2007). While this treatment is considered low risk in terms of the development of resistance by
pathogens, it is limited by the requirement for donor cells to come into physical contact with the bacteria. Therefore, this treatment may only be effective for the treatment of surface wound infections (MiHu & Martínez, 2011).

**Probiotics**

Probiotics, such as those of the *Lactobacillus* spp., can enhance the host immune response, produce antimicrobial compounds, and inhibit quorum sensing in other bacterial species (Alexandre, Le Berre, Barbier, & Le Blay, 2014; Chatterjee et al., 2016). Studies have indicated that some lactobaccilli can inhibit the growth, cytotoxicity, elastin production, and biofilm formation of certain strains of *P. aeruginosa* (Valdés, Peral, Rachid, Santana, & Perdigón, 2005; Varma, Nisha, Dinesh, Kumar, & Biswas, 2011). Additional studies in larger test populations are needed to determine the utility of probiotics in preventing and treating infections in humans (Forestier et al., 2008).

**Plant Compounds**

Treatment with plant and other naturally derived compounds, potentially in combination with conventional antimicrobial therapies, are currently being investigated (MiHu & Martínez, 2011; Taneja & Kaur, 2016). For instance, ginger-derived antioxidant compounds (6-dehydrogingerdione, 10-gingerol, 6-shogaol, and 6-gingerol) have been shown to inhibit the growth of a multidrug-resistant strain of *A. baumannii* in vitro, particularly when combined with the antibiotic tetracycline (Wang et al., 2010). Similar results have been observed for propolis extract when used in combination with antibiotics against *S. aureus* (Fernandes Júnior et al., 2005). Other compounds, such as arylomycin, mannopeptimycin, and nocathiacin, are also currently under investigation (Taneja & Kaur, 2016).

Additional potential alternative prevention and treatment approaches for drug-resistant pathogens (Chatterjee et al., 2016; García-Quintanilla et al., 2013) include anti-adhesion therapeutics (Krachler, Mende, Murray, & Orth, 2012; Thomas, 2010), electric field application (Golberg et al., 2015; Khan et al., 2016), efflux pump inhibitors (Gill, Franco, & Hancock, 2015), radioimmunotherapy (MiHu & Martínez, 2011), and nitric oxide (NO)-based therapies (MiHu et al., 2010; MiHu & Martínez, 2011).

**Clinical Trials**

Additional detail about clinical trials described above for potential alternative treatment therapies for wound infection can be found below in Table 10.
### Table 10. Clinical Trials of Alternative Therapies for Wound Infection

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Title</th>
<th>Purpose</th>
<th>Status (as of July 2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00413218</td>
<td>Isavuconazole (BAL8557) in the Treatment of Candidemia and Other Invasive Candida Infections</td>
<td>Compare the safety and efficacy of Isavuconazole versus caspofungin followed by voriconazole in the treatment of candidemia and other invasive Candida infections.</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT02244606</td>
<td>Oral SCY-078 vs Standard-of-Care Following IV Echinocandin in the Treatment of Invasive Candidiasis</td>
<td>Compare the safety, pharmacokinetics, and efficacy of oral SCY-078 vs. standard-of-care following initial intravenous echinocandin therapy in the treatment of invasive candidiasis.</td>
<td>Recruiting participants</td>
</tr>
<tr>
<td>NCT00876252</td>
<td>Study Assessing Immunogenicity and Safety of IC43 In Intensive Care Patients</td>
<td>Randomized, placebo-controlled, partially blinded phase 2 pilot study. Multicenter study (approximately 50 centers) in approximately 9 countries. Proposed start date is December 2008.</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT01563263</td>
<td>Confirmatory Phase II/III Study Assessing Efficacy, Immunogenicity and Safety of IC43</td>
<td>Confirmatory, randomized, placebo-controlled, multi-center, double-blinded phase II/III study. The study population consists of male or female intensive care unit (ICU) patients with a need for mechanical ventilation for more than 48 hours, aged between 18 and 80 years.</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT02492958</td>
<td>SA4Ag Safety, Tolerability, and Immunogenicity Study in Japanese Adults</td>
<td>Evaluate the safety, tolerability, and immunogenicity of a single dose of Staphylococcus aureus 4 antigen vaccine in Japanese adults aged 20 to &lt;86 years.</td>
<td>Ongoing; not recruiting</td>
</tr>
<tr>
<td>NCT02388165</td>
<td>Safety and Efficacy of SA4Ag Vaccine in Adults Having Elective Posterior Instrumented Lumbar Spinal Fusion Procedure (STRIVE)</td>
<td>Determine whether the SA4Ag vaccine can prevent postoperative Staphylococcus aureus infections in patients who are undergoing elective spinal fusion; evaluate the safety of SA4Ag in patients who are undergoing elective spinal surgery.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02296320</td>
<td>Study of the Efficacy and Safety of MEDI4893 (SAATELLITE)</td>
<td>Safety and efficacy of MEDI4893 in prevention of pneumonia caused by S. aureus in high-risk patients.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02357966</td>
<td>A Study of the Safety and Efficacy of 514G3 in Subjects Hospitalized With Bacteremia Due to Staphylococcus Aureus</td>
<td>Evaluate the maximum safe dose of the true human monoclonal antibody, 514G3, in the treatment of patients with Staphylococcus Aureus bacteremia. Preliminary evidence of efficacy will be evaluated as well. Patients will receive 514G3 plus antibiotics or placebo plus antibiotics in approximately a 3 to 1 ratio.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01589185</td>
<td>Safety, Pharmacokinetics and Efficacy of KBSA301 in Severe Pneumonia (S. Aureus)</td>
<td>Assess the safety, tolerability, pharmacokinetics, pharmacodynamics and clinical outcome of patients who have severe pneumonia caused by S. aureus after a single intravenous administration of KBSA301 in addition of standard of care antibiotic treatment.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01273922</td>
<td>Safety and Immunogenicity Study of a Recombinant Protein Vaccine (NDV-3) Against S. Aureus and Candida</td>
<td>Evaluate the safety, tolerability and immunogenicity of the investigational vaccine, NDV-3.</td>
<td>Completed</td>
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<tr>
<td>NCT02096315</td>
<td>Safety, Efficacy and PK/PD of POL7080 in Patients With Exacerbation of Non-cystic Fibrosis Bronchiectasis.</td>
<td>Test whether POL7080 is effective in patients with exacerbation of non-cystic fibrosis bronchiectasis caused by Pseudomonas aeruginosa infection.</td>
<td>Completed</td>
</tr>
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</table>
Discussion

The emergence of advanced therapeutic approaches has significantly reduced the incidence and impact of infections following combat injuries (Eardley et al., 2011). However, wound infection following combat- and blast-related injuries continues to be a significant source of morbidity and mortality in the modern era of military healthcare (Blyth et al., 2015; Hospenthal & Murray, 2011; Weintrob et al., 2015). The increasing role of nosocomial transmission in the military healthcare system (Burns et al., 2012; Johnson et al., 2007; Kaspar et al., 2009; Keen et al., 2010; Mende et al., 2014; Mody et al., 2009; Murray, Hospenthal, et al., 2011; Petersen et al., 2007; Sheppard et al., 2010; Wallum et al., 2015; Weintrob et al., 2013) and the emergence of drug-resistant organisms (Calhoun et al., 2008; Hospenthal, Crouch, et al., 2011; Murray, 2008a; Scott et al., 2007; Vento et al., 2013) present significant challenges for improving infection control, as well as for diagnosis, prevention, and treatment approaches. Further improving existing knowledge about infection epidemiology and risk factors can inform and advance existing approaches for diagnosis, prevention, and treatment.

Improved approaches to diagnosing and detecting infection would promote better prediction of infection, earlier diagnosis, earlier treatment application, individually tailored treatments, and improved understanding of the epidemiology of wound infection in US military Service Members. While CPGs are in place to guide detection and diagnosis of wound infection (Hospenthal, Murray, et al., 2011; JTTS, 2012), limited information is available about specific diagnostic capabilities at each level of the JTTS, and there are variations in the diagnostic capacity and availability of diagnostic resources in MTFs. US and international researchers from government, private, and non-profit organizations are seeking to develop infection biomarkers (Brown, Safford, Caramanica, & Elster, 2010; Hahm et al., 2011; Tegl et al., 2015). These emerging approaches could be applied to detection and diagnosis of combat wound infection in US military Service Members. Development of novel objective biomarkers is needed to enable faster and more precise wound infection diagnosis capabilities.

Existing CPGs, including infection control recommendations and resources for MTFs, provide an evidence-based framework for prevention and treatment of combat-related wound infection in the military healthcare system during the modern era of drug resistance (Hospenthal, Murray, et al., 2011; JTTS, 2012). Medical experts continue to explore ways to improve existing infection control practices and medical treatment approaches, including antimicrobials. Research efforts are also underway to develop new prevention and treatment approaches, including vaccines, as alternatives to antimicrobials (Chatterjee et al., 2016; Chen, 2015; Garcia-Quintanilla et al., 2013, 2013; Medici & Del Poeta, 2015; Sause et al., 2016).

Research Needs

Diagnosis, prevention, and treatment of wound infection is subject to the pathophysiology of infection and underlying mechanisms of immunological response. Experts have identified specific basic science research needs relevant to minimizing wound infection following blast-related injury (Akers et al., 2015; Brown & Wright, 2016;
Chen, 2015; Hospenthal, Murray, et al., 2011; Hurlow et al., 2015). These research needs include studies directed at achieving a better understanding of:

- The pathophysiology of infection and the host immune response to infection
- The association between biofilms and infection
- The microbiome associated with wound infection
- The mechanism of action of existing antibiotics, including how bacteria impede the permeability of antibiotics
- The underlying mechanisms of immunoprotection against pathogens, particularly A. baumannii
- Topical wound therapies, including decolonization and cleansing interventions
- The pharmacokinetics and wound penetration of antifungals

Drawing upon the experiences of the military healthcare system can greatly inform advances in the identification and care of wound infection following blast-related injuries. Analysis of identified research literature, including the recommendations put forth by military healthcare experts (Hospenthal, Murray, et al., 2011), highlights research needs that, if achieved, would contribute to minimizing wound infection following blast-related injury:

- Additional epidemiological study of bacterial and fungal infection in the military healthcare system
- Identifying the evidence-base for use of clinical signs for diagnosis of infection to support current JTTS CPGs
- Assessment of post-injury antimicrobial delivery and subsequent infection rates in military populations
- Assessment of the availability and use of molecular techniques for detection and diagnosis of wound infection in the military healthcare system

Existing capability gaps in research, as well as diagnosis, prevention, and treatment approaches, could potentially be met with inception of novel products or methods. Bridging these gaps would provide researchers and clinicians with new tools that would minimize wound infection following combat- or blast-related injury. Experts have identified these research needs (Brown & Wright, 2016; Hospenthal, Murray, et al., 2011; Hurlow et al., 2015; Priebe & Goldberg, 2014; Sause et al., 2016; Wang et al., 2015), which include development of:

- Robust, disposable, and economically viable intelligent wound dressings that generate clinically useful information
- Techniques to confirm the presence of a wound biofilm
- Algorithms for the most effective post-injury antimicrobials, including timing of antimicrobial application and determination of shortest effective duration
More relevant measures of antibiotic effectiveness

Vaccine candidates tailored toward specific patient populations and specific types of infections

Approaches to strengthen immune responses to vaccines against *C. albicans* and other pathogens as appropriate, including targeting cell wall proteins, developing new or modified adjuvants, and delivery of vaccine by dendritic cells

Passive immunotherapy approaches that: 1) combine antibodies to target different antigens and 2) attack *S. aureus* or other appropriate pathogens in both extracellular and intracellular environments

Animal models that reflect the comorbid conditions associated with wound infections in humans, such as immunosuppression

Wound infection is an international concern, affecting military Service Members and civilians globally. Understanding wound infection as a global issue is especially important considering the increasing role of nosocomial transmission and the international rise of drug-resistant infections. Emphasizing the need for advances in infection diagnosis and treatment, including vaccines or other alternative approaches, in national and international research agendas will encourage stronger consideration by policymakers. US military researchers can continue to partner with national and international collaborators to address this issue of mutual significance.
## Appendices

### Appendix 1: Search Terms

<table>
<thead>
<tr>
<th>Injury and Environment</th>
<th>Infection and Pathogens</th>
<th>Strategies and Challenges</th>
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<tbody>
<tr>
<td>military</td>
<td>infection</td>
<td>drug resistance</td>
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<tr>
<td>combat</td>
<td>pathogen</td>
<td>antibiotic resistance</td>
</tr>
<tr>
<td>combat-related</td>
<td>nosocomial</td>
<td>antimicrobial resistance</td>
</tr>
<tr>
<td>wound</td>
<td>microbial*, polymicrobial</td>
<td>multidrug-resistant bacteria</td>
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<tr>
<td>burn</td>
<td>contamination, contaminant</td>
<td>multidrug-resistant organisms (MDR, MRDO)</td>
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<tr>
<td>extremity injury</td>
<td>colonization, coloniz*</td>
<td>carbapenem-resistant</td>
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<tr>
<td>soft tissue injury</td>
<td>soft tissue infection</td>
<td>biomarker, biomarkers</td>
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<td>laceration</td>
<td>bacteria* (bacteria, bacterial)</td>
<td>treatment</td>
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<td>trauma</td>
<td>Staphylococcus aureus</td>
<td>prevention, infection</td>
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<tr>
<td>infect* (infection, infectious, infected)</td>
<td>methicillin-resistant Staphylococcus aureus (MRSA)</td>
<td>prevention</td>
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<td>dismounted complex blast injury</td>
<td>Acinetobacter baumannii</td>
<td>microbial profiling</td>
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<td>complex battle injury</td>
<td>Pseudomonas aeruginosa</td>
<td>biofilm, biofilms</td>
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<td>blast</td>
<td>extended-spectrum beta-lactamase-producing Enterobacteriaceae</td>
<td>negative pressure wound dressings</td>
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<td>blast injury</td>
<td>Enterobacteriaceae</td>
<td>monoclonal antib* (antibody, antibodies), mAB</td>
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<td>chronic injury</td>
<td>Escherichia coli</td>
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<td>sepsis</td>
<td>Klebsiella pneumoniae</td>
<td>debridement</td>
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<td>wound healing</td>
<td>fung* (fungal, fungus)</td>
<td>irrigation</td>
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<td>wound exudate</td>
<td>invasive fungal infection (IFI)</td>
<td>wound management</td>
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<td>acute infection</td>
<td>mold, mould</td>
<td>detection</td>
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<td>chronic wound</td>
<td>Mucor* (Mucorales. Mucoralean)</td>
<td>diagnosis, diagnostic</td>
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<td>medical transport</td>
<td>Aspergillus</td>
<td>bacteriophage</td>
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<td>en route care</td>
<td>Fusarium</td>
<td>microbiome</td>
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<td>extended evacuation</td>
<td>Scedosporium</td>
<td>microbiota</td>
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<td>blast debris</td>
<td>Saksenaea erythrospora</td>
<td>diagnostic equipment, devices</td>
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<td>casualty</td>
<td>Candida</td>
<td>antimicrobial textiles</td>
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<td>deployed</td>
<td>osteomyelitis</td>
<td>immune response</td>
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<td>Iraq, OIF, Afghanistan, OEF</td>
<td>anaerobic, anaerobes</td>
<td>infection control</td>
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<td>bomb</td>
<td>secondary infections</td>
<td>Dakins</td>
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<td></td>
<td>Zygomycye*</td>
<td>fixation</td>
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<td></td>
<td>Rhizopus</td>
<td>fracture</td>
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<td></td>
<td>enterococcus</td>
<td>amputation</td>
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<td></td>
<td>microorganism</td>
<td>salvage</td>
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</table>
|                       | vancomycin-resistant enterococi (VRE) | }
Appendix 2: Selected Acronyms and Abbreviations

ABC  
Acinetobacter baumannii-calcoaceticus complex

CAT G  
Cathepsin G

CRP  
C-reactive protein

CDC  
Centers for Disease Control and Prevention

CPGs  
Clinical practice guidelines

DoD  
Department of Defense

DoDTR  
Department of Defense Trauma Registry

DTIC  
Defense Technical Information Center

ED  
Emergency Department

FDA  
US Food and Drug Administration

HHS  
Department of Health and Human Services

HICPAC  
Healthcare Infection Control Practices Advisory Committee

HNE  
Human neutrophil elastase

ICO  
Infection control officers

ICU  
Intensive care unit

IED  
Improvised explosive device

IFIs  
Invasive fungal infections

ISS  
Injury Severity Scores

JTTS  
Joint Theater Trauma System

JTTR  
Joint Theater Trauma Registry

LLMDA  
Lawrence Livermore Microbial Detection Array

LYS  
Lysozyme

MADM  
Matrix-assisted laser desorption/ionization-time of flight

MALDI-TOF  
Multiplexed automated digital microscopy

MDRO  
Multidrug-resistant organisms

MMP  
Matrix metalloproteinases

MRSA  
methicillin-resistant Staphylococcus aureus

MTFs  
Military treatment facilities

MPO  
Myeloperoxidase

MS  
Mass spectroscopy

NATO  
North Atlantic Treaty Organization

NGS  
Next-generation sequencing

NPWT  
Negative pressure wound therapy

OEF  
Operation Enduring Freedom

OIF  
Operation Iraqi Freedom

PCO  
Program Coordinating Office

PCR  
Polymerase chain reaction

PCT  
Procalcitonin

PEG  
Polyethylene glycol

SBIR  
Small Business Innovation Research

SoS  
State of the science

USAISR  
US Army Institute of Surgical Research
Appendix 3: References


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