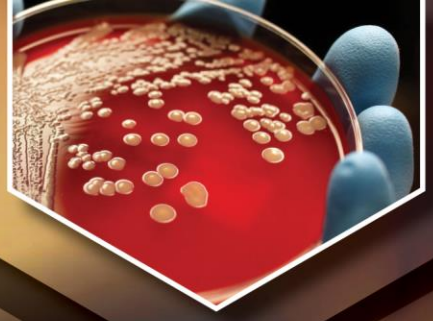
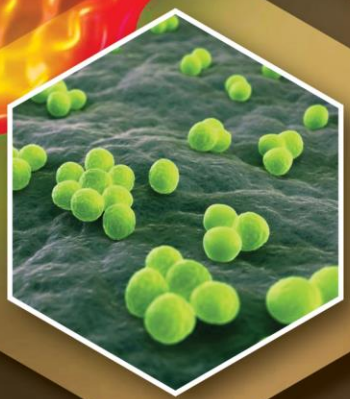
DEPARTMENT OF DEFENSE
BLAST INJURY RESEARCH PROGRAM
COORDINATING OFFICE



2016 INTERNATIONAL STATE-OF-THE-SCIENCE MEETING

MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

LITERATURE REVIEW



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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

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

* 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

Military/ Civilian	Study sample	Site	Infection rate	Outcome	Most common organism	Study
Iraq						
British military	48	Open femur fractures	8%	4% underwent amputation	*S. aureus*	Bennett, Sargeant, Myatt, & Penn-Barwell, 2015
US military	300	Lower extremity amputations	27%	53% underwent reoperation	not stated	Tintle et al., 2014
British military	182	Chest	43%	4.9% overall mortality	not stated	Senanayake, Poon, Graham, & Midwinter, 2014
US military	192	Diaphyseal tibia fractures	27%	22% underwent amputation	ABC (surveillance) *S. aureus* (infected)	Burns et al., 2012
US military	16,742	Variable	5.5%	0.6% overall mortality	Gram negatives	Murray, Wilkins, et al., 2011
Civilian	137	Chronic osteomyelitis	78%	not stated	*S. aureus*	Murphy et al., 2011
Military and civilian	211	Variable	26.5%	3.57% mortality among infected	ABC	Petersen et al., 2007
US military	49	Variable	49%	not stated	Coagulase-negative staphylococci	Murray et al., 2006
Syria						
Civilian	100	Variable	12%	2% overall mortality	not stated	Biswas et al., 2016
Military and civilian	66	Cranial trauma	10.6%	4.5% overall mortality	not stated	Barhoum et al., 2015
Military and civilian	345	Variable	18%	not stated	*P. aeruginosa*	Teicher et al., 2014
Israel						
Civilian	21	Variable	30%	43% mortality rate	Candida	Wolf et al., 2000
Military	Group 1982:184 Group 1973:130	Extremities	Group 1982: 30.5% Group 1973: 31.5%	not stated	*P. aeruginosa*	Simchen, Raz, Stein, & Danon, 1991
Military and civilian	142	Chest trauma	4.9%	not stated	not stated	Romanoff, 1975
Military	624	Variable	12.5%	6 cases of bacterial sepsis	*P. aeruginosa*	Klein, Berger, & Yekutiel, 1975
Lebanon						
Military and civilian	350	Total body cluster munitions	19.4%	not stated	*P. aeruginosa*	Fares, El-Zaatari, Fares, Bedrosian, & Yared, 2013
Military and civilian	1021	Head and neck injuries	12%	not stated	*S. aureus*	Zaytoun, Shikhani, & Salman, 1986

"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

Wound Culture Results Before Surgical Intervention			Wound Culture Results on Follow-up Evaluation		
	OIF	**OEF***	**OIF/OEF****	**OIF/OEF‡**	**OEF***
Reference	Murray et al., 2006	Wallum et al., 2015	Burns et al., 2012	Sheppard et al., 2010	Wallum et al., 2015
Dates of Study	2004	2013-2014	2003-2007	2007-2008	2013-2014
No. Patients	49	10	192	34	10
No. wounds evaluated	61	13	145	91	11
No. bacteria isolated	37	27	133	33	13
Timing of culture	Within ED	Day of injury	Within 72h of Level V admission	Within 72h of Level V admission	Day 3-12 after injury
Gram positive, %	93	44	19	15	54
S. aureus, %	11	0	8	0	0
Coagulase-negative Staphylococcus species, %	86	8	28	0	0
Enterococcus species, %	0	42	56	60	57
Gram negative, %	7	56	81	85	46
P. aeruginosa, %	0	0	12	0	17
Acinetobacter baumannii complex, %	0	0	53	71	0
E. coli, %	33	0	6	4	34
Proteus species, %	0	0	0	0	0
Enterobacter species, %	0	7	16	7	17
Klebsiella species, %	0	0	12	0	0
Serratia marcescens, %	0	0	0	0	0

* 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

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

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. pneumoniae* (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 patents 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 Landsthul 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 IIIC 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 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, Sarıosmanoğlu, & 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; Radowsky, 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 acidotic 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; Misic, 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, Heinzle, & 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, Thurig, 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) (Misic 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; Misic 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 5. Clinical Trials of Infection Diagnosis Approaches

Trial ID	Title	Purpose	Status (as of July 2016)
NCT00287599	Rapid Identification of Key Pathogens in Wound Infection by Molecular Means	Evaluate real-time PCR methods for the rapid identification and quantification of wound pathogens in military and civilian populations	Completed; no study results posted
NCT01198262	Rapid Test to Detect Staphylococcus Aureus in Blood and Wound Infections	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	Completed; no study results posted
NCT02508272	Transcriptomic Profiling in Severely Injured Patients	Link defined clinical phenotypes with RNA data obtained by high throughput technologies in polytrauma patients with systemic inflammation	Completed; no study results posted
NCT01379053	Volatile Organic Compounds in Staphylococcus Aureus Patients (MRSAVOC)	Evaluate the ability of the noninvasive zNose® MRSA test to detect the presence of S. aureus in patients with suspected infection or colonization	Completed; no study results posted
NCT01875692	Can we Better Understand the Development of VAP and Eventually Predict and Prevent it?	Determine biomarkers of VAP in the oropharyngeal juice and tracheal aspirates in adult ICU patients	Completed; no study results posted
NCT01496014	Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage (Bioburden)	Characterize wound bioburden at the time of definitive wound closure of severe tibia fractures in military and civilian populations	Active, not recruiting
NCT02457663	Identification and Validation of Biomarkers for Infections in Burns	Validate previously identified biomarkers and identify novel biomarkers of infections in burn patients using discovery proteomics	Active, not recruiting
NCT02323165	A New Method for Detection of Bacteria in the Bloodstream	Evaluate the use of PCR with universal bacterial 16S primers for detection and identification of bacteria in burn patients	Recruiting
NCT02753608	Early Detection of Ventilator-associated Pneumonia (VAP) (cheqVAP)	Identify biomarkers for VAP in exhaled breath condensates from patients receiving invasive ventilation	Not yet recruiting
NCT00258869	Observational Study of Sepsis and Pneumonia to Develop Diagnostic Tests	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	Unknown

Source: 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).

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

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

Level of Care*	Care Category	Recommendations
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 produce resuscitation • Use only topical antimicrobials for burns
	Debridement and irrigation	• Irrigate wounds to remove gross contamination with normal saline, sterile, or potable water, under low pressure (bulb syringe or equivalent) without additives • Do not attempt to remove retained deep soft tissue fragments if criteria met.† Provide cefazolin 2 g IV × 1 dose
Role 2/Level II with surgical support (IIb)/ Role 3/Level III	Post-injury antimicrobials	• 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 • Antimicrobial beads or pouches may be used • Provide post-splenectomy immunizations if indicated
	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 wound management	• 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 splint immobilization of open humerus/forearm fractures
Role 4/Level IV	Post-injury antimicrobials	• Complete course of post-injury antimicrobials • Antimicrobial beads or pouches may be used • Provide post-splenectomy immunizations, if indicated
	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 wound management	• 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 splint immobilization of open humerus/forearm fractures

IV, intravenous; PSI, pounds per square inch.
* 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 [USMC]); 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, USMC); 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

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

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

Challenges	Solutions
Efforts to identify and exploit new antibiotic targets utilizing genomics approaches have not been successful.	Efforts should be directed towards identifying novel targets and approaches in antibiotic discovery.
The mechanisms by which current antibiotics work is not well enough elucidated.	Additional efforts to understand the mechanism of action of existing antibiotics is needed.
Conventional methods of drug discovery (e.g., development of synthetic chemicals) are unlike the natural products initially discovered during the golden era.	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.
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.	Additional, more relevant measures of antibiotic effectiveness need to be developed.

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 (Kostyanev 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 yearsfor 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

Vaccine Type	Advantages	Disadvantages
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. albicas*, 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 pursed 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* (Kaźmierczak, Górski, & Dąbrowska, 2014; Magana et al., 2015), and *K. pneumoniae* (Taneja & Kaur, 2016).

A study by Lin et al. (2010) indicated that *A. baumanni*-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. baumanni*, 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

(Pires et al., 2015). 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, Shirtliff, & 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* wse 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 mannosides 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 azetreonam, 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. baumanii*-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 & Martinez, 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* (Valdéz, 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 & Martinez, 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 & Martinez, 2011), and nitric oxide (NO)-based therapies (Mihu et al., 2010; Mihu & Martinez, 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

Trial ID	Title	Purpose	Status (as of July 2016)
NCT00413218	Isavuconazole (BAL8557) in the Treatment of Candidemia and Other Invasive Candida Infections	Compare the safety and efficacy of Isavuconazole versus caspofungin followed by voriconazole in the treatment of candidemia and other invasive Candida infections.	Completed
NCT02244606	Oral SCY-078 vs Standard-of-Care Following IV Echinocandin in the Treatment of Invasive Candidiasis	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.	Recruiting participants
NCT00876252	Study Assessing Immunogenicity and Safety of IC43 In Intensive Care Patients	Randomized, placebo-controlled, partially blinded phase 2 pilot study. Multicenter study (approximately 50 centers) in approximately 9 countries. Proposed start date is December 2008.	Completed
NCT01563263	Confirmatory Phase II/III Study Assessing Efficacy, Immunogenicity and Safety of IC43	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.	Completed
NCT02492958	SA4Ag Safety, Tolerability, and Immunogenicity Study in Japanese Adults	Evaluate the safety, tolerability, and immunogenicity of a single dose of Staphylococcus aureus 4 antigen vaccine in Japanese adults aged 20 to <86 years.	Ongoing; not recruiting
NCT02388165	Safety and Efficacy of SA4Ag Vaccine in Adults Having Elective Posterior Instrumented Lumbar Spinal Fusion Procedure (STRIVE)	Determine whether the SA4Ag vaccine can prevent postoperative Staphylococcus aureus infections in patients who are undergoing elective spinal fusion surgery; evaluate the safety of SA4Ag in patients who are undergoing elective spinal surgery.	Recruiting
NCT02296320	Study of the Efficacy and Safety of MEDI4893 (SAATELLITE)	Safety and efficacy of MEDI4893 in prevention of pneumonia caused by *S. aureus* in high-risk patients.	Recruiting
NCT02357966	A Study of the Safety and Efficacy of 514G3 in Subjects Hospitalized With Bacteremia Due to Staphylococcus Aureus	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.	Recruiting
NCT01589185	Safety, Pharmacokinetics and Efficacy of KBSA301 in Severe Pneumonia (*S. Aureus*)	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.	Recruiting
NCT01273922	Safety and Immunogenicity Study of a Recombinant Protein Vaccine (NDV-3) Against *S. Aureus* and Candida	Evaluate the safety, tolerability and immunogenicity of the investigational vaccine, NDV-3.	Completed
NCT02096315	Safety, Efficacy and PK/PD of POL7080 in Patients With Exacerbation of Non-cystic Fibrosis Bronchiectasis.	Test whether POL7080 is effective in patients with exacerbation of non-cystic fibrosis bronchiectasis caused by Pseudomonas aeruginosa infection.	Completed

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. baumanni*
- 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 policy makers. US military researchers can continue to partner with national and international collaborators to address this issue of mutual significance.

Appendices

Appendix 1: Search Terms

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

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

Abat, C., Kerbaj, J., Dubourg, G., Garcia, V., & Rolain, J.-M. (2015). Human Infection with Sporolactobacillus laevolacticus, Marseille, France. *Emerging Infectious Diseases*, *21*(11), 2106–2108. http://doi.org/10.3201/eid2111.151197

Ahmad, T. A., El-Sayed, L. H., Haroun, M., Hussein, A. A., & El Ashry, E. S. H. (2012). Development of immunization trials against Klebsiella pneumoniae. *Vaccine*, *30*(14), 2411–2420. http://doi.org/10.1016/j.vaccine.2011.11.027

Akers, K. S., Mende, K., Cheatle, K. A., Zera, W. C., Yu, X., Beckius, M. L., … Murray, C. K. (2014). Biofilms and persistent wound infections in United States military trauma patients: a case–control analysis. *BMC Infectious Diseases*, *14*, 190. http://doi.org/10.1186/1471-2334-14-190

Akers, K. S., Rowan, M. P., Niece, K. L., Graybill, J. C., Mende, K., Chung, K. K., & Murray, C. K. (2015). Antifungal wound penetration of amphotericin and voriconazole in combat-related injuries: case report. *BMC Infectious Diseases*, *15*, 184. http://doi.org/10.1186/s12879-015-0918-8

Al-Arabi, Y. B., Nader, M., Nader, M., Hamidian-Jahromi, A. R., & Woods, D. A. (2007). The effect of the timing of antibiotics and surgical treatment on infection rates in open long-bone fractures: a 9-year prospective study from a district general hospital. *Injury*, *38*(8), 900–905. http://doi.org/10.1016/j.injury.2007.02.043

Alexandre, Y., Le Berre, R., Barbier, G., & Le Blay, G. (2014). Screening of Lactobacillus spp. for the prevention of Pseudomonas aeruginosa pulmonary infections. *BMC Microbiology*, *14*, 107. http://doi.org/10.1186/1471-2180-14-107

Anderson, A. S., Miller, A. A., Donald, R. G. K., Scully, I. L., Nanra, J. S., Cooper, D., & Jansen, K. U. (2012). Development of a multicomponent Staphylococcus aureus vaccine designed to counter multiple bacterial virulence factors. *Human Vaccines & Immunotherapeutics*, *8*(11), 1585–1594. http://doi.org/10.4161/hv.21872

Andersson, J. A., Fitts, E. C., Kirtley, M. L., Ponnusamy, D., Peniche, A. G., Dann, S. M., … Chopra, A. K. (2016). New role for FDA-approved drugs in combating antibiotic-resistant bacteria. *Antimicrobial Agents and Chemotherapy*. http://doi.org/10.1128/AAC.00326-16

Antunes, L. C. S., Imperi, F., Minandri, F., & Visca, P. (2012). In Vitro and In Vivo Antimicrobial Activities of Gallium Nitrate against Multidrug-Resistant Acinetobacter baumannii. *Antimicrobial Agents and Chemotherapy*, *56*(11), 5961–5970. http://doi.org/10.1128/AAC.01519-12

Aridis Pharmaceuticals, Inc. (2016). Safety, Pharmacokinetics and Efficacy of KBSA301 in Severe Pneumonia (S. Aureus) - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT01589185?term=AR-301&rank=1

Aronson, N. E., Sanders, J. W., & Moran, K. A. (2006). In Harm's Way: Infections in Deployed American Military Forces. *Clinical Infectious Diseases*, *43*(8), 1045–1051. http://doi.org/10.1086/507539

Astellas Pharma Inc. (2016). Isavuconazole (BAL8557) in the Treatment of Candidemia and Other Invasive Candida Infections - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT00413218?term=isavuconazole+candida&rank=1

Baer, M., Sawa, T., Flynn, P., Luehrsen, K., Martinez, D., Wiener-Kronish, J. P., … Bebbington, C. (2009). An Engineered Human Antibody Fab Fragment Specific for Pseudomonas aeruginosa PcrV Antigen Has Potent Antibacterial Activity. *Infection and Immunity*, *77*(3), 1083–1090. http://doi.org/10.1128/IAI.00815-08

Balazs, G. C., Blais, M. B., Bluman, E. M., Andersen, R. C., & Potter, B. K. (2015). Blurred front lines: triage and initial management of blast injuries. *Current Reviews in Musculoskeletal Medicine*, *8*(3), 304–311. http://doi.org/10.1007/s12178-015-9288-5

Baltazar, L. M., Ray, A., Santos, D. A., Cisalpino, P. S., Friedman, A. J., & Nosanchuk, J. D. (2015). Antimicrobial photodynamic therapy: an effective alternative approach to control fungal infections. *Frontiers in Microbiology*, *6*. http://doi.org/10.3389/fmicb.2015.00202

Banin, E., Vasil, M. L., & Greenberg, E. P. (2005). Iron and Pseudomonas aeruginosa biofilm formation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11076–11081. http://doi.org/10.1073/pnas.0504266102

Barenfanger, J., Drake, C., & Kacich, G. (1999). Clinical and Financial Benefits of Rapid Bacterial Identification and Antimicrobial Susceptibility Testing. *Journal of Clinical Microbiology*, *37*(5), 1415–1418.

Barhoum, M., Tobias, S., Elron, M., Sharon, A., Heija, T., & Soustiel, J. F. (2015). Syria civil war: Outcomes of humanitarian neurosurgical care provided to Syrian wounded refugees in Israel. *Brain Injury*, *29*(11), 1370–1375. http://doi.org/10.3109/02699052.2015.1043346

Barillo, D. J., Pozza, M., & Margaret-Brandt, M. (2014). A literature review of the military uses of silver-nylon dressings with emphasis on wartime operations. *Burns: Journal of the International Society for Burn Injuries*, *40 Suppl 1*, S24-29. http://doi.org/10.1016/j.burns.2014.09.017

Barsoumian, A., Sanchez, C. J., Mende, K., Tully, C. C., Beckius, M. L., Akers, K. S., … Murray, C. K. (2013). In vitro toxicity and activity of Dakin's solution, mafenide acetate, and amphotericin B on filamentous fungi and human cells. *Journal of Orthopaedic Trauma*, *27*(8), 428–436. http://doi.org/10.1097/BOT.0b013e3182830bf9

Be, N. A., Allen, J. E., Brown, T. S., Gardner, S. N., McLoughlin, K. S., Forsberg, J. A., … Jaing, C. J. (2014). Microbial profiling of combat wound infection through detection

microarray and next-generation sequencing. *Journal of Clinical Microbiology*, *52*(7), 2583–2594. http://doi.org/10.1128/JCM.00556-14

Belmont, P. J., McCriskin, B. J., Hsiao, M. S., Burks, R., Nelson, K. J., & Schoenfeld, A. J. (2013). The nature and incidence of musculoskeletal combat wounds in Iraq and Afghanistan (2005-2009). *Journal of Orthopaedic Trauma*, *27*(5), e107-113. http://doi.org/10.1097/BOT.0b013e3182703188

Bennett, P. M., Sargeant, I. D., Myatt, R. W., & Penn-Barwell, J. G. (2015). The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period. *The Bone & Joint Journal*, *97–B*(6), 842–846. http://doi.org/10.1302/0301-620X.97B6.34962

Berríos-Torres, S. I. (2016). Evidence-Based Update to the U.S. Centers for Disease Control and Prevention and Healthcare Infection Control Practices Advisory Committee Guideline for the Prevention of Surgical Site Infection: Developmental Process. *Surgical Infections*, *17*(2), 256–261. http://doi.org/10.1089/sur.2015.264

Bertesteanu, S., Triaridis, S., Stankovic, M., Lazar, V., Chifiriuc, M. C., Vlad, M., & Grigore, R. (2014). Polymicrobial wound infections: pathophysiology and current therapeutic approaches. *International Journal of Pharmaceutics*, *463*(2), 119–126. http://doi.org/10.1016/j.ijpharm.2013.12.012

Biel, M. A. (2015). Antimicrobial photodynamic therapy for treatment of biofilm-based infections. *Advances in Experimental Medicine and Biology*, *831*, 119–136. http://doi.org/10.1007/978-3-319-09782-4_8

Biswas, S., Waksman, I., Baron, S., Fuchs, D., Rechnitzer, H., Dally, N., … Hadary, A. (2016). Analysis of the First 100 Patients From the Syrian Civil War Treated in an Israeli District Hospital. *Annals of Surgery*, *263*(1), 205–209. http://doi.org/10.1097/SLA.0000000000001230

Blokhuis-Arkes, M. H. E., Haalboom, M., van der Palen, J., Heinzle, A., Sigl, E., Guebitz, G., & Beuk, R. (2015). Rapid enzyme analysis as a diagnostic tool for wound infection: Comparison between clinical judgment, microbiological analysis, and enzyme analysis. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *23*(3), 345–352. http://doi.org/10.1111/wrr.12282

Blyth, D. M., Yun, H. C., Tribble, D. R., & Murray, C. K. (2015). Lessons of war: Combat-related injury infections during the Vietnam War and Operation Iraqi and Enduring Freedom. *The Journal of Trauma and Acute Care Surgery*, *79*(4 Suppl 2), S227-235. http://doi.org/10.1097/TA.0000000000000768

Brocklesby, K. L., Johns, S. C., Jones, A. E., Sharp, D., & Smith, R. B. (2013). Smart bandages--a colourful approach to early stage infection detection & control in wound care. *Medical Hypotheses*, *80*(3), 237–240. http://doi.org/10.1016/j.mehy.2012.11.037

Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature*, *529*(7586), 336–343. http://doi.org/10.1038/nature17042

Brown, K. V., Murray, C. K., & Clasper, J. C. (2010). Infectious complications of combat-related mangled extremity injuries in the British military. *The Journal of Trauma*, *69 Suppl 1*, S109-115. http://doi.org/10.1097/TA.0b013e3181e4b33d

Brown, T. S., Safford, S., Caramanica, J., & Elster, E. A. (2010). Biomarker use in tailored combat casualty care. *Biomarkers in Medicine*, *4*(3), 465–473. http://doi.org/10.2217/bmm.10.13

Burns, T. C., Stinner, D. J., Mack, A. W., Potter, B. K., Beer, R., Eckel, T. T., … Skeletal Trauma Research Consortium. (2012). Microbiology and injury characteristics in severe open tibia fractures from combat. *The Journal of Trauma and Acute Care Surgery*, *72*(4), 1062–1067. http://doi.org/10.1097/TA.0b013e318241f534

Byun, H.-G., Persaud, K. C., & Pisanelli, A. M. (2010). Wound-State Monitoring for Burn Patients Using E-Nose/SPME System. *ETRI Journal*, *32*(3), 440–446. http://doi.org/10.4218/etrij.10.0109.0300

Calhoun, J. H., Murray, C. K., & Manring, M. M. (2008). Multidrug-resistant Organisms in Military Wounds from Iraq and Afghanistan. *Clinical Orthopaedics and Related Research*, *466*(6), 1356–1362. http://doi.org/10.1007/s11999-008-0212-9

Cannon, J. W., Hofmann, L. J., Glasgow, S. C., Potter, B. K., Rodriguez, C. J., Cancio, L. C., … Elster, E. A. (2016). Dismounted Complex Blast Injuries: A Comprehensive Review of the Modern Combat Experience. *Journal of the American College of Surgeons*. http://doi.org/10.1016/j.jamcollsurg.2016.07.009

Carpenter, R. J., Hartzell, J. D., Forsberg, J. A., Babel, B. S., & Ganesan, A. (2008). Pseudomonas putida war wound infection in a US Marine: a case report and review of the literature. *The Journal of Infection*, *56*(4), 234–240. http://doi.org/10.1016/j.jinf.2008.01.004

Casey, K., Demers, P., Deben, S., Nelles, M. E., & Weiss, J. S. (2015). Outcomes after long-term follow-up of combat-related extremity injuries in a multidisciplinary limb salvage clinic. *Annals of Vascular Surgery*, *29*(3), 496–501. http://doi.org/10.1016/j.avsg.2014.09.035

Castillo-Juárez, I., Maeda, T., Mandujano-Tinoco, E. A., Tomás, M., Pérez-Eretza, B., García-Contreras, S. J., … García-Contreras, R. (2015). Role of quorum sensing in bacterial infections. *World Journal of Clinical Cases : WJCC*, *3*(7), 575–598. http://doi.org/10.12998/wjcc.v3.i7.575

CDC. (2016). CDC - HICPAC. Retrieved July 11, 2016, from https://www.cdc.gov/hicpac/index.html

Chantell, C. (2015). Multiplexed Automated Digital Microscopy for Rapid Identification and Antimicrobial Susceptibility Testing of Bacteria and Yeast Directly from Clinical

Samples. *Clinical Microbiology Newsletter*, *37*(20), 161–167. http://doi.org/10.1016/j.clinmicnews.2015.10.001

Chatterjee, M., Anju, C. P., Biswas, L., Anil Kumar, V., Gopi Mohan, C., & Biswas, R. (2016). Antibiotic resistance in Pseudomonas aeruginosa and alternative therapeutic options. *International Journal of Medical Microbiology: IJMM*, *306*(1), 48–58. http://doi.org/10.1016/j.ijmm.2015.11.004

Chemani, C., Imberty, A., de Bentzmann, S., Pierre, M., Wimmerová, M., Guery, B. P., & Faure, K. (2009). Role of LecA and LecB Lectins in Pseudomonas aeruginosa-Induced Lung Injury and Effect of Carbohydrate Ligands. *Infection and Immunity*, *77*(5), 2065–2075. http://doi.org/10.1128/IAI.01204-08

Chen, S. L., Hung, C. S., Pinkner, J. S., Walker, J. N., Cusumano, C. K., Li, Z., … Hultgren, S. J. (2009). Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(52), 22439–22444. http://doi.org/10.1073/pnas.0902179106

Chen, W. (2015). Current advances and challenges in the development of Acinetobacter vaccines. *Human Vaccines & Immunotherapeutics*, *11*(10), 2495–2500. http://doi.org/10.1080/21645515.2015.1052354

Chromy, B. A., Eldridge, A., Forsberg, J. A., Brown, T. S., Kirkup, B. C., Jaing, C., … Luciw, P. A. (2013). Wound outcome in combat injuries is associated with a unique set of protein biomarkers. *Journal of Translational Medicine*, *11*, 281. http://doi.org/10.1186/1479-5876-11-281

Ciani, I., Schulze, H., Corrigan, D. K., Henihan, G., Giraud, G., Terry, J. G., … Mount, A. R. (2012). Development of immunosensors for direct detection of three wound infection biomarkers at point of care using electrochemical impedance spectroscopy. *Biosensors & Bioelectronics*, *31*(1), 413–418. http://doi.org/10.1016/j.bios.2011.11.004

Clooney, A. G., Fouhy, F., Sleator, R. D., O' Driscoll, A., Stanton, C., Cotter, P. D., & Claesson, M. J. (2016). Comparing Apples and Oranges?: Next Generation Sequencing and Its Impact on Microbiome Analysis. *PLoS ONE*, *11*(2). http://doi.org/10.1371/journal.pone.0148028

Conger, N. G., Landrum, M. L., Jenkins, D. H., Martin, R. R., Dunne, J. R., & Hirsch, E. F. (2008). Prevention and management of infections associated with combat-related thoracic and abdominal cavity injuries. *The Journal of Trauma*, *64*(3 Suppl), S257-264. http://doi.org/10.1097/TA.0b013e318163d2c8

Crouch, H. K., Murray, C. K., & Hospenthal, D. R. (2010). Development of a deployment infection control course. *Military Medicine*, *175*(12), 983–989.

Cutting, K. F., & White, R. (2004). Defined and refined: criteria for identifying wound infection revisited. *British Journal of Community Nursing*, *9*(3), S6-15. http://doi.org/10.12968/bjcn.2004.9.Sup1.12495

Dai, T., Huang, Y.-Y., & Hamblin, M. R. (2009). Photodynamic therapy for localized infections – state of the art. *Photodiagnosis and Photodynamic Therapy*, *6*(3–4), 170–188. http://doi.org/10.1016/j.pdpdt.2009.10.008

Datta, K., & Hamad, M. (2015). Immunotherapy of Fungal Infections. *Immunological Investigations*, *44*(8), 738–776. http://doi.org/10.3109/08820139.2015.1093913

D'Avignon, L. C., Saffle, J. R., Chung, K. K., & Cancio, L. C. (2008). Prevention and management of infections associated with burns in the combat casualty. *The Journal of Trauma*, *64*(3 Suppl), S277-286. http://doi.org/10.1097/TA.0b013e318163c3e4

Davis, K. A., Moran, K. A., McAllister, C. K., & Gray, P. J. (2005). Multidrug-resistant Acinetobacter extremity infections in soldiers. *Emerging Infectious Diseases*, *11*(8), 1218–1224. http://doi.org/10.3201/eid1108.050103

De Bernardis, F., Amacker, M., Arancia, S., Sandini, S., Gremion, C., Zurbriggen, R., … Cassone, A. (2012). A virosomal vaccine against candidal vaginitis: immunogenicity, efficacy and safety profile in animal models. *Vaccine*, *30*(30), 4490–4498. http://doi.org/10.1016/j.vaccine.2012.04.069

De Bruyne, K., Slabbinck, B., Waegeman, W., Vauterin, P., De Baets, B., & Vandamme, P. (2011). Bacterial species identification from MALDI-TOF mass spectra through data analysis and machine learning. *Systematic and Applied Microbiology*, *34*(1), 20–29. http://doi.org/10.1016/j.syapm.2010.11.003

De Keukeleire, S., De Bel, A., Jansen, Y., Janssens, M., Wauters, G., & Piérard, D. (2014). Yersinia ruckeri, an unusual microorganism isolated from a human wound infection. *New Microbes and New Infections*, *2*(4), 134–135. http://doi.org/10.1002/nmi2.56

de Léséleuc, L., Harris, G., KuoLee, R., & Chen, W. (2012). In Vitro and In Vivo Biological Activities of Iron Chelators and Gallium Nitrate against Acinetobacter baumannii. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5397–5400. http://doi.org/10.1128/AAC.00778-12

Debarbieux, L., Leduc, D., Maura, D., Morello, E., Criscuolo, A., Grossi, O., … Touqui, L. (2010). Bacteriophages can treat and prevent Pseudomonas aeruginosa lung infections. *The Journal of Infectious Diseases*, *201*(7), 1096–1104. http://doi.org/10.1086/651135

Dickens, J. F., Kilcoyne, K. G., Kluk, M. W., Gordon, W. T., Shawen, S. B., & Potter, B. K. (2013). Risk factors for infection and amputation following open, combat-related calcaneal fractures. *The Journal of Bone and Joint Surgery. American Volume*, *95*(5), e24. http://doi.org/10.2106/JBJS.L.00003

Dix, A., Hünniger, K., Weber, M., Guthke, R., Kurzai, O., & Linde, J. (2015). Biomarker-based classification of bacterial and fungal whole-blood infections in a genome-wide expression study. *Infectious Diseases*, *6*, 171. http://doi.org/10.3389/fmicb.2015.00171

Doern, G. V., Vautour, R., Gaudet, M., & Levy, B. (1994). Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *Journal of Clinical Microbiology*, *32*(7), 1757–1762.

Douraiswami, B., Dilip, P. K., Harish, B. N., & Jagdish, M. (2012). C-reactive protein and interleukin-6 levels in the early detection of infection after open fractures. *Journal of Orthopaedic Surgery (Hong Kong)*, *20*(3), 381–385.

Drinka, P., Bonham, P., & Crnich, C. J. (2012). Swab culture of purulent skin infection to detect infection or colonization with antibiotic-resistant bacteria. *Journal of the American Medical Directors Association*, *13*(1), 75–79. http://doi.org/10.1016/j.jamda.2011.04.012

Dunne, J. R., Hawksworth, J. S., Stojadinovic, A., Gage, F., Tadaki, D. K., Perdue, P. W., … Elster, E. A. (2009). Perioperative blood transfusion in combat casualties: a pilot study. *The Journal of Trauma*, *66*(4 Suppl), S150-156. http://doi.org/10.1097/TA.0b013e31819d9561

Dupuy, A.-M., Philippart, F., Péan, Y., Lasocki, S., Charles, P.-E., Chalumeau, M., … Maurice Rapin Institute Biomarkers Group. (2013). Role of biomarkers in the management of antibiotic therapy: an expert panel review: I - currently available biomarkers for clinical use in acute infections. *Annals of Intensive Care*, *3*(1), 22. http://doi.org/10.1186/2110-5820-3-22

D'Alleyrand, J.-C. G., Lewandowski, L. R., Forsberg, J. A., Gordon, W. T., Fleming, M. E., Mullis, B. H., … Potter, B. K. (2015). Combat-Related Hemipelvectomy: 14 Cases, a Review of the Literature and Lessons Learned. *Journal of Orthopaedic Trauma*, *29*(12), e493-498. http://doi.org/10.1097/BOT.0000000000000398

D'Avignon, L. C., Chung, K. K., Saffle, J. R., Renz, E. M., & Cancio, L. C. (2011). Prevention of Infections Associated With Combat-Related Burn Injuries: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S282–S289. http://doi.org/10.1097/TA.0b013e318227adc2

Eardley, W. G. P., Brown, K. V., Bonner, T. J., Green, A. D., & Clasper, J. C. (2011). Infection in conflict wounded. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*(1562), 204–218. http://doi.org/10.1098/rstb.2010.0225

Eastridge, B. J., Costanzo, G., Jenkins, D., Spott, M. A., Wade, C., Greydanus, D., … Blackbourne, L. H. (2009). Impact of joint theater trauma system initiatives on battlefield injury outcomes. *American Journal of Surgery*, *198*(6), 852–857. http://doi.org/10.1016/j.amjsurg.2009.04.029

Eastridge, B. J., Jenkins, D., Flaherty, S., Schiller, H., & Holcomb, J. B. (2006). Trauma system development in a theater of war: Experiences from Operation Iraqi Freedom and Operation Enduring Freedom. *The Journal of Trauma*, *61*(6), 1366-1372-1373. http://doi.org/10.1097/01.ta.0000245894.78941.90

Eastridge, B. J., Wade, C. E., Spott, M. A., Costanzo, G., Dunne, J., Flaherty, S., … Casscells, S. W. (2010). Utilizing a trauma systems approach to benchmark and improve combat casualty care. *The Journal of Trauma*, *69 Suppl 1*, S5-9. http://doi.org/10.1097/TA.0b013e3181e421f3

Edwards, J. E. (2012). Fungal cell wall vaccines: an update. *Journal of Medical Microbiology*, *61*(Pt 7), 895–903. http://doi.org/10.1099/jmm.0.041665-0

Elder, I. (2013, February 28). A wound management system. Retrieved from http://www.google.com/patents/WO2013026999A1

El-Shanshory, A. A., Chen, W., El-Hamshary, H. A., Al-Deyab, S. S., & Mo, X. (2015). Antibacterial ciprofloxacin hydrochloride incorporated PVA/regenerated silk fibroin nanofibers composite for wound dressing applications. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, *213*, e8-9. http://doi.org/10.1016/j.jconrel.2015.05.010

Evriviades, D., Jeffery, S., Cubison, T., Lawton, G., Gill, M., & Mortiboy, D. (2011). Shaping the military wound: issues surrounding the reconstruction of injured servicemen at the Royal Centre for Defence Medicine. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1562), 219–230. http://doi.org/10.1098/rstb.2010.0237

Fanfair, R., Benedict, K., Bos, J., Bennett, S. D., Lo, Y.-C., Adebanjo, T., … Park, B. J. (2012). Necrotizing cutaneous mucormycosis after a tornado in Joplin, Missouri, in 2011. *The New England Journal of Medicine*, *367*(23), 2214–2225. http://doi.org/10.1056/NEJMoa1204781

Fares, Y., El-Zaatari, M., Fares, J., Bedrosian, N., & Yared, N. (2013). Trauma-related infections due to cluster munitions. *Journal of Infection and Public Health*, *6*(6), 482–486. http://doi.org/10.1016/j.jiph.2013.05.006

Farmer, A. R., Murray, C. K., Driscoll, I. R., Wickes, B. L., Wiederhold, N., Sutton, D. A., … Vento, T. J. (2015). Combat-Related Pythium aphanidermatum Invasive Wound Infection: Case Report and Discussion of Utility of Molecular Diagnostics. *Journal of Clinical Microbiology*, *53*(6), 1968–1975. http://doi.org/10.1128/JCM.00410-15

Fernandes Júnior, A., Balestrin, E. C., Betoni, J. E. C., Orsi, R. de O., Cunha, M. de L. R. de S. da, & Montelli, A. C. (2005). Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. *Memórias Do Instituto Oswaldo Cruz*, *100*(5), 563–566. http://doi.org/10.1590/S0074-02762005000500018

Ficke, J. R., Eastridge, Brian J., Butler, Frank K., Alvarez, John, Brown, Tommy, Pasquina, Paul, … Caravalho, Joseph Jr. (2012). Dismounted complex blast injury report of the army dismounted complex blast injury task force. *Journal of Trauma and Acute Care Surgery*, *73*(6), S520–S534.

Fleming, M., Waterman, S., Dunne, J., D'Alleyrand, J.-C., & Andersen, R. C. (2012). Dismounted complex blast injuries: patterns of injuries and resource utilization

associated with the multiple extremity amputee. *Journal of Surgical Orthopaedic Advances*, *21*(1), 32–37.

Fletcher, T. E., Hutley, E., Adcock, C. J., Martin, N., & Wilson, D. R. (2013). Deployed antimicrobial stewardship: an audit of antimicrobial use at Role 3. *Journal of the Royal Army Medical Corps*, *159*(3), 237–239. http://doi.org/10.1136/jramc-2013-000116

Flores, A. R., Sahasrabhojane, P., Saldaña, M., Galloway-Peña, J., Olsen, R. J., Musser, J. M., & Shelburne, S. A. (2014). Molecular Characterization of an Invasive Phenotype of Group A Streptococcus Arising During Human Infection Using Whole Genome Sequencing of Multiple Isolates From the Same Patient. *The Journal of Infectious Diseases*, *209*(10), 1520–1523. http://doi.org/10.1093/infdis/jit674

Foley, T. L., & Simeonov, A. (2012). Targeting iron assimilation to develop new antibacterials. *Expert Opinion on Drug Discovery*, *7*(9), 831–847. http://doi.org/10.1517/17460441.2012.708335

Forestier, C., Guelon, D., Cluytens, V., Gillart, T., Sirot, J., & De Champs, C. (2008). Oral probiotic and prevention of Pseudomonas aeruginosa infections: a randomized, double-blind, placebo-controlled pilot study in intensive care unit patients. *Critical Care*, *12*(3), R69. http://doi.org/10.1186/cc6907

Forgione, M. A., Moores, L. E., & Wortmann, G. W. (2011). Prevention of Infections Associated With Combat-Related Central Nervous System Injuries: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S258–S263. http://doi.org/10.1097/TA.0b013e318227ad86

Forsberg, J., Elster, E. A., Andersen, R. C., Nylen, E., Brown, T. S., Rose, M. W., … McGuigan, F. X. (2008). Correlation of procalcitonin and cytokine expression with dehiscence of wartime extremity wounds. *The Journal of Bone and Joint Surgery. American Volume*, *90*(3), 580–588. http://doi.org/10.2106/JBJS.G.00265

Forsberg, J., Potter, B. K., Polfer, E. M., Safford, S. D., & Elster, E. A. (2014). Do Inflammatory Markers Portend Heterotopic Ossification and Wound Failure in Combat Wounds? *Clinical Orthopaedics and Related Research*, *472*(9), 2845–2854. http://doi.org/10.1007/s11999-014-3694-7

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules (Basel, Switzerland)*, *20*(5), 8856–8874. http://doi.org/10.3390/molecules20058856

García-Quintanilla, M., Pulido, M. R., López-Rojas, R., Pachón, J., & McConnell, M. J. (2013). Emerging therapies for multidrug resistant Acinetobacter baumannii. *Trends in Microbiology*, *21*(3), 157–163. http://doi.org/10.1016/j.tim.2012.12.002

Garcia-Quintanilla, M., Pulido, M. R., & McConnell, M. J. (2013). First steps towards a vaccine against Acinetobacter baumannii. *Current Pharmaceutical Biotechnology*, *14*(10), 897–902.

Gardner, J., Craven, M., Dow, C., & Hines, E. (1998). The prediction of bacteria type and culture growth phase by an electronic nose with a multi-layer perceptron network. *Measurement Science and Technology*, *9*(1), 120.

Gardner, S. E., Frantz, R. A., & Doebbeling, B. N. (2001). The validity of the clinical signs and symptoms used to identify localized chronic wound infection. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *9*(3), 178–186.

Gardner, S. E., Hillis, S. L., Heilmann, K., Segre, J. A., & Grice, E. A. (2013). The Neuropathic Diabetic Foot Ulcer Microbiome Is Associated With Clinical Factors. *Diabetes*, *62*(3), 923–930. http://doi.org/10.2337/db12-0771

Gardner, S. N., Jaing, C. J., McLoughlin, K. S., & Slezak, T. R. (2010). A microbial detection array (MDA) for viral and bacterial detection. *BMC Genomics*, *11*, 668. http://doi.org/10.1186/1471-2164-11-668

Georgi, E., Guellec, S. L., Vecellio, L., Fichant, E., Stordeur, P., Bordeau, P., & Perraudin, J. P. (2011). 68* Feasibility study of OSCN⁻ and lactoferrin (Meveol®) nebulization for cystic fibrosis patients. *Journal of Cystic Fibrosis*, *10*, S18. http://doi.org/10.1016/S1569-1993(11)60087-8

Giersing, B. K., Dastgheyb, S. S., Modjarrad, K., & Moorthy, V. (2016). Status of vaccine research and development of vaccines for Staphylococcus aureus. *Vaccine*, *34*(26), 2962–2966. http://doi.org/10.1016/j.vaccine.2016.03.110

Gilbert, L. J., Li, P., Murray, C. K., Yun, H. C., Aggarwal, D., Weintrob, A. C., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2016). Multidrug-resistant gram-negative bacilli colonization risk factors among trauma patients. *Diagnostic Microbiology and Infectious Disease*, *84*(4), 358–360. http://doi.org/10.1016/j.diagmicrobio.2015.12.014

Gill, E. E., Franco, O. L., & Hancock, R. E. W. (2015). Antibiotic Adjuvants: Diverse Strategies for Controlling Drug-Resistant Pathogens. *Chemical Biology & Drug Design*, *85*(1), 56–78. http://doi.org/10.1111/cbdd.12478

Golberg, A., Broelsch, G. F., Vecchio, D., Khan, S., Hamblin, M. R., Austen, W. G., … Yarmush, M. L. (2015). Pulsed Electric Fields for Burn Wound Disinfection in a Murine Model. *Journal of Burn Care & Research : Official Publication of the American Burn Association*, *36*(1), 7–13. http://doi.org/10.1097/BCR.0000000000000157

Gomez, R., Murray, C. K., Hospenthal, D. R., Cancio, L. C., Renz, E. M., Holcomb, J. B., … Wolf, S. E. (2009). Causes of mortality by autopsy findings of combat casualties and civilian patients admitted to a burn unit. *Journal of the American College of Surgeons*, *208*(3), 348–354. http://doi.org/10.1016/j.jamcollsurg.2008.11.012

Gou, P., Kraut, N. D., Feigel, I. M., Bai, H., Morgan, G. J., Chen, Y., … Star, A. (2014). Carbon nanotube chemiresistor for wireless pH sensing. *Scientific Reports*, *4*, 4468. http://doi.org/10.1038/srep04468

Grandclément, C., Tannières, M., Moréra, S., Dessaux, Y., & Faure, D. (2016). Quorum quenching: role in nature and applied developments. *FEMS Microbiology Reviews*, *40*(1), 86–116. http://doi.org/10.1093/femsre/fuv038

Greidanus, N. V., Masri, B. A., Garbuz, D. S., Wilson, S. D., McAlinden, M. G., Xu, M., & Duncan, C. P. (2007). Use of erythrocyte sedimentation rate and C-reactive protein level to diagnose infection before revision total knee arthroplasty. A prospective evaluation. *The Journal of Bone and Joint Surgery. American Volume*, *89*(7), 1409–1416. http://doi.org/10.2106/JBJS.D.02602

Grice, E. A., & Segre, J. A. (2012). Interaction of Microbiome and the Innate Immune Response in Chronic Wounds. *Advances in Experimental Medicine and Biology*, *946*, 55–68. http://doi.org/10.1007/978-1-4614-0106-3_4

Grishin, A. V., Krivozubov, M. S., Karyagina, A. S., & Gintsburg, A. L. (2015). Pseudomonas Aeruginosa Lectins As Targets for Novel Antibacterials. *Acta Naturae*, *7*(2), 29–41.

Grumaz, S., Stevens, P., Grumaz, C., Decker, S. O., Weigand, M. A., Hofer, S., … Sohn, K. (2016). Next-generation sequencing diagnostics of bacteremia in septic patients. *Genome Medicine*, *8*. http://doi.org/10.1186/s13073-016-0326-8

Guthrie, H. C., Martin, K. R., Taylor, C., Spear, A. M., Whiting, R., Macildowie, S., … Watts, S. A. (2014). A pre-clinical evaluation of silver, iodine and Manuka honey based dressings in a model of traumatic extremity wounds contaminated with Staphylococcus aureus. *Injury*, *45*(8), 1171–1178. http://doi.org/10.1016/j.injury.2014.05.007

Hagens, S., Habel, A., & Bläsi, U. (2006). Augmentation of the antimicrobial efficacy of antibiotics by filamentous phage. *Microbial Drug Resistance (Larchmont, N.Y.)*, *12*(3), 164–168. http://doi.org/10.1089/mdr.2006.12.164

Hahm, G., Glaser, J. J., & Elster, E. A. (2011). Biomarkers to predict wound healing: the future of complex war wound management. *Plastic and Reconstructive Surgery*, *127 Suppl 1*, 21S–26S. http://doi.org/10.1097/PRS.0b013e3181fbe291

Hajdu, S., Obradovic, A., Presterl, E., & Vécsei, V. (2009). Invasive mycoses following trauma. *Injury*, *40*(5), 548–554. http://doi.org/10.1016/j.injury.2008.03.034

Hall, A. R., De Vos, D., Friman, V.-P., Pirnay, J.-P., & Buckling, A. (2012). Effects of Sequential and Simultaneous Applications of Bacteriophages on Populations of Pseudomonas aeruginosa In Vitro and in Wax Moth Larvae. *Applied and Environmental Microbiology*, *78*(16), 5646–5652. http://doi.org/10.1128/AEM.00757-12

Halwani, M., Yebio, B., Suntres, Z. E., Alipour, M., Azghani, A. O., & Omri, A. (2008). Co-encapsulation of gallium with gentamicin in liposomes enhances antimicrobial activity of gentamicin against Pseudomonas aeruginosa. *The Journal of Antimicrobial Chemotherapy*, *62*(6), 1291–1297. http://doi.org/10.1093/jac/dkn422

Han, A., Zenilman, J. M., Melendez, J. H., Shirtliff, M. E., Agostinho, A., James, G., … Lazarus, G. S. (2011). THE IMPORTANCE OF A MULTI-FACETED APPROACH TO

CHARACTERIZING THE MICROBIAL FLORA OF CHRONIC WOUNDS. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *19*(5), 532–541. http://doi.org/10.1111/j.1524-475X.2011.00720.x

Hasmann, A., Wehrschuetz-Sigl, E., Kanzler, G., Gewessler, U., Hulla, E., Schneider, K. P., … Guebitz, G. M. (2011). Novel peptidoglycan-based diagnostic devices for detection of wound infection. *Diagnostic Microbiology and Infectious Disease*, *71*(1), 12–23. http://doi.org/10.1016/j.diagmicrobio.2010.09.009

Hasmann, A., Wehrschuetz-Sigl, E., Marold, A., Wiesbauer, H., Schoeftner, R., Gewessler, U., … Guebitz, G. M. (2013). Analysis of myeloperoxidase activity in wound fluids as a marker of infection. *Annals of Clinical Biochemistry*, *50*(Pt 3), 245–254. http://doi.org/10.1258/acb.2011.010249

Hauber, H.-P., Schulz, M., Pforte, A., Mack, D., Zabel, P., & Schumacher, U. (2008). Inhalation with Fucose and Galactose for Treatment of Pseudomonas Aeruginosa in Cystic Fibrosis Patients. *International Journal of Medical Sciences*, *5*(6), 371–376.

Hawkins, C., Harper, D., Burch, D., Anggård, E., & Soothill, J. (2010). Topical treatment of Pseudomonas aeruginosa otitis of dogs with a bacteriophage mixture: a before/after clinical trial. *Veterinary Microbiology*, *146*(3–4), 309–313. http://doi.org/10.1016/j.vetmic.2010.05.014

Hawksworth, J. S., Stojadinovic, A., Gage, F. A., Tadaki, D. K., Perdue, P. W., Forsberg, J., … Elster, E. A. (2009). Inflammatory biomarkers in combat wound healing. *Annals of Surgery*, *250*(6), 1002–1007.

Heaton, S. M., Weintrob, A. C., Downing, K., Keenan, B., Aggarwal, D., Shaikh, F., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2016). Histopathological techniques for the diagnosis of combat-related invasive fungal wound infections. *BMC Clinical Pathology*, *16*, 11. http://doi.org/10.1186/s12907-016-0033-9

Heilborn, J. D., Nilsson, M. F., Sørensen, O., Ståhle-Bäckdahl, M., Kratz, G., Weber, G., & Borregaard, N. (2003). The Cathelicidin Anti-Microbial Peptide LL-37 is Involved in Re-Epithelialization of Human Skin Wounds and is Lacking in Chronic Ulcer Epithelium. *Journal of Investigative Dermatology*, *120*(3), 379–389. http://doi.org/10.1046/j.1523-1747.2003.12069.x

Heinzle, A., Papen-Botterhuis, N. E., Schiffer, D., Schneider, K. P., Binder, B., Schintler, M., … Sigl, E. (2013). Novel protease-based diagnostic devices for detection of wound infection. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *21*(3), 482–489. http://doi.org/10.1111/wrr.12040

Heo, Y.-J., Lee, Y.-R., Jung, H.-H., Lee, J., Ko, G., & Cho, Y.-H. (2009). Antibacterial Efficacy of Phages against Pseudomonas aeruginosa Infections in Mice and Drosophila

melanogaster. *Antimicrobial Agents and Chemotherapy*, *53*(6), 2469–2474. http://doi.org/10.1128/AAC.01646-08

Hinck, D., Franke, A., & Gatzka, F. (2010). Use of vacuum-assisted closure negative pressure wound therapy in combat-related injuries--literature review. *Military Medicine*, *175*(3), 173–181.

Hodkinson, B. P., & Grice, E. A. (2015a). Next-Generation Sequencing: A Review of Technologies and Tools for Wound Microbiome Research. *Advances in Wound Care*, *4*(1), 50–58. http://doi.org/10.1089/wound.2014.0542

Hodkinson, B. P., & Grice, E. A. (2015b). Next-Generation Sequencing: A Review of Technologies and Tools for Wound Microbiome Research. *Advances in Wound Care*, *4*(1), 50–58. http://doi.org/10.1089/wound.2014.0542

Hoppe, S., Bier, F. F., & von Nickisch-Rosenegk, M. (2014). Identification of Antigenic Proteins of the Nosocomial Pathogen Klebsiella pneumoniae. *PLoS ONE*, *9*(10). http://doi.org/10.1371/journal.pone.0110703

Hospenthal, D. R., & Crouch, H. K. (2009). Infection control challenges in deployed US military treatment facilities. *The Journal of Trauma*, *66*(4 Suppl), S120-128. http://doi.org/10.1097/TA.0b013e31819cdd96

Hospenthal, D. R., Crouch, H. K., English, J. F., Leach, F., Pool, J., Conger, N. G., … Gamble, W. B. (2010). Response to infection control challenges in the deployed setting: Operations Iraqi and Enduring Freedom. *The Journal of Trauma*, *69 Suppl 1*, S94-101. http://doi.org/10.1097/TA.0b013e3181e44b3f

Hospenthal, D. R., Crouch, H. K., English, J. F., Leach, F., Pool, J., Conger, N. G., … Murray, C. K. (2011). Multidrug-resistant bacterial colonization of combat-injured personnel at admission to medical centers after evacuation from Afghanistan and Iraq. *The Journal of Trauma*, *71*(1 Suppl), S52-57. http://doi.org/10.1097/TA.0b013e31822118fb

Hospenthal, D. R., Green, A. D., Crouch, H. K., English, J. F., Pool, J., Yun, H. C., & Murray, C. K. (2011). Infection Prevention and Control in Deployed Military Medical Treatment Facilities: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S290–S298. http://doi.org/10.1097/TA.0b013e318227add8

Hospenthal, D. R., & Murray, C. K. (2011). Preface: Guidelines for the Prevention of Infections Associated With Combat-Related Injuries: 2011 Update: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S197–S201. http://doi.org/10.1097/TA.0b013e318227ac23

Hospenthal, D. R., Murray, C. K., Andersen, R. C., Bell, R. B., Calhoun, J. H., Cancio, L. C., … Wortmann, G. W. (2011). Guidelines for the Prevention of Infections Associated With Combat-Related Injuries: 2011 Update: Endorsed by the Infectious Diseases Society of America and the Surgical Infection Society. *The Journal of Trauma: Injury,*

Infection, and Critical Care, *71*, S210–S234. http://doi.org/10.1097/TA.0b013e318227ac4b

Hospenthal, D. R., Murray, C. K., Andersen, R. C., Blice, J. P., Calhoun, J. H., Cancio, L. C., … Holcomb, J. B. (2008). Guidelines for the prevention of infection after combat-related injuries. *The Journal of Trauma*, *64*(3 Suppl), S211-220. http://doi.org/10.1097/TA.0b013e318163c421

Hossain, M. A., Lalloz, A., Benhaddou, A., Pagniez, F., Raymond, M., Le Pape, P., … Leblond, J. (2016). Econazole imprinted textiles with antifungal activity. *European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik e.V*, *101*, 137–144. http://doi.org/10.1016/j.ejpb.2016.02.003

Huang, W., Yao, Y., Wang, S., Xia, Y., Yang, X., Long, Q., … Ma, Y. (2016). Immunization with a 22-kDa outer membrane protein elicits protective immunity to multidrug-resistant Acinetobacter baumannii. *Scientific Reports*, *6*. http://doi.org/10.1038/srep20724

Hundenborn, J., Thurig, S., Kommerell, M., Haag, H., & Nolte, O. (2013). Severe Wound Infection with Photobacterium damselae ssp. damselae and Vibrio harveyi, following a Laceration Injury in Marine Environment: A Case Report and Review of the Literature. *Case Reports in Medicine*, *2013*. http://doi.org/10.1155/2013/610632

Hurley, M. N., Cámara, M., & Smyth, A. R. (2012). Novel approaches to the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. *The European Respiratory Journal*, *40*(4), 1014–1023. http://doi.org/10.1183/09031936.00042012

Hurlow, J., Couch, K., Laforet, K., Bolton, L., Metcalf, D., & Bowler, P. (2015). Clinical Biofilms: A Challenging Frontier in Wound Care. *Advances in Wound Care*, *4*(5), 295–301. http://doi.org/10.1089/wound.2014.0567

Insa, R., Marín, M., Martín, A., Martín-Rabadán, P., Alcalá, L., Cercenado, E., … Bouza, E. (2012). Systematic use of universal 16S rRNA gene polymerase chain reaction (PCR) and sequencing for processing pleural effusions improves conventional culture techniques. *Medicine*, *91*(2), 103–110. http://doi.org/10.1097/MD.0b013e31824dfdb0

Jain, A., Duvvuri, L. S., Farah, S., Beyth, N., Domb, A. J., & Khan, W. (2014). Antimicrobial polymers. *Advanced Healthcare Materials*, *3*(12), 1969–1985. http://doi.org/10.1002/adhm.201400418

Johnson, E. N., Burns, T. C., Hayda, R. A., Hospenthal, D. R., & Murray, C. K. (2007). Infectious complications of open type III tibial fractures among combat casualties. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *45*(4), 409–415. http://doi.org/10.1086/520029

JTTS. (2012). Treatment of Suspected Invasive Fungal Infection inWar Wounds. Joint Theater Trauma System. Retrieved from

http://www.usaisr.amedd.army.mil/cpgs/Invasive_Fungal_Infection_in_War_Wounds_1_Nov_12.pdf

Kallstrom, G. (2014). Are Quantitative Bacterial Wound Cultures Useful? *Journal of Clinical Microbiology*, *52*(8), 2753–2756. http://doi.org/10.1128/JCM.00522-14

Kaneko, Y., Thoendel, M., Olakanmi, O., Britigan, B. E., & Singh, P. K. (2007). The transition metal gallium disrupts Pseudomonas aeruginosa iron metabolism and has antimicrobial and antibiofilm activity. *The Journal of Clinical Investigation*, *117*(4), 877–888. http://doi.org/10.1172/JCI30783

Kaspar, R. L., Griffith, M. E., Mann, P. B., Lehman, D. J., Conger, N. G., Hospenthal, D. R., & Murray, C. K. (2009). Association of bacterial colonization at the time of presentation to a combat support hospital in a combat zone with subsequent 30-day colonization or infection. *Military Medicine*, *174*(9), 899–903.

Kassal, P., Kim, J., Kumar, R., de Araujo, W. R., Steinberg, I. M., Steinberg, M. D., & Wang, J. (2015). Smart bandage with wireless connectivity for uric acid biosensing as an indicator of wound status. *Electrochemistry Communications*, *56*, 6–10. http://doi.org/10.1016/j.elecom.2015.03.018

Kaźmierczak, Z., Górski, A., & Dąbrowska, K. (2014). Facing Antibiotic Resistance: Staphylococcus aureus Phages as a Medical Tool. *Viruses*, *6*(7), 2551–2570. http://doi.org/10.3390/v6072551

Keeling, J. J., Gwinn, D. E., Tintle, S. M., Andersen, R. C., & McGuigan, F. X. (2008). Short-term outcomes of severe open wartime tibial fractures treated with ring external fixation. *The Journal of Bone and Joint Surgery. American Volume*, *90*(12), 2643–2651. http://doi.org/10.2106/JBJS.G.01326

Keen, E. F., Robinson, B. J., Hospenthal, D. R., Aldous, W. K., Wolf, S. E., Chung, K. K., & Murray, C. K. (2010). Incidence and bacteriology of burn infections at a military burn center. *Burns: Journal of the International Society for Burn Injuries*, *36*(4), 461–468. http://doi.org/10.1016/j.burns.2009.10.012

Kelson, A. B., Carnevali, M., & Truong-Le, V. (2013). Gallium-based anti-infectives: targeting microbial iron-uptake mechanisms. *Current Opinion in Pharmacology*, *13*(5), 707–716. http://doi.org/10.1016/j.coph.2013.07.001

Khairnar, K., Raut, M. P., Chandekar, R. H., Sanmukh, S. G., & Paunikar, W. N. (2013). Novel bacteriophage therapy for controlling metallo-beta-lactamase producing Pseudomonas aeruginosa infection in Catfish. *BMC Veterinary Research*, *9*, 264. http://doi.org/10.1186/1746-6148-9-264

Khan, B. A., Yeh, A. J., Cheung, G. Y. C., & Otto, M. (2015). Investigational therapies targeting quorum-sensing for the treatment of Staphylococcus aureus infections. *Expert Opinion on Investigational Drugs*, *24*(5), 689–704. http://doi.org/10.1517/13543784.2015.1019062

Khan, S. I., Blumrosen, G., Vecchio, D., Golberg, A., McCormack, M. C., Yarmush, M. L., … Austen, W. G. (2016). Eradication of multidrug-resistant pseudomonas biofilm with pulsed electric fields. *Biotechnology and Bioengineering*, *113*(3), 643–650. http://doi.org/10.1002/bit.25818

Kirketerp-Møller, K., Jensen, P. Ø., Fazli, M., Madsen, K. G., Pedersen, J., Moser, C., … Bjarnsholt, T. (2008). Distribution, Organization, and Ecology of Bacteria in Chronic Wounds. *Journal of Clinical Microbiology*, *46*(8), 2717–2722. http://doi.org/10.1128/JCM.00501-08

Klein, R. S., Berger, S. A., & Yekutiel, P. (1975). Wound infection during the Yom Kippur war: observations concerning antibiotic prophylaxis and therapy. *Annals of Surgery*, *182*(1), 15–21.

Kniemeyer, O., Ebel, F., Krüger, T., Bacher, P., Scheffold, A., Luo, T., … Brakhage, A. A. (2016). Immunoproteomics of Aspergillus for the development of biomarkers and immunotherapies. *Proteomics. Clinical Applications*. http://doi.org/10.1002/prca.201600053

Kolomiets, E., Swiderska, M. A., Kadam, R. U., Johansson, E. M. V., Jaeger, K.-E., Darbre, T., & Reymond, J.-L. (2009). Glycopeptide dendrimers with high affinity for the fucose-binding lectin LecB from Pseudomonas aeruginosa. *ChemMedChem*, *4*(4), 562–569. http://doi.org/10.1002/cmdc.200800380

Kostyanev, T., Bonten, M. J. M., O'Brien, S., Steel, H., Ross, S., François, B., … Goossens, H. (2016). The Innovative Medicines Initiative's New Drugs for Bad Bugs programme: European public-private partnerships for the development of new strategies to tackle antibiotic resistance. *The Journal of Antimicrobial Chemotherapy*, *71*(2), 290–295. http://doi.org/10.1093/jac/dkv339

Krachler, A. M., Mende, K., Murray, C., & Orth, K. (2012). In vitro characterization of multivalent adhesion molecule 7-based inhibition of multidrug-resistant bacteria isolated from wounded military personnel. *Virulence*, *3*(4), 389–399. http://doi.org/10.4161/viru.20816

Krylov, V., Shaburova, O., Krylov, S., & Pleteneva, E. (2012). A Genetic Approach to the Development of New Therapeutic Phages to Fight Pseudomonas Aeruginosa in Wound Infections. *Viruses*, *5*(1), 15–53. http://doi.org/10.3390/v5010015

Kumar, P., Chen, K., & Kolls, J. K. (2013). Th17 cell based vaccines in mucosal immunity. *Current Opinion in Immunology*, *25*(3), 373–380. http://doi.org/10.1016/j.coi.2013.03.011

Lack, W. D., Karunakar, M. A., Angerame, M. R., Seymour, R. B., Sims, S., Kellam, J. F., & Bosse, M. J. (2015). Type III open tibia fractures: immediate antibiotic prophylaxis minimizes infection. *Journal of Orthopaedic Trauma*, *29*(1), 1–6. http://doi.org/10.1097/BOT.0000000000000262

Langan, N. R., Eckert, M., & Martin, M. J. (2014). Changing patterns of in-hospital deaths following implementation of damage control resuscitation practices in US forward military treatment facilities. *JAMA Surgery*, *149*(9), 904–912. http://doi.org/10.1001/jamasurg.2014.940

Leber, A. L. (Ed.). (2016). *Clinical Microbiology Procedures Handbook, Fourth Edition* (Vol. 2). American Society of Microbiology. Retrieved from http://www.asmscience.org/content/book/10.1128/9781555818814

Lee, W.-H., Choi, H.-I., Hong, S.-W., Kim, K., Gho, Y. S., & Jeon, S. G. (2015). Vaccination with Klebsiella pneumoniae-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. *Experimental & Molecular Medicine*, *47*(9), e183. http://doi.org/10.1038/emm.2015.59

Leli, C., Cenci, E., Cardaccia, A., Moretti, A., D'Alò, F., Pagliochini, R., … Mencacci, A. (2013). Rapid identification of bacterial and fungal pathogens from positive blood cultures by MALDI-TOF MS. *International Journal of Medical Microbiology: IJMM*, *303*(4), 205–209. http://doi.org/10.1016/j.ijmm.2013.03.002

Lesho, E., Craft, D., Kirkup, B. C., Waterman, P., Summers, A., Vahey, M. T., … Bowden, R. (2011). Surveillance, characterisation, and preservation of multidrug-resistant bacteria. *The Lancet. Infectious Diseases*, *11*(1), 8–10. http://doi.org/10.1016/S1473-3099(10)70261-9

Lewandowski, L., Purcell, R., Fleming, M., & Gordon, W. T. (2013). The use of dilute Dakin's solution for the treatment of angioinvasive fungal infection in the combat wounded: a case series. *Military Medicine*, *178*(4), e503-507. http://doi.org/10.7205/MILMED-D-12-00322

Lewandowski, L., Weintrob, A. C., Tribble, D. R., Rodriguez, C. J., Petfield, J., Lloyd, B. A., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2016). Early Complications and Outcomes in Combat Injury-Related Invasive Fungal Wound Infections: A Case-Control Analysis. *Journal of Orthopaedic Trauma*, *30*(3), e93-99. http://doi.org/10.1097/BOT.0000000000000447

Li, J., Topaz, M., Tan, H., Li, Y., Li, W., Xun, W., … Li, X. (2013). Treatment of infected soft tissue blast injury in swine by regulated negative pressure wound therapy. *Annals of Surgery*, *257*(2), 335–344. http://doi.org/10.1097/SLA.0b013e318269d1ca

Li, X., Kong, H., Mout, R., Saha, K., Moyano, D. F., Robinson, S. M., … Rotello, V. M. (2014). Rapid Identification of Bacterial Biofilms and Biofilm Wound Models Using a Multichannel Nanosensor. *ACS Nano*, *8*(12), 12014–12019. http://doi.org/10.1021/nn505753s

Lin, N.-T., Chiou, P.-Y., Chang, K.-C., Chen, L.-K., & Lai, M.-J. (2010). Isolation and characterization of phi AB2: a novel bacteriophage of Acinetobacter baumannii. *Research in Microbiology*, *161*(4), 308–314. http://doi.org/10.1016/j.resmic.2010.03.007

Lisboa, F. A., Forsberg, J. A., Brown, T. S., Gage, F. A., Potter, B. K., & Elster, E. A. (2013). Bilateral lower-extremity amputation wounds are associated with distinct local and systemic cytokine response. *Surgery*, *154*(2), 282–290. http://doi.org/10.1016/j.surg.2013.04.024

Liu, Y., Zhou, Q., Wang, Y., Liu, Z., Dong, M., Wang, Y., … Hu, D. (2014). Negative Pressure Wound Therapy Decreases Mortality in a Murine Model of Burn-Wound Sepsis Involving Pseudomonas aeruginosa Infection. *PLoS ONE*, *9*(2). http://doi.org/10.1371/journal.pone.0090494

Lloyd, B., Weintrob, A. C., Hinkle, M. K., Fortuna, G. R., Murray, C. K., Bradley, W., … Tribble, D. R. (2014). Adherence to published antimicrobial prophylaxis guidelines for wounded service members in the ongoing conflicts in Southwest Asia. *Military Medicine*, *179*(3), 324–328. http://doi.org/10.7205/MILMED-D-13-00424

Lloyd, B., Weintrob, A. C., Rodriguez, C., Dunne, J. R., Weisbrod, A. B., Hinkle, M., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2014). Effect of early screening for invasive fungal infections in U.S. service members with explosive blast injuries. *Surgical Infections*, *15*(5), 619–626. http://doi.org/10.1089/sur.2012.245

Lundberg, U., Senn, B. M., Schüler, W., Meinke, A., & Hanner, M. (2013). Identification and characterization of antigens as vaccine candidates against Klebsiella pneumoniae. *Human Vaccines & Immunotherapeutics*, *9*(3), 497–505. http://doi.org/10.4161/hv.23225

Madhava Charyulu, E., Gnanamani, A., & Mandal, A. B. (2012). Identification and Discrimination of Methicillin Resistant Staphylococcus aureus Strains Isolated from Burn Wound Sites Using PCR and Authentication with MALDI-TOF–MS. *Indian Journal of Microbiology*, *52*(3), 337–345. http://doi.org/10.1007/s12088-011-0245-8

Magana, M., Ioannidis, A., Magiorkinis, E., Ursu, O., Bologa, C. G., Chatzipanagiotou, S., … Tegos, G. P. (2015). Therapeutic options and emerging alternatives for multidrug resistant staphylococcal infections. *Current Pharmaceutical Design*, *21*(16), 2058–2072.

Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., & Jarvis, W. R. (1999). Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infection Control and Hospital Epidemiology*, *20*(4), 250-278-280. http://doi.org/10.1086/501620

Marin, S., Vlasceanu, G. M., Tiplea, R. E., Bucur, I. R., Lemnaru, M., Marin, M. M., & Grumezescu, A. M. (2015). Applications and toxicity of silver nanoparticles: a recent review. *Current Topics in Medicinal Chemistry*, *15*(16), 1596–1604.

Martin, G. J., Dunne, J. R., Cho, J. M., & Solomkin, J. S. (2011). Prevention of Infections Associated With Combat-Related Thoracic and Abdominal Cavity Injuries: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S270–S281. http://doi.org/10.1097/TA.0b013e318227adae

Marza, J. A. S., Soothill, J. S., Boydell, P., & Collyns, T. A. (2006). Multiplication of therapeutically administered bacteriophages in Pseudomonas aeruginosa infected patients. *Burns: Journal of the International Society for Burn Injuries*, *32*(5), 644–646. http://doi.org/10.1016/j.burns.2006.02.012

McCullough, M., & Carlson, G. W. (2014). Dakin's solution: historical perspective and current practice. *Annals of Plastic Surgery*, *73*(3), 254–256. http://doi.org/10.1097/SAP.0b013e3182a634f7

McLister, A., Phair, J., Cundell, J., & Davis, J. (2014). Electrochemical approaches to the development of smart bandages: A mini-review. *Electrochemistry Communications*, *40*, 96–99. http://doi.org/10.1016/j.elecom.2014.01.003

McVay, C. S., Velásquez, M., & Fralick, J. A. (2007). Phage Therapy of Pseudomonas aeruginosa Infection in a Mouse Burn Wound Model. *Antimicrobial Agents and Chemotherapy*, *51*(6), 1934–1938. http://doi.org/10.1128/AAC.01028-06

Medici, N. P., & Del Poeta, M. (2015). New insights on the development of fungal vaccines: from immunity to recent challenges. *Memórias Do Instituto Oswaldo Cruz*, *110*(8), 966–973. http://doi.org/10.1590/0074-02760150335

MedImmune LLC. (2016). Study of the Efficacy and Safety of MEDI4893 - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02296320?term=MEDI4893&rank=1

Mehmood, N., Hariz, A., Fitridge, R., & Voelcker, N. H. (2014). Applications of modern sensors and wireless technology in effective wound management. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials*, *102*(4), 885–895. http://doi.org/10.1002/jbm.b.33063

Melvin, J. A., Lashua, L. P., Kiedrowski, M. R., Yang, G., Deslouches, B., Montelaro, R. C., & Bomberger, J. M. (2016). Simultaneous Antibiofilm and Antiviral Activities of an Engineered Antimicrobial Peptide during Virus-Bacterium Coinfection. *mSphere*, *1*(3). http://doi.org/10.1128/mSphere.00083-16

Mende, K., Beckius, M. L., Zera, W. C., Yu, X., Cheatle, K. A., Aggarwal, D., … Murray, C. K. (2014). Phenotypic and Genotypic Changes over Time and across Facilities of Serial Colonizing and Infecting Escherichia coli Isolates Recovered from Injured Service Members. *Journal of Clinical Microbiology*, *52*(11), 3869–3877. http://doi.org/10.1128/JCM.00821-14

Mérens, A., Rapp, C., Delaune, D., Danis, J., Berger, F., & Michel, R. (2014). Prevention of combat-related infections: antimicrobial therapy in battlefield and barrier measures in French military medical treatment facilities. *Travel Medicine and Infectious Disease*, *12*(4), 318–329. http://doi.org/10.1016/j.tmaid.2014.04.013

Metcalf, D. G., Bowler, P. G., & Hurlow, J. (2014). A clinical algorithm for wound biofilm identification. *Journal of Wound Care*, *23*(3), 137–138, 140–142. http://doi.org/10.12968/jowc.2014.23.3.137

Metzger, S., Frobel, R. A., & Dunne, W. M. (2014). Rapid simultaneous identification and quantitation of Staphylococcus aureus and Pseudomonas aeruginosa directly from bronchoalveolar lavage specimens using automated microscopy. *Diagnostic Microbiology and Infectious Disease*, *79*(2), 160–165. http://doi.org/10.1016/j.diagmicrobio.2013.11.029

Mihu, M. R., & Martinez, L. R. (2011). Novel therapies for treatment of multi-drug resistant Acinetobacter baumannii skin infections. *Virulence*, *2*(2), 97–102.

Mihu, M. R., Sandkovsky, U., Han, G., Friedman, J. M., Nosanchuk, J. D., & Martinez, L. R. (2010). The use of nitric oxide releasing nanoparticles as a treatment against Acinetobacter baumannii in wound infections. *Virulence*, *1*(2), 62–67. http://doi.org/10.4161/viru.1.2.10038

Misic, A. M., Gardner, S. E., & Grice, E. A. (2014). The Wound Microbiome: Modern Approaches to Examining the Role of Microorganisms in Impaired Chronic Wound Healing. *Advances in Wound Care*, *3*(7), 502–510. http://doi.org/10.1089/wound.2012.0397

Mody, R. M., Zapor, M., Hartzell, J. D., Robben, P. M., Waterman, P., Wood-Morris, R., … Wortmann, G. (2009). Infectious complications of damage control orthopedics in war trauma. *The Journal of Trauma*, *67*(4), 758–761. http://doi.org/10.1097/TA.0b013e3181af6aa6

Mokline, A., Garsallah, L., Rahmani, I., Jerbi, K., Oueslati, H., Tlaili, S., … Messadi, A. A. (2015). Procalcitonin: a diagnostic and prognostic biomarker of sepsis in burned patients. *Annals of Burns and Fire Disasters*, *28*(2), 116–120.

Moreau-Marquis, S., Coutermarsh, B., & Stanton, B. A. (2015). Combination of hypothiocyanite and lactoferrin (ALX-109) enhances the ability of tobramycin and aztreonam to eliminate Pseudomonas aeruginosa biofilms growing on cystic fibrosis airway epithelial cells. *Journal of Antimicrobial Chemotherapy*, *70*(1), 160–166. http://doi.org/10.1093/jac/dku357

Moreau-Marquis, S., O'Toole, G. A., & Stanton, B. A. (2009). Tobramycin and FDA-Approved Iron Chelators Eliminate Pseudomonas aeruginosa Biofilms on Cystic Fibrosis Cells. *American Journal of Respiratory Cell and Molecular Biology*, *41*(3), 305–313. http://doi.org/10.1165/rcmb.2008-0299OC

Morello, E., Saussereau, E., Maura, D., Huerre, M., Touqui, L., & Debarbieux, L. (2011). Pulmonary Bacteriophage Therapy on Pseudomonas aeruginosa Cystic Fibrosis Strains: First Steps Towards Treatment and Prevention. *PLOS ONE*, *6*(2), e16963. http://doi.org/10.1371/journal.pone.0016963

Moriyama, B., Gordon, L. A., McCarthy, M., Henning, S. A., Walsh, T. J., & Penzak, S. R. (2014). Emerging Drugs and Vaccines for Candidemia. *Mycoses*, *57*(12), 718–733. http://doi.org/10.1111/myc.12265

Moultrie, D., Hawker, J., & Cole, S. (2011). Factors associated with multidrug-resistant Acinetobacter transmission: an integrative review of the literature. *AORN Journal*, *94*(1), 27–36. http://doi.org/10.1016/j.aorn.2010.12.026

Murphy, R. A., Ronat, J.-B., Fakhri, R. M., Herard, P., Blackwell, N., Abgrall, S., & Anderson, D. J. (2011). Multidrug-resistant chronic osteomyelitis complicating war injury in Iraqi civilians. *The Journal of Trauma*, *71*(1), 252–254. http://doi.org/10.1097/TA.0b013e31821b8622

Murray, C. K. (2008a). Epidemiology of infections associated with combat-related injuries in Iraq and Afghanistan. *The Journal of Trauma*, *64*(3 Suppl), S232-238. http://doi.org/10.1097/TA.0b013e318163c3f5

Murray, C. K. (2008b). Infectious disease complications of combat-related injuries. *Critical Care Medicine*, *36*(7 Suppl), S358-364. http://doi.org/10.1097/CCM.0b013e31817e2ffc

Murray, C. K., Griffith, M. E., Mende, K., Guymon, C. H., Ellis, M. W., Beckius, M., … Hospenthal, D. R. (2010). Methicillin-resistant Staphylococcus aureus in wound cultures recovered from a combat support hospital in Iraq. *The Journal of Trauma*, *69 Suppl 1*, S102-108. http://doi.org/10.1097/TA.0b013e3181e44b57

Murray, C. K., Hospenthal, D. R., Kotwal, R. S., & Butler, F. K. (2011). Efficacy of point-of-injury combat antimicrobials. *The Journal of Trauma*, *71*(2 Suppl 2), S307-313. http://doi.org/10.1097/TA.0b013e318227af79

Murray, C. K., Hsu, J. R., Solomkin, J. S., Keeling, J. J., Andersen, R. C., Ficke, J. R., & Calhoun, J. H. (2008). Prevention and management of infections associated with combat-related extremity injuries. *The Journal of Trauma*, *64*(3 Suppl), S239-251. http://doi.org/10.1097/TA.0b013e318163cd14

Murray, C. K., Loo, F. L., Hospenthal, D. R., Cancio, L. C., Jones, J. A., Kim, S. H., … Wolf, S. E. (2008). Incidence of systemic fungal infection and related mortality following severe burns. *Burns: Journal of the International Society for Burn Injuries*, *34*(8), 1108–1112. http://doi.org/10.1016/j.burns.2008.04.007

Murray, C. K., Obremskey, W. T., Hsu, J. R., Andersen, R. C., Calhoun, J. H., Clasper, J. C., … Ficke, J. R. (2011). Prevention of Infections Associated With Combat-Related Extremity Injuries: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S235–S257. http://doi.org/10.1097/TA.0b013e318227ac5f

Murray, C. K., Roop, S. A., Hospenthal, D. R., Dooley, D. P., Wenner, K., Hammock, J., … Gourdine, E. (2006). Bacteriology of war wounds at the time of injury. *Military Medicine*, *171*(9), 826–829.

Murray, C. K., Wilkins, K., Molter, N. C., Li, F., Yu, L., Spott, M. A., … Hospenthal, D. R. (2011). Infections complicating the care of combat casualties during operations Iraqi Freedom and Enduring Freedom. *The Journal of Trauma*, *71*(1 Suppl), S62-73. http://doi.org/10.1097/TA.0b013e3182218c99

Murray, C. K., Wilkins, K., Molter, N. C., Yun, H. C., Dubick, M. A., Spott, M. A., … Hospenthal, D. R. (2009). Infections in combat casualties during Operations Iraqi and Enduring Freedom. *The Journal of Trauma*, *66*(4 Suppl), S138-144. http://doi.org/10.1097/TA.0b013e31819d894c

Naccache, S. N., Peggs, K. S., Mattes, F. M., Phadke, R., Garson, J. A., Grant, P., … Chiu, C. Y. (2015). Diagnosis of Neuroinvasive Astrovirus Infection in an Immunocompromised Adult With Encephalitis by Unbiased Next-Generation Sequencing. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *60*(6), 919–923. http://doi.org/10.1093/cid/ciu912

Nanjappa, S. G., & Klein, B. S. (2014). Vaccine Immunity against Fungal Infections. *Current Opinion in Immunology*, *0*, 27–33. http://doi.org/10.1016/j.coi.2014.01.014

NovaDigm Therapeutics, Inc. (2016). Safety and Immunogenicity Study of a Recombinant Protein Vaccine (NDV-3) Against S.Aureus and Candida - Full Text View - ClinicalTrials.gov. In *In: ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT01273922?term=NDV3&rank=2

Ostorhazi, E., Rozgonyi, F., Sztodola, A., Harmos, F., Kovalszky, I., Szabo, D., … Otvos, L. (2010). Preclinical advantages of intramuscularly administered peptide A3-APO over existing therapies in Acinetobacter baumannii wound infections. *Journal of Antimicrobial Chemotherapy*, *65*(11), 2416–2422. http://doi.org/10.1093/jac/dkq337

Otvos, L., & Ostorhazi, E. (2015). Therapeutic utility of antibacterial peptides in wound healing. *Expert Review of Anti-Infective Therapy*, *13*(7), 871–881. http://doi.org/10.1586/14787210.2015.1033402

Palacios, G., Quan, P.-L., Jabado, O. J., Conlan, S., Hirschberg, D. L., Liu, Y., … Lipkin, W. I. (2007). Panmicrobial Oligonucleotide Array for Diagnosis of Infectious Diseases. *Emerging Infectious Diseases*, *13*(1), 73–81. http://doi.org/10.3201/eid1301.060837

Parker, A. C., Beenken, K. E., Jennings, J. A., Hittle, L., Shirtliff, M. E., Bumgardner, J. D., … Haggard, W. O. (2015). Characterization of local delivery with amphotericin B and vancomycin from modified chitosan sponges and functional biofilm prevention evaluation. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society*, *33*(3), 439–447. http://doi.org/10.1002/jor.22760

Patel, R. (2015). MALDI-TOF MS for the Diagnosis of Infectious Diseases. *Clinical Chemistry*, *61*(1), 100–111. http://doi.org/10.1373/clinchem.2014.221770

Penn-Barwell, J. G., Bennett, P. M., Fries, C. A., Kendrew, J. M., Midwinter, M. J., & Rickard, R. F. (2013). Severe open tibial fractures in combat trauma: management and

preliminary outcomes. *The Bone & Joint Journal*, *95–B*(1), 101–105. http://doi.org/10.1302/0301-620X.95B1.30580

Penn-Barwell, J. G., Bennett, P. M., Mortiboy, D. E., Fries, C. A., Groom, A. F. G., & Sargeant, I. D. (2016). Factors influencing infection in 10 years of battlefield open tibia fractures. *Strategies in Trauma and Limb Reconstruction*, *11*(1), 13–18. http://doi.org/10.1007/s11751-016-0250-x

Penn-Barwell, J. G., Murray, C. K., & Wenke, J. C. (2014). Local antibiotic delivery by a bioabsorbable gel is superior to PMMA bead depot in reducing infection in an open fracture model. *Journal of Orthopaedic Trauma*, *28*(6), 370–375. http://doi.org/10.1097/BOT.0b013e3182a7739e

Percival, S. L., McCarty, S. M., & Lipsky, B. (2015). Biofilms and Wounds: An Overview of the Evidence. *Advances in Wound Care*, *4*(7), 373–381. http://doi.org/10.1089/wound.2014.0557

Peters, B. M., Shirtliff, M. E., & Jabra-Rizk, M. A. (2010). Antimicrobial Peptides: Primeval Molecules or Future Drugs? *PLoS Pathogens*, *6*(10). http://doi.org/10.1371/journal.ppat.1001067

Petersen, K., Colyer, M. H., Hayes, D. K., Hale, R. G., & Bell, R. B. (2011). Prevention of Infections Associated With Combat-Related Eye, Maxillofacial, and Neck Injuries: *The Journal of Trauma: Injury, Infection, and Critical Care*, *71*, S264–S269. http://doi.org/10.1097/TA.0b013e318227ad9a

Petersen, K., Hayes, D. K., Blice, J. P., & Hale, R. G. (2008). Prevention and management of infections associated with combat-related head and neck injuries. *The Journal of Trauma*, *64*(3 Suppl), S265-276. http://doi.org/10.1097/TA.0b013e318163d2a6

Petersen, K., Riddle, M. S., Danko, J. R., Blazes, D. L., Hayden, R., Tasker, S. A., & Dunne, J. R. (2007). Trauma-related infections in battlefield casualties from Iraq. *Annals of Surgery*, *245*(5), 803–811. http://doi.org/10.1097/01.sla.0000251707.32332.c1

Pfaller, M. A., Richter, S. S., Funke, G., Jorgensen, J. H., Landry, M. L., Carroll, K. C., & Warnock, D. W. (Eds.). (2015). *Manual of Clinical Microbiology, 11th Edition*. American Society of Microbiology. Retrieved from http://www.asmscience.org/content/book/10.1128/9781555817381

Pfizer. (2016a). SA4Ag Safety, Tolerability, and Immunogenicity Study in Japanese Adults - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02492958?term=SA4Ag&rank=5

Pfizer. (2016b). Safety and Efficacy of SA4Ag Vaccine in Adults Having Elective Posterior Instrumented Lumbar Spinal Fusion Procedure - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02388165

Pherecydes Pharma. (2016). Evaluation of Phage Therapy for the Treatment of Escherichia Coli and Pseudomonas Aeruginosa Wound Infections in Burned Patients - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02116010?term=P.+aeruginosa&rank=1

Pires, D. P., Vilas Boas, D., Sillankorva, S., & Azeredo, J. (2015). Phage Therapy: a Step Forward in the Treatment of Pseudomonas aeruginosa Infections. *Journal of Virology*, *89*(15), 7449–7456. http://doi.org/10.1128/JVI.00385-15

Polfer, E. M., Hoyt, B. W., Senchak, L. T., Murphey, M. D., Forsberg, J. A., & Potter, B. K. (2014). Fluid collections in amputations are not indicative or predictive of infection. *Clinical Orthopaedics and Related Research*, *472*(10), 2978–2983. http://doi.org/10.1007/s11999-014-3586-x

Polkade, A. V., Mantri, S. S., Patwekar, U. J., & Jangid, K. (2016). Quorum Sensing: An Under-Explored Phenomenon in the Phylum Actinobacteria. *Frontiers in Microbiology*, *7*. http://doi.org/10.3389/fmicb.2016.00131

Pollak, A. N., Jones, A. L., Castillo, R. C., Bosse, M. J., MacKenzie, E. J., & LEAP Study Group. (2010). The relationship between time to surgical debridement and incidence of infection after open high-energy lower extremity trauma. *The Journal of Bone and Joint Surgery. American Volume*, *92*(1), 7–15. http://doi.org/10.2106/JBJS.H.00984

Polyphor Ltd. (2016). Safety, Efficacy and PK/PD of POL7080 in Patients With Exacerbation of Non-cystic Fibrosis Bronchiectasis. - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02096315?term=POL7080&rank=3

Price, C. S., Kon, S. E., & Metzger, S. (2014). Rapid antibiotic susceptibility phenotypic characterization of Staphylococcus aureus using automated microscopy of small numbers of cells. *Journal of Microbiological Methods*, *98*, 50–58. http://doi.org/10.1016/j.mimet.2013.12.021

Price, L. B., Liu, C. M., Melendez, J. H., Frankel, Y. M., Engelthaler, D., Aziz, M., … Zenilman, J. M. (2009). Community Analysis of Chronic Wound Bacteria Using 16S rRNA Gene-Based Pyrosequencing: Impact of Diabetes and Antibiotics on Chronic Wound Microbiota. *PLoS ONE*, *4*(7). http://doi.org/10.1371/journal.pone.0006462

Priebe, G. P., & Goldberg, J. B. (2014). Vaccines for Pseudomonas aeruginosa: A long and winding road. *Expert Review of Vaccines*, *13*(4), 507–519. http://doi.org/10.1586/14760584.2014.890053

Qadir, M. I. (2015). Review: phage therapy: a modern tool to control bacterial infections. *Pakistan Journal of Pharmaceutical Sciences*, *28*(1), 265–270.

Radowsky, J. S., Strawn, A. A., Sherwood, J., Braden, A., & Liston, W. (2011). Invasive mucormycosis and aspergillosis in a healthy 22-year-old battle casualty: case report. *Surgical Infections*, *12*(5), 397–400. http://doi.org/10.1089/sur.2010.065

Rangel-Vega, A., Bernstein, L. R., Mandujano-Tinoco, E. A., García-Contreras, S. J., & García-Contreras, R. (2015). Drug repurposing as an alternative for the treatment of recalcitrant bacterial infections. *Frontiers in Microbiology*, *6*. http://doi.org/10.3389/fmicb.2015.00282

Reinhart, K., Bauer, M., Riedemann, N. C., & Hartog, C. S. (2012). New Approaches to Sepsis: Molecular Diagnostics and Biomarkers. *Clinical Microbiology Reviews*, *25*(4), 609–634. http://doi.org/10.1128/CMR.00016-12

Rhoads, D. D., Wolcott, R. D., Sun, Y., & Dowd, S. E. (2012). Comparison of Culture and Molecular Identification of Bacteria in Chronic Wounds. *International Journal of Molecular Sciences*, *13*(3), 2535–2550. http://doi.org/10.3390/ijms13032535

Robson, M. C., Duke, W. F., & Krizek, T. J. (1973). Rapid bacterial screening in the treatment of civilian wounds. *The Journal of Surgical Research*, *14*(5), 426–430.

Rodriguez, C., Weintrob, A. C., Dunne, J. R., Weisbrod, A. B., Lloyd, B., Warkentien, T., … the Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Investigative Team. (2014). Clinical relevance of mold culture positivity with and without recurrent wound necrosis following combat-related injuries. *The Journal of Trauma and Acute Care Surgery*, *77*(5), 769–773. http://doi.org/10.1097/TA.0000000000000438

Rodriguez, C., Weintrob, A. C., Shah, J., Malone, D., Dunne, J. R., Weisbrod, A. B., … Tribble, D. R. (2014). Risk Factors Associated with Invasive Fungal Infections in Combat Trauma. *Surgical Infections*, *15*(5), 521–526. http://doi.org/10.1089/sur.2013.123

Rodríguez-Sánchez, B., Sánchez-Carrillo, C., Ruiz, A., Marín, M., Cercenado, E., Rodríguez-Créixems, M., & Bouza, E. (2014). Direct identification of pathogens from positive blood cultures using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *20*(7), O421-427. http://doi.org/10.1111/1469-0691.12455

Romanoff, H. (1975). Prevention of infection in war chest injuries. *Annals of Surgery*, *182*(2), 144–149.

Rose, T., Verbeken, G., Vos, D. D., Merabishvili, M., Vaneechoutte, M., Lavigne, R., … Pirnay, J.-P. (2014). Experimental phage therapy of burn wound infection: difficult first steps. *International Journal of Burns and Trauma*, *4*(2), 66–73.

Rota, P. A., Trees, E., MacCannell, D., & Gerner-Smidt, P. (2015). Molecular Epidemiology. In M. A. Pfaller, S. S. Richter, G. Funke, J. H. Jorgensen, M. L. Landry, K. C. Carroll, & D. W. Warnock (Eds.), *Manual of Clinical Microbiology, 11th Edition* (pp.

131–160). American Society of Microbiology. Retrieved from http://www.asmscience.org/content/book/10.1128/9781555817381.mcm11.ch10

Rutala, W., Weber, D., Healthcare, & Infection Control Practices Advisory Committee (HICPAC). (2008). Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. The Centers for Disease Control and Prevention.

Sacco, P., Travan, A., Borgogna, M., Paoletti, S., & Marsich, E. (2015). Silver-containing antimicrobial membrane based on chitosan-TPP hydrogel for the treatment of wounds. *Journal of Materials Science. Materials in Medicine*, *26*(3), 128. http://doi.org/10.1007/s10856-015-5474-7

Saffert, R. T., Cunningham, S. A., Ihde, S. M., Jobe, K. E. M., Mandrekar, J., & Patel, R. (2011). Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. *Journal of Clinical Microbiology*, *49*(3), 887–892. http://doi.org/10.1128/JCM.01890-10

Sahli, Z. T., Bizri, A. R., & Abu-Sittah, G. S. (2016). Microbiology and risk factors associated with war-related wound infections in the Middle East. *Epidemiology and Infection*, 1–10. http://doi.org/10.1017/S0950268816000431

Santos, E., & Levitz, S. M. (2014). Fungal vaccines and immunotherapeutics. *Cold Spring Harbor Perspectives in Medicine*, *4*(11), a019711. http://doi.org/10.1101/cshperspect.a019711

Sause, W. E., Buckley, P. T., Strohl, W. R., Lynch, A. S., & Torres, V. J. (2016). Antibody-Based Biologics and Their Promise to Combat Staphylococcus aureus Infections. *Trends in Pharmacological Sciences*, *37*(3), 231–241. http://doi.org/10.1016/j.tips.2015.11.008

SBIR Source. (2016). SBIR Open Funding Opportunities - SBIR Source. Retrieved July 15, 2016, from http://sbirsource.com/sbir/topics/86930

Schmidt, C. S., White, C. J., Ibrahim, A. S., Filler, S. G., Fu, Y., Yeaman, M. R., … Hennessey, J. P. (2012). NDV-3, a Recombinant Alum-Adjuvanted Vaccine for Candida and Staphylococcus aureus is Safe and Immunogenic in Healthy Adults. *Vaccine*, *30*(52), 7594–7600. http://doi.org/10.1016/j.vaccine.2012.10.038

Scott, P., Deye, G., Srinivasan, A., Murray, C., Moran, K., Hulten, E., … Petruccelli, B. (2007). An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *44*(12), 1577–1584. http://doi.org/10.1086/518170

Scynexis, Inc. (2016). Oral SCY-078 vs Standard-of-Care Following IV Echinocandin in the Treatment of Invasive Candidiasis - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02244606?term=SCY-078+candida&rank=1

Sehulster, L., Chinn, R. Y. W., CDC, & HICPAC. (2003). Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR. Recommendations and Reports: Morbidity and Mortality Weekly Report. Recommendations and Reports / Centers for Disease Control*, *52*(RR-10), 1–42.

Senanayake, E. L., Poon, H., Graham, T. R., & Midwinter, M. J. (2014). UK specialist cardiothoracic management of thoracic injuries in military casualties sustained in the wars in Iraq and Afghanistan. *European Journal of Cardio-Thoracic Surgery: Official Journal of the European Association for Cardio-Thoracic Surgery*, *45*(6), e202-3207. http://doi.org/10.1093/ejcts/ezu076

Serena, T. E., Hanft, J. R., & Snyder, R. (2008). The lack of reliability of clinical examination in the diagnosis of wound infection: preliminary communication. *The International Journal of Lower Extremity Wounds*, *7*(1), 32–35. http://doi.org/10.1177/1534734607313984

Sevgi, M., Toklu, A., Vecchio, D., & Hamblin, M. R. (2013). Topical Antimicrobials for Burn Infections – An Update. *Recent Patents on Anti-Infective Drug Discovery*, *8*(3), 161–197.

Shankar, R., He, L.-K., Szilagyi, A., Muthu, K., Gamelli, R. L., Filutowicz, M., … Dominguez, M. (2007). A novel antibacterial gene transfer treatment for multidrug-resistant Acinetobacter baumannii-induced burn sepsis. *Journal of Burn Care & Research: Official Publication of the American Burn Association*, *28*(1), 6–12. http://doi.org/10.1097/BCR.0b013e31802c8861

Sheean, A. J., Tintle, S. M., & Rhee, P. C. (2015). Soft tissue and wound management of blast injuries. *Current Reviews in Musculoskeletal Medicine*, *8*(3), 265–271. http://doi.org/10.1007/s12178-015-9275-x

Sheppard, F. R., Keiser, P., Craft, D. W., Gage, F., Robson, M., Brown, T. S., … Elster, E. (2010). The majority of US combat casualty soft-tissue wounds are not infected or colonized upon arrival or during treatment at a continental US military medical facility. *American Journal of Surgery*, *200*(4), 489–495. http://doi.org/10.1016/j.amjsurg.2010.03.001

Siegel, J. D., Rhinehart, E., Jackson, M., Chiarello, L., & Healthcare Infection Control Practices Advisory Committee. (2007). Management of multidrug-resistant organisms in health care settings, 2006. *American Journal of Infection Control*, *35*(10 Suppl 2), S165-193. http://doi.org/10.1016/j.ajic.2007.10.006

Simchen, E., Raz, R., Stein, H., & Danon, Y. (1991). Risk factors for infection in fracture war wounds (1973 and 1982 wars, Israel). *Military Medicine*, *156*(10), 520–527.

Simon, L., Gauvin, F., Amre, D. K., Saint-Louis, P., & Lacroix, J. (2004). Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clinical Infectious Diseases: An Official*

Publication of the Infectious Diseases Society of America, *39*(2), 206–217. http://doi.org/10.1086/421997

Şişli, E., Kavala, A. A., Mavi, M., Sarıosmanoğlu, O. N., & Oto, Ö. (2016). Single centre experience of combat-related vascular injury in victims of Syrian conflict: Retrospective evaluation of risk factors associated with amputation. *Injury*, *47*(9), 1945–1950. http://doi.org/10.1016/j.injury.2016.03.030

Sismaet, H. J., Banerjee, A., McNish, S., Choi, Y., Torralba, M., Lucas, S., … Goluch, E. D. (2016). Electrochemical detection of Pseudomonas in wound exudate samples from patients with chronic wounds. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *24*(2), 366–372. http://doi.org/10.1111/wrr.12414

Song, Z., Sun, H., Yang, Y., Jing, H., Yang, L., Tong, Y., … Zeng, H. (2016). Enhanced efficacy and anti-biofilm activity of novel nanoemulsions against skin burn wound multi-drug resistant MRSA infections. *Nanomedicine: Nanotechnology, Biology, and Medicine*. http://doi.org/10.1016/j.nano.2016.01.015

Sprockett, D. D., Ammons, C. G., & Tuttle, M. S. (2015). Use of 16S rRNA sequencing and quantitative PCR to correlate venous leg ulcer bacterial bioburden dynamics with wound expansion, antibiotic therapy, and healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *23*(5), 765–771. http://doi.org/10.1111/wrr.12309

Srour, M., Inaba, K., Okoye, O., Chan, C., Skiada, D., Schnüriger, B., … Demetriades, D. (2015). Prospective evaluation of treatment of open fractures: effect of time to irrigation and debridement. *JAMA Surgery*, *150*(4), 332–336. http://doi.org/10.1001/jamasurg.2014.2022

Stewart, E. J. (2012). Growing Unculturable Bacteria. *Journal of Bacteriology*, *194*(16), 4151–4160. http://doi.org/10.1128/JB.00345-12

Stinner, D. J., Hsu, J. R., & Wenke, J. C. (2012). Negative pressure wound therapy reduces the effectiveness of traditional local antibiotic depot in a large complex musculoskeletal wound animal model. *Journal of Orthopaedic Trauma*, *26*(9), 512–518. http://doi.org/10.1097/BOT.0b013e318251291b

Strimbu, K., & Tavel, J. A. (2010). What are Biomarkers? *Current Opinion in HIV and AIDS*, *5*(6), 463–466. http://doi.org/10.1097/COH.0b013e32833ed177

Taneja, N., & Kaur, H. (2016). Insights into Newer Antimicrobial Agents Against Gram-negative Bacteria. *Microbiology Insights*, *9*, 9–19. http://doi.org/10.4137/MBI.S29459

Tatum, O. L., & Dowd, S. E. (2012). Wound Healing Finally Enters the Age of Molecular Diagnostic Medicine. *Advances in Wound Care*, *1*(3), 115–119. http://doi.org/10.1089/wound.2011.0303

Tay, W. H., Chong, K. K. L., & Kline, K. A. (2016). Polymicrobial-Host Interactions during Infection. *Journal of Molecular Biology*. http://doi.org/10.1016/j.jmb.2016.05.006

Tchesnokova, V., Aprikian, P., Kisiela, D., Gowey, S., Korotkova, N., Thomas, W., & Sokurenko, E. (2011). Type 1 Fimbrial Adhesin FimH Elicits an Immune Response That Enhances Cell Adhesion of Escherichia coli ▽. *Infection and Immunity*, *79*(10), 3895–3904. http://doi.org/10.1128/IAI.05169-11

Tegl, G., Schiffer, D., Sigl, E., Heinzle, A., & Guebitz, G. M. (2015). Biomarkers for infection: enzymes, microbes, and metabolites. *Applied Microbiology and Biotechnology*, *99*(11), 4595–4614. http://doi.org/10.1007/s00253-015-6637-7

Teicher, C. L., Ronat, J.-B., Fakhri, R. M., Basel, M., Labar, A. S., Herard, P., & Murphy, R. A. (2014). Antimicrobial Drug–Resistant Bacteria Isolated from Syrian War–Injured Patients, August 2011–March 2013. *Emerging Infectious Diseases*, *20*(11), 1949–1951. http://doi.org/10.3201/eid2011.140835

Thet, N. T., Alves, D. R., Bean, J. E., Booth, S., Nzakizwanayo, J., Young, A. E. R., … Jenkins, A. T. A. (2015). Prototype Development of the Intelligent Hydrogel Wound Dressing and Its Efficacy in the Detection of Model Pathogenic Wound Biofilms. *ACS Applied Materials & Interfaces*. http://doi.org/10.1021/acsami.5b07372

Thomas, R. J. (2010). Receptor mimicry as novel therapeutic treatment for biothreat agents. *Bioengineered Bugs*, *1*(1), 17–30. http://doi.org/10.4161/bbug.1.1.10049

Thomas-Virnig, C. L., Centanni, J. M., Johnston, C. E., He, L.-K., Schlosser, S. J., Van Winkle, K. F., … Allen-Hoffmann, B. L. (2009). Inhibition of Multidrug-resistant Acinetobacter baumannii by Nonviral Expression of hCAP-18 in a Bioengineered Human Skin Tissue. *Molecular Therapy*, *17*(3), 562–569. http://doi.org/10.1038/mt.2008.289

Thompson, M. G., Corey, B. W., Si, Y., Craft, D. W., & Zurawski, D. V. (2012). Antibacterial Activities of Iron Chelators against Common Nosocomial Pathogens. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5419–5421. http://doi.org/10.1128/AAC.01197-12

Tillotson, G. S., & Theriault, N. (2013). New and alternative approaches to tackling antibiotic resistance. *F1000Prime Reports*, *5*. http://doi.org/10.12703/P5-51

Tintle, S. M., Shawen, S. B., Forsberg, J. A., Gajewski, D. A., Keeling, J. J., Andersen, R. C., & Potter, B. K. (2014). Reoperation after combat-related major lower extremity amputations. *Journal of Orthopaedic Trauma*, *28*(4), 232–237. http://doi.org/10.1097/BOT.0b013e3182a53130

Tiwari, B. R., Kim, S., Rahman, M., & Kim, J. (2011). Antibacterial efficacy of lytic Pseudomonas bacteriophage in normal and neutropenic mice models. *Journal of Microbiology (Seoul, Korea)*, *49*(6), 994–999. http://doi.org/10.1007/s12275-011-1512-4

Torres-Barceló, C., Arias-Sánchez, F. I., Vasse, M., Ramsayer, J., Kaltz, O., & Hochberg, M. E. (2014). A Window of Opportunity to Control the Bacterial Pathogen Pseudomonas aeruginosa Combining Antibiotics and Phages. *PLoS ONE*, *9*(9). http://doi.org/10.1371/journal.pone.0106628

Tribble, D. R., Conger, N. G., Fraser, S., Gleeson, T. D., Wilkins, K., Antonille, T., … Murray, C. K. (2011). Infection-Associated Clinical Outcomes in Hospitalized Medical Evacuees following Traumatic Injury- Trauma Infectious Disease Outcome Study (TIDOS). *The Journal of Trauma*, *71*(1 0), S33–S42. http://doi.org/10.1097/TA.0b013e318221162e

Tribble, D. R., Lloyd, B., Weintrob, A., Ganesan, A., Murray, C. K., Li, P., … IDCRP TIDOS group. (2011). Antimicrobial prescribing practices following publication of guidelines for the prevention of infections associated with combat-related injuries. *The Journal of Trauma*, *71*(2 Suppl 2), S299-306. http://doi.org/10.1097/TA.0b013e318227af64

Tribble, D. R., & Rodriguez, C. J. (2014). Combat-Related Invasive Fungal Wound Infections. *Current Fungal Infection Reports*, *8*(4), 277–286. http://doi.org/10.1007/s12281-014-0205-y

Tribble, D. R., Rodriguez, C. J., Weintrob, A. C., Shaikh, F., Aggarwal, D., Carson, M. L., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2015). Environmental Factors Related to Fungal Wound Contamination after Combat Trauma in Afghanistan, 2009-2011. *Emerging Infectious Diseases*, *21*(10), 1759–1769. http://doi.org/10.3201/eid2110.141759

Utz, E. R., Elster, E. A., Tadaki, D. K., Gage, F., Perdue, P. W., Forsberg, J. A., … Brown, T. S. (2010). Metalloproteinase expression is associated with traumatic wound failure. *The Journal of Surgical Research*, *159*(2), 633–639. http://doi.org/10.1016/j.jss.2009.08.021

Valdéz, J. C., Peral, M. C., Rachid, M., Santana, M., & Perdigón, G. (2005). Interference of Lactobacillus plantarum with Pseudomonas aeruginosa in vitro and in infected burns: the potential use of probiotics in wound treatment. *Clinical Microbiology and Infection*, *11*(6), 472–479. http://doi.org/10.1111/j.1469-0691.2005.01142.x

Valneva Austria GmbH. (2016a). Confirmatory Phase II/III Study Assessing Efficacy, Immunogenicity and Safety of IC43 - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT01563263?term=pseudomonas+aeruginosa+vaccine&rank=2

Valneva Austria GmbH. (2016b). Study Assessing Immunogenicity and Safety of IC43 In Intensive Care Patients - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT00876252?term=OpRF%2FI&rank=1

Varma, P., Nisha, N., Dinesh, K. R., Kumar, A. V., & Biswas, R. (2011). Anti-infective properties of Lactobacillus fermentum against Staphylococcus aureus and Pseudomonas aeruginosa. *Journal of Molecular Microbiology and Biotechnology*, *20*(3), 137–143. http://doi.org/10.1159/000328512

Velázquez-Velázquez, J. L., Santos-Flores, A., Araujo-Meléndez, J., Sánchez-Sánchez, R., Velasquillo, C., González, C., … Martinez-Gutierrez, F. (2015). Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles. *Materials Science & Engineering. C, Materials for Biological Applications*, *49*, 604–611. http://doi.org/10.1016/j.msec.2014.12.084

Vento, T. J., Cole, D. W., Mende, K., Calvano, T. P., Rini, E. A., Tully, C. C., … Murray, C. K. (2013). Multidrug-resistant gram-negative bacteria colonization of healthy US military personnel in the US and Afghanistan. *BMC Infectious Diseases*, *13*, 68. http://doi.org/10.1186/1471-2334-13-68

Vijaya Satya, R., Zavaljevski, N., Kumar, K., & Reifman, J. (2008). A high-throughput pipeline for designing microarray-based pathogen diagnostic assays. *BMC Bioinformatics*, *9*, 185. http://doi.org/10.1186/1471-2105-9-185

Vlek, A. L. M., Bonten, M. J. M., & Boel, C. H. E. (2012). Direct Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry Improves Appropriateness of Antibiotic Treatment of Bacteremia. *PLoS ONE*, *7*(3). http://doi.org/10.1371/journal.pone.0032589

Vyas, K. S., & Wong, L. K. (2016). Detection of Biofilm in Wounds as an Early Indicator for Risk for Tissue Infection and Wound Chronicity. *Annals of Plastic Surgery*, *76*(1), 127–131. http://doi.org/10.1097/SAP.0000000000000440

Wallum, T. E., Yun, H. C., Rini, E. A., Carter, K., Guymon, C. H., Akers, K. S., … Murray, C. K. (2015). Pathogens present in acute mangled extremities from Afghanistan and subsequent pathogen recovery. *Military Medicine*, *180*(1), 97–103. http://doi.org/10.7205/MILMED-D-14-00301

Wang, H.-M., Chen, C.-Y., Chen, H.-A., Huang, W.-C., Lin, W.-R., Chen, T.-C., … Chen, Y.-H. (2010). Zingiber officinale (ginger) compounds have tetracycline-resistance modifying effects against clinical extensively drug-resistant Acinetobacter baumannii. *Phytotherapy Research*, *24*(12), 1825–1830. http://doi.org/10.1002/ptr.3201

Wang, X., Sui, X., Yan, L., Wang, Y., Cao, Y., & Jiang, Y. (2015). Vaccines in the treatment of invasive candidiasis. *Virulence*, *6*(4), 309–315. http://doi.org/10.4161/21505594.2014.983015

Wang, X.-W., Zhang, L., Jin, L.-Q., Jin, M., Shen, Z.-Q., An, S., … Li, J.-W. (2007). Development and application of an oligonucleotide microarray for the detection of food-borne bacterial pathogens. *Applied Microbiology and Biotechnology*, *76*(1), 225–233. http://doi.org/10.1007/s00253-007-0993-x

Warkentien, T., Rodriguez, C., Lloyd, B., Wells, J., Weintrob, A., Dunne, J. R., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2012). Invasive mold infections following combat-related injuries. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *55*(11), 1441–1449. http://doi.org/10.1093/cid/cis749

Warkentien, T., Shaikh, F., Weintrob, A. C., Rodriguez, C. J., Murray, C. K., Lloyd, B. A., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2015). Impact of Mucorales and Other Invasive Molds on Clinical Outcomes of Polymicrobial Traumatic Wound Infections. *Journal of Clinical Microbiology*, *53*(7), 2262–2270. http://doi.org/10.1128/JCM.00835-15

Watanabe, R., Matsumoto, T., Sano, G., Ishii, Y., Tateda, K., Sumiyama, Y., … Yamaguchi, K. (2007). Efficacy of Bacteriophage Therapy against Gut-Derived Sepsis Caused by Pseudomonas aeruginosa in Mice. *Antimicrobial Agents and Chemotherapy*, *51*(2), 446–452. http://doi.org/10.1128/AAC.00635-06

Weber, D., Dulai, S. K., Bergman, J., Buckley, R., & Beaupre, L. A. (2014). Time to initial operative treatment following open fracture does not impact development of deep infection: a prospective cohort study of 736 subjects. *Journal of Orthopaedic Trauma*, *28*(11), 613–619. http://doi.org/10.1097/BOT.0000000000000197

Weile, J., & Knabbe, C. (2009). Current applications and future trends of molecular diagnostics in clinical bacteriology. *Analytical and Bioanalytical Chemistry*, *394*(3), 731–742. http://doi.org/10.1007/s00216-009-2779-8

Weintrob, A. C., Murray, C. K., Lloyd, B., Li, P., Lu, D., Miao, Z., … Tribble, D. R. (2013). Active Surveillance for Asymptomatic Colonization with Multidrug-Resistant Gram-Negative Bacilli among Injured Service Members – A Three-Year Evaluation. *MSMR*, *20*(8), 17–22.

Weintrob, A. C., Roediger, M. P., Barber, M., Summers, A., Fieberg, A. M., Dunn, J., … Wortmann, G. W. (2010). Natural history of colonization with gram-negative multidrug-resistant organisms among hospitalized patients. *Infection Control and Hospital Epidemiology*, *31*(4), 330–337. http://doi.org/10.1086/651304

Weintrob, A. C., Weisbrod, A. B., Dunne, J. R., Rodriguez, C. J., Malone, D., Lloyd, B. A., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group. (2015). Combat trauma-associated invasive fungal wound infections: epidemiology and clinical classification. *Epidemiology and Infection*, *143*(1), 214–224. http://doi.org/10.1017/S095026881400051X

Westgeest, J., Weber, D., Dulai, S. K., Bergman, J. W., Buckley, R., & Beaupre, L. A. (2016). Factors Associated With Development of Nonunion or Delayed Healing After an Open Long Bone Fracture: A Prospective Cohort Study of 736 Subjects. *Journal of Orthopaedic Trauma*, *30*(3), 149–155. http://doi.org/10.1097/BOT.0000000000000488

White, B. K., Mende, K., Weintrob, A. C., Beckius, M. L., Zera, W. C., Lu, D., … Infectious Disease Clinical Research Program Trauma Infectious Disease Outcome Study Group. (2016). Epidemiology and antimicrobial susceptibilities of wound isolates of obligate anaerobes from combat casualties. *Diagnostic Microbiology and Infectious Disease*, *84*(2), 144–150. http://doi.org/10.1016/j.diagmicrobio.2015.10.010

Wilson, M. R., Naccache, S. N., Samayoa, E., Biagtan, M., Bashir, H., Yu, G., … Chiu, C. Y. (2014). Actionable Diagnosis of Neuroleptospirosis by Next-Generation

Sequencing. *New England Journal of Medicine*, *370*(25), 2408–2417. http://doi.org/10.1056/NEJMoa1401268

Wittebole, X., De Roock, S., & Opal, S. M. (2014). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence*, *5*(1), 226–235. http://doi.org/10.4161/viru.25991

Wolcott, R. D., Cox, S. B., & Dowd, S. E. (2010). Healing and healing rates of chronic wounds in the age of molecular pathogen diagnostics. *Journal of Wound Care*, *19*(7), 272–278, 280–281.

Wolf, D. G., Polacheck, I., Block, C., Sprung, C. L., Muggia-Sullam, M., Wolf, Y. G., … Shapiro, M. (2000). High rate of candidemia in patients sustaining injuries in a bomb blast at a marketplace: a possible environmental source. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *31*(3), 712–716. http://doi.org/10.1086/314024

Wortmann, G. W., Valadka, A. B., & Moores, L. E. (2008). Prevention and management of infections associated with combat-related central nervous system injuries. *The Journal of Trauma*, *64*(3 Suppl), S252-256. http://doi.org/10.1097/TA.0b013e318163d2b7

XBiotech, Inc. (2016). A Study of the Safety and Efficacy of 514G3 in Subjects Hospitalized With Bacteremia Due to Staphylococcus Aureus - Full Text View - ClinicalTrials.gov. In *ClinicalTrials.gov [Internet]*. Bethesda, MD: National Library of Medicine (US). Retrieved from https://clinicaltrials.gov/ct2/show/NCT02357966?term=514G3&rank=1

Yah, C. S., & Simate, G. S. (2015). Nanoparticles as potential new generation broad spectrum antimicrobial agents. *DARU Journal of Pharmaceutical Sciences*, *23*. http://doi.org/10.1186/s40199-015-0125-6

Yan, S., Tsurumi, A., Que, Y.-A., Ryan, C. M., Bandyopadhaya, A., Morgan, A. A., … Rahme, L. G. (2015). Prediction of Multiple Infections After Severe Burn Trauma: a Prospective Cohort Study. *Annals of Surgery*, *261*(4), 781–792. http://doi.org/10.1097/SLA.0000000000000759

Yang, Q., Larose, C., Della Porta, A. C., Schultz, G. S., & Gibson, D. J. (2016). A surfactant-based wound dressing can reduce bacterial biofilms in a porcine skin explant model. *International Wound Journal*. http://doi.org/10.1111/iwj.12619

Yun, H. C., Branstetter, J. G., & Murray, C. K. (2008). Osteomyelitis in military personnel wounded in Iraq and Afghanistan. *The Journal of Trauma*, *64*(2 Suppl), S163–168; discussion S168. http://doi.org/10.1097/TA.0b013e318160868c

Zaytoun, G. M., Shikhani, A. H., & Salman, S. D. (1986). Head and neck war injuries: 10-year experience at the American University of Beirut Medical Center. *The Laryngoscope*, *96*(8), 899–903.

Zhang, B., & Powers, R. (2012). Analysis of bacterial biofilms using NMR-based metabolomics. *Future Medicinal Chemistry*, *4*(10), 1273–1306. http://doi.org/10.4155/fmc.12.59

Zhang, X., Yang, T., Cao, J., Sun, J., Dai, W., & Zhang, L. (2016). Mucosal immunization with purified OmpA elicited protective immunity against infections caused by multidrug-resistant Acinetobacter baumannii. *Microbial Pathogenesis*, *96*, 20–25. http://doi.org/10.1016/j.micpath.2016.04.019

Zhang, Y., Zhu, Y., Gupta, A., Huang, Y., Murray, C. K., Vrahas, M. S., … Dai, T. (2014). Antimicrobial Blue Light Therapy for Multidrug-Resistant Acinetobacter baumannii Infection in a Mouse Burn Model: Implications for Prophylaxis and Treatment of Combat-related Wound Infections. *The Journal of Infectious Diseases*, *209*(12), 1963–1971. http://doi.org/10.1093/infdis/jit842

Zhou, J., Tun, T. N., Hong, S., Mercer-Chalmers, J. D., Laabei, M., Young, A. E. R., & Jenkins, A. T. A. (2011). Development of a prototype wound dressing technology which can detect and report colonization by pathogenic bacteria. *Biosensors & Bioelectronics*, *30*(1), 67–72. http://doi.org/10.1016/j.bios.2011.08.028