ORIGINAL ARTICLE

Evaluation of Potential Environmental Contamination Sources for the Presence of Multidrug-Resistant Bacteria Linked to Wound Infections in Combat Casualties

Edward F. Keen III, PhD;[1] Katrin Mende, PhD;[1,2] Heather C. Yun, MD;[1,3] Wade K. Aldous, PhD;[4] Timothy E. Wallum, MSc;[5] Charles H. Guymon, MA;[5] David W. Cole, MD;[6] Helen K. Crouch, MPH, CIC;[1] Matthew E. Griffith, MD;[1,3] Bernadette L. Thompson, RN, BSN, CIC;[1] Joel T. Rose, BS;[4] Clinton K. Murray, MD[1,3]

OBJECTIVE. To determine whether multidrug-resistant (MDR) gram-negative organisms are present in Afghanistan or Iraq soil samples, contaminate standard deployed hospital or modular operating rooms (ORs), or aerosolize during surgical procedures.

DESIGN. Active surveillance.

SETTING. US military hospitals in the United States, Afghanistan, and Iraq.

METHODS. Soil samples were collected from sites throughout Afghanistan and Iraq and analyzed for presence of MDR bacteria. Environmental sampling of selected newly established modular and deployed OR high-touch surfaces and equipment was performed to determine the presence of bacterial contamination. Gram-negative bacteria aerosolization during OR surgical procedures was determined by microbiological analysis of settle plate growth.

RESULTS. Subsurface soil sample isolates recovered in Afghanistan and Iraq included various pansusceptible members of Enterobacteriaceae, *Vibrio* species, *Pseudomonas* species, *Acinetobacter lwoffii*, and coagulase-negative *Staphylococcus* (CNS). OR contamination studies in Afghanistan revealed 1 surface with a *Micrococcus luteus*. Newly established US-based modular ORs and the colocated fixed-facility ORs revealed no gram-negative bacterial contamination prior to the opening of the modular OR and 5 weeks later. Bacterial aerosolization during surgery in a deployed fixed hospital revealed a mean gram-negative bacteria colony count of 12.8 colony-forming units (CFU)/dm^2/h (standard deviation [SD], 17.0) during surgeries and 6.5 CFU/dm^2/h (SD, 7.5; $P = .14$) when the OR was not in use.

CONCLUSION. This study demonstrates no significant gram-negative bacilli colonization of modular and fixed-facility ORs or dirt and no significant aerosolization of these bacilli during surgical procedures. These results lend additional support to the role of nosocomial transmission of MDR pathogens or the colonization of the patient themselves prior to injury.

Infect Control Hosp Epidemiol 2012;33(9):905-911

Increasing antibiotic resistance of commonly recovered bacterial isolates is recognized as a global threat, including the isolation of multidrug-resistant (MDR) bacteria during disaster relief and humanitarian missions in the developing world and during armed conflicts.[1-3] Cross-contamination of medical treatment facilities with these pathogens and subsequent nosocomial transmission to susceptible patients is a significant source of morbidity and mortality.[4,5] The most recent example of this trend is the spread of Enterobacteriaceae containing the New Delhi metallo-beta-lactamase 1 (NDM-1) gene from patients obtaining medical care in India and Pakistan.[6] NDM-1-containing isolates are not limited to medical treatment facilities; researchers have also recovered these organisms from drinking-water supplies and seepage pools in the environment.[7] Treatment of host-country patients infected with MDR bacteria or asymptomatic carriers returning from leisure travel represents a significant infection control challenge.[8]

Since the beginning of combat operations in Iraq and Afghanistan, the US military healthcare system has noted an increase in the number of antibiotic-resistant organisms isolated from wounded service members.[9-11] Potential sources of these MDR organisms include inoculation of the wound at the time of injury with the host normal flora or with dirt from the environment and nosocomial transmission during evacuation to US tertiary care facilities. Although the majority

Affiliations: 1. San Antonio Military Medical Center, Brooke Army Medical Center, Fort Sam Houston, Texas; 2. Infectious Disease Clinical Research Program, Uniformed Services University of the Health Science, Bethesda, Maryland; 3. Uniformed Services University of the Health Science, Bethesda, Maryland; 4. AMEDD (Army Medical Department) Center and School, Fort Sam Houston, Texas; 5. US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas; 6. Blanchfield Army Community Hospital, Fort Campbell, Kentucky.

Received March 7, 2012; accepted April 15, 2012; electronically published July 23, 2012.

 0899-823X/2012/3309-0007. DOI: 10.1086/667382

of this work was focused on *Acinetobacter baumannii*, recent studies linked treatment of local national patients in Iraq and Afghanistan as the likely source of cross-contamination in deployed US hospitals, as opposed to colonization of casualties prior to injury.[11-15] Knowing the source of the MDR bacteria allows clinicians to enhance infection control strategies and institute earlier empiric therapy, if necessary.

We undertook this study to determine whether the standard deployed hospital or modular operating rooms (ORs), set up within International Organization for Standardization (ISO) shelters (http://www.natick.army.mil/soldier/media/fact/shelter/ISO.htm; accessed February 2012), can remain clean in austere environments without significant contamination during routine use and to determine whether there is increased aerosolization of gram-negative bacteria during surgical procedures. In addition, we expanded the review of sources in Afghanistan and Iraq to determine whether MDR gram-negative organisms were present in superficial as well as deep soil samples.

METHODS

Environmental Soil

Studies to determine whether MDR organisms are present in soil were performed using samples obtained from Afghanistan and Iraq. Two locations were chosen in Afghanistan (Bagram and eastern Afghanistan) and 6 in northern, central, and southern Iraq (Mosul, Tikrit, 2 locations in Baghdad, Tallil, and Basrah). To account for seasonal differences in the types of bacteria recovered from soil, samples were collected at different times of the year. Afghanistan soil samples were collected during August 2011 (3 in the Andar District and 3 in the Band E Sarde District) and November 2011 (4 locations at Bagram). Iraq soil samples were collected during June 2010 (3 locations on Sather Airbase) and September 2010 (6 locations at Sather, 3 locations at Tallil, and 3 locations at Basrah). Three samples were taken at each site: 1 each from the surface, 6 inches below the surface, and 1 foot below the surface. The deeper depths were selected because improvised explosive devices (IEDs) can be buried below the surface for concealment. Dirt from below the surface is likely dispersed during IED detonations and may represent a potential source of MDR pathogens. Dirt samples were stored in sterile containers and then analyzed for MDR bacteria in theater within 2–3 weeks of collection. Soil was added to a 15-mL conical tube and mixed with an equal volume of thioglycolate broth. Each sample was vortexed to allow the material to settle before a 50-μL aliquot was plated on a MacConkey agar plate and incubated at 35°C for 24–48 hours. Recovered bacterial colonies were restreaked on sheep blood agar plates and subsequently identified with the MicroScan AutoSCAN 4 instrument and NBPC30 Gram-Negative Panels (Siemens Healthcare Diagnostics). Single surface and 1-foot samples were obtained (1 g of dirt in 9 mL of sterile saline, with serial dilutions on MacConkey plates) in June 2009 from Baghdad (downtown) and 2 northern Iraq locations, Mosul and Tikrit, in an assessment for only *A. baumannii*.

Field Hospital OR

Surface swabs (BD CultureSwab MaxV(+); Becton, Dickinson) containing Amies medium and a unique blend of non-animal proteins embedded in the swab fibers, which provided additional nutrients for maintenance of microorganisms during transport, were collected from an Afghanistan theater hospital OR during January 2011. The hospital was a combined hardened structure with attached modular field-type ORs commonly used in the deployed combat environment. At the time, it had three ORs, 13 intensive care unit (ICU) beds, and an inpatient ward capacity of 30–40 patients. Beds on the ward and ICU were commonly arranged in open bays with 4–8 beds per bay. Swabs were used to screen 13 surfaces (Table 2) for contamination. Swabs were then mailed to the United States (1–4-week transit times), and pathogens were evaluated by means of standard clinical microbiology techniques, with pathogens identified using the BD Phoenix Automated Microbiology System, with PMIC/ID-107 panels for gram-positive bacteria and NMIC/ID-123 panels for gram-negative bacteria (Becton, Dickinson). Previous studies that examined the viability of bacteria collected on culture swabs by mimicking typical transport times with exposure to a variety of temperatures demonstrated the efficacy of this approach.[16]

Modular/Fixed-Facility ORs

A study was performed in modular ORs set up within ISO shelters in a parking lot located at the San Antonio Military Medical Center, Texas, during February and March 2011. Culture swabs with Stuart liquid media (Becton, Dickinson) were used to screen a total of 14 surfaces (Table 3) for contamination prior to these ORs' opening for surgeries and 5 weeks into their initial use. In parallel, swabs from ORs within the colocated US-based hospital were collected using identical methods and surfaces. Pathogens were evaluated within hours using standard clinical microbiology techniques, with pathogens identified using the VITEK 2 (bioMérieux).

Gram-Negative Bacilli Aerosolization

A study to determine the amount of gram-negative bacilli aerosolization during trauma surgery in an Iraq theater hospital was performed between December 2007 and February 2008. The OR was located in the Ibn Sina fixed hospital within the Green Zone, Baghdad, Iraq. Settle plates were evaluated during 21 surgical procedures and 21 periods without surgery. Standard traditional clinical microbiology, including gram stain morphology and growth on MacConkey agar plates, was used to evaluate bacterial growth on settle plates within hours of collection.

Statistics

Bacterial isolates were considered MDR if they were resistant to 3 or more classes of antimicrobial agents (penicillins/cephalosporins, carbapenems, aminoglycosides, and quinolones), not including tetracyclines or colistin.[17] Categorical values were compared using Pearson χ^2 analysis. All statistical operations were performed in SISA (http://www.quantitativeskills.com/sisa/; accessed May 2011). All P values less than .05 were considered significant, and all reported P values were two-tailed.

RESULTS

Environmental Soil

Soil samples collected around Bagram in November 2011 yielded a number of different types of gram-negative bacteria and coagulase-negative staphylococci (CNS) but no MDR pathogens, especially those commonly associated with wounded warriors (Table 1). The isolates included various pansensitive members of Enterobacteriaceae, *Vibrio* species, and *Pseudomonas* species. The soil samples in eastern Afghanistan revealed only CNS isolates and a pansensitive *Pseudomonas* isolate.

The first set of samples collected from northern and central Iraq in 2009 (Baghdad, Tikrit, and Mosul) revealed a few isolates of *Ralstonia*, *Pseudomonas*, and pansusceptible *Acinetobacter lwoffii* in subsurface soil samples, with no MDR bacteria recovered (Table 1). A second set of samples from central and southern Iraq in 2010 recovered no gram-negative organisms from surface samples collected in Baghdad, Tallil, and Basrah or from subsurface samples from Tallil and Basrah. Multiple colonies of pansusceptible *A. lwoffii* were recovered from subsurface soil samples collected in Baghdad.

Field Hospital OR

Sampling of 13 surfaces in an Afghanistan fixed theater hospital OR during 1 time period revealed only 1 contaminated surface, a liquid warmer unit with *Micrococcus luteus* (Table 2).

TABLE 1. Recovery of Gram-Negative Bacilli from Soil Samples Collected in Afghanistan and Iraq

	Depth of sample		
Country, site	Surface	6 inches	12 inches
Afghanistan			
Bagram 1	CNS, *Enterobacter cloacae*	*Pseudomonas aeruginosa*	NG
Bagram 2	CNS, ABC	*Pseudomonas fluorescens*	ABC
Bagram 3	*Escherichia hermannii*	CNS, *Vibrio* spp.	*Serratia rubidae*
Bagram 4	*P. aeruginosa*	CNS, *E. cloacae*	NG
East 1	NG	NG	CNS
East 2	NG	NG	CNS
East 3	NG	NG	CNS
East 4	NG	NG	CNS
East 5	NG	NG	CNS
East 6	NG	NG	*Pseudomonas* spp.
Iraq			
Tallil 1	NG	NG	NG
Tallil 2	NG	NG	NG
Tallil 3	NG	NG	NG
Mosul 1	NG	NG	*Ralstonia* spp.
Tikrit 1	NG	NG	NG
Baghdad 1	NG	NG	*Pseudomonas* spp.
Sather 1	NG	NG	NG
Sather 2	NG	NG	NG
Sather 3	NG	*Acinetobacter lwoffii*	*A. lwoffii*, fungus
Sather 4	NG	*A. lwoffii*	*A. lwoffii*
Sather 5	NG	NG	NG
Sather 6	NG	NG	NG
Basrah 1	NG	NG	NG
Basrah 2	NG	NG	NG
Basrah 3	NG	NG	NG

NOTE. ABC, *Acinetobacter baumannii-calcoaceticus* complex; CNS, coagulase-negative *Staphylococcus*; NG, no growth.

https://doi.org/10.1086/667382 Published online by Cambridge University Press

TABLE 2. Bagram Theater Hospital Operating Room Surveillance Data

Site	Results
Room 2 anesthesia machine	NG
Room 2 Bowie machine	NG
Room 2 anesthesia cart	NG
Room 2 pulse ox and monitor leads	NG
Liquid warmer unit	*Micrococcus luteus*
Room 3 patient monitor	NG
Room 2 door	NG
Storage room sterile core drawers	NG
Endoscopy machine	NG
Blanket warmer unit × 2	NG
Scrub sink × 2	NG

NOTE. NG, no growth.

Modular/Fixed-Facility ORs

Of the 28 surfaces from which swab samples were collected in the two new modular ORs at the San Antonio Army Medical Center, 2 were colonized with CNS, 1 with *Bacillus* species, and 1 with a fungus (Table 3). In contrast, the 28 surfaces screened in fixed-facility ORs at the same colocated hospital included 4 surfaces with CNS. An additional 28 swabs were collected from the same surfaces in the modular ORs 5 weeks after the hospital began using them to perform surgeries. Two surfaces in the modular OR were colonized with CNS, compared to 4 fixed-facility OR surfaces colonized with CNS. No gram-negative pathogens were recovered in the ORs.

Gram-Negative Bacilli Aerosolization

During surgeries performed in a fixed Iraq theater hospital OR in 2007 and 2008, the mean aerosolization gram-negative bacilli colony count on settle plates was 12.8 colony-forming units (CFU)/dm^2/h (standard deviation [SD], 17.0; Table 4). Excluding the 3 burn patients undergoing surgical debridement, the mean settle plates colony counts were 8.7 CFU/dm^2/h with a (SD, 9.1). The colony count collected in ORs not during surgery was 6.5 CFU/dm^2/h (SD, 7.5; $P = .14$). The average length of time for surgeries in the study was 99.8 minutes. Average surgical time for burn debridement procedures was 48.3 minutes, while all other surgeries averaged 108.8 minutes.

DISCUSSION

Development of infectious complications with MDR bacteria contributes significantly to increased morbidity and mortality in battlefield medicine and humanitarian response missions.[18,19] Epidemiological data from Iraq and Afghanistan implicated nosocomial transmission of *A. baumannii* and other MDR gram-negative bacilli within the deployed military treatment facility as the most likely source of infection among injured US service members transferred to higher echelons of care.[20,21] Previous limited studies assessed the environment and the deployed medical facilities as a source for other MDR pathogens.[11-13] In this study, we showed no substantial contamination of gram-negative bacilli in the OR environments in a deployed setting or in modular field ORs set up in the United States. In addition, no MDR gram-negative bacilli were found in the soil, and no substantial increase in bacteria aerosolization was noted in the OR during surgical procedures.

Ongoing studies to identify sources of MDR bacteria are necessary during a deployed military or humanitarian mission to ensure that the appropriate antimicrobial agents are im-

TABLE 3. Continental US Operating Room (OR) Surveillance Data for Initial and 5-Week Follow-up Surface Samples

		Fixed-facility ORs				Modular ORs			
		OR4		OR10		OR18		OR19	
Swab	Site	Initial	Follow-up	Initial	Follow-up	Initial[a]	Follow-up	Initial[a]	Follow-up
1	Patient arm board	NG	CNS	NG	NG	NG	NG	NG	NG
2	Patient main bed	NG	NG	NG	NG	NG	NG	NG	NG
3	Anesthesia cart handle, inside	NG	CNS	NG	NG	NG	NG	NG	NG
4	Anesthesia keyboard	CNS	NG	*Bacillus*	CNS	CNS	CNS, CB	NG	CNS
5	Nursing keyboard	NG	CNS	CNS	CNS	NG	CNS	NG	NG
6	Back wall	CNS	NG	NG	NG	CNS	*Bacillus*, CB	NG	NG
7	OR wall, left side	CNS	NG	NG	NG	*Bacillus*	NG	NG	NG
8	OR wall, right side	NG	NG	CNS	NG	Fungus	CB	NG	NG
9	Door handles, inside	NG	NG	NG	NG	NG	CNS	NG	NG
10	Mayo stands	NG	NG	NG	NG	NG	CNS, CB	NG	NG
11	Back table	NG	NG	CNS, CB	CNS	NG	NG	NG	NG
12	Inside of patient safety strap	NG	NG	NG	NG	NG	NG	NG	NG
13	Black anesthesia monitor	CNS	NG	NG	NG	NG	NG	NG	NG
14	Ambu bag holder	NG	NG	CNS	NG	NG	NG	NG	NG

NOTE. CB, *Corynebacterium* spp.; CNS, coagulase-negative *Staphylococcus*; NG, no growth.
[a] Initial surface samples collected in the modular ORs before use as a surgical suite began.

https://doi.org/10.1086/667382 Published online by Cambridge University Press

TABLE 4. Aerosolization of Gram-Negative Bacilli during Surgical Procedures in an Iraqi Theater Hospital

Settle plate[a]	Mean colony counts (SD), CFU/dm²/h	GNB growth	Procedure	Surgery time, min	Control plate	Mean colony counts (SD), CFU/dm²/h	GNB growth	Control time, min
SURG-1	22.35	N	I&D, ortho	120	CTL-1	3.82	N	
SURG-2	5.28	N	I&D	10	CTL-2	15.89	N	120
SURG-3	4.1	N	Ortho	95	CTL-3	5.6	N	25
SURG-4	2.78	N	Ortho	305	CTL-4	8.44	N	130
SURG-5	36.87	N	I&D, ortho	175	CTL-5	6.32	N	120
SURG-6	7.9	N	I&D, ortho	65	CTL-6	2.35	N	125
SURG-7	5.71	N	I&D	40	CTL-7	0.44	N	120
SURG-8	4.14	N	Ortho	115	CTL-8	4.42	N	128
SURG-9	2.51	N	I&D	70	CTL-9	3.09	N	118
SURG-10	12.94	N	I&D, ortho	120	CTL-10	1.22	N	125
SURG-11	3.52	N	I&D	40	CTL-11	2.21	N	120
SURG-12	4.63	N	I&D	65	CTL-12	1.27	N	125
SURG-13		N	I&D, ortho	220	CTL-13	0.71	N	125
SURG-14	72.35	Y	I&D, BD	30	CTL-14	0.54	N	130
SURG-15	12.01	N	I&D	85	CTL-15		N	130
SURG-17	6.41	Y	BD	80	CTL-17	15.33	N	70
SURG-18	31.44	Y	BD	35	CTL-18	31.17	Y	70
SURG-19	5.21	N	I&D	115	CTL-19	7.03	N	120
SURG-20	7.56	Y	I&D	105	CTL-20	8.13	N	130
SURG-21	2.18	N	I&D	105	CTL-21	5.99	N	100
Total	12.8 (17.0)	4 plates		97 (69.0)		6.5 (7.5)	1 plate	112.2 (27.7)

NOTE. BD, burn debridement; CFU, colony-forming unit; GNB, gram-negative bacilli; I&D, incision and drainage; SD, standard deviation.

[a] Settle plates were evaluated during 21 surgical procedures and control plates during 21 periods without surgery. Mean colony count was computed as colony counts divided by the size of the plate, divided by the time of the surgery.

plemented and that adequate infection control is instituted. Typically, pathogens contaminate patient wounds through the patient's own skin flora, environmental contact at the point of injury, or nosocomial transmission during treatment within the medical facility. Despite reports of MDR organisms being recovered from contaminated water sources and the soil, initial studies in Iraq did not demonstrate that MDR *A. baumannii* is a common dirt pathogen.[7,11] This study indicates that soil is not the likely source of MDR gram-negative bacteria, since none were isolated from multiple samples of superficial or subsurface soil collected across Iraq and Afghanistan. Although previous studies identified some soil bacteria with novel resistance genes, they are not always clinically relevant pathogens, as shown in this study.[22,23]

Despite reports of challenges to maintain an adequate sterile environment within ORs under austere conditions, the limited environmental sampling of OR surfaces in an Afghanistan field hospital did not reveal gram-negative bacteria contamination.[24] Comparative environmental sampling in modular field-type ORs that are used in the deployed combat environment and fixed-facility ORs in the United States also demonstrated no significant contamination. Although studies have demonstrated a correlation between length of surgery and aerosolization of bacteria during wound care of burn patients with MRSA infections, we did not show appreciable aerosolization of bacteria during surgery, except maybe in the case of burn patients.[25-27] This is likely related to the increased burden of bacteria in burn patients, in comparison to patients undergoing other surgeries, as shown previously.[28,29]

Despite a broad array of investigations across various combat environments, we were limited by our ability to perform a more thorough evaluation of sources of pathogens or to establish standard collection and testing methods at every location, but this falls within the challenges of conducting research in the combat environment with limited local microbiology capability and changing personnel. Increased transportation times for samples shipped to the United States for additional testing could have affected the recovery of gram-negative bacteria from swabs with low numbers of organisms, but this is not likely the case, according to prior studies.[16] It is notable that MDR gram-negative bacteria were infecting our casualties throughout this study in both the deployed hospitals and the fixed US facility, lending support that the periods of sampling across this study were appropriate for identifying potential sources of MDR pathogens.[28,30,31] Overall, continued focus on adequately powered studies, especially ones assessing aerosolization of bacteria during surgical procedures, is needed.

Our study demonstrates no significant gram-negative bacilli colonization of modular and fixed-facility ORs and no significant aerosolization of these bacilli during surgical procedures or of soil from the environment. The lack of bacterial

contamination in US modular ORs prior to their use in this study suggests that similar ISO shelters deployed to austere locations should be clean at the time of initial use. In addition, this study provides further strength to the argument that the potential sources of gram-negative bacterial infections are (1) nosocomial transmission between host-nation patients and US personnel treated in military healthcare facilities through direct patient care or patient's contact with the hospital environment and (2) the patient's own bacterial flora. Future studies should focus on these two potential sources of transmission, including studies examining skin colonization and wound contamination of patients/equipment evacuated from the combat zone to Landstuhl Regional Medical Center in Germany and on to the United States for definitive care. Treatment should still be directed to standard therapy, as recommended for standard surgical procedures in the United States, and to a continued focus on aggressive infection control practices in the hospital infrastructure.[32,33]

ACKNOWLEDGMENTS

Financial support. This work was supported by the Armed Forces Health Surveillance Center, including the Global Emerging Infectious System.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

Address correspondence to Clinton K. Murray, LTC, MC, USA, Infectious Disease Service, San Antonio Military Medical Center, Brooke Army Medical Center, 3551 Roger Brooke Drive, Fort Sam Houston, TX 78234 (clinton.murray@amedd.army.mil).

Presented in part: 49th Annual Meeting of the Infectious Diseases Society of America; Boston, Massachusetts; October 20–23, 2011.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army, the Department of the Air Force, the Department of Defense, or the US government. This work was prepared as part of their official duties.

REFERENCES

1. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! an update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009;48(1):1–12.
2. Amundson D, Dadekian G, Etienne M, et al. Practicing internal medicine onboard the USNS *Comfort* in the aftermath of the Haitian earthquake. *Ann Intern Med* 2010;152(11):733–737.
3. Elston JW, Bannan CL, Chih DT, Boutlis CS. *Acinetobacter* spp. in gunshot injuries. *Emerg Infect Dis* 2008;14(1):178–180.
4. Öncül O, Keskin Ö, Acar HV, et al. Hospital-acquired infections following the 1999 Marmara earthquake. *J Hosp Infect* 2002; 51(1):47–51.
5. Hussey SM, Dukette PJ, Dunn SH, et al. The 2010 Haiti earthquake: a pathology perspective aboard the USNS *Comfort*. *Arch Pathol Lab Med* 2011;135(4):417–421.
6. Moellering RC Jr. NDM-1: a cause for worldwide concern. *N Engl J Med* 2010;363(25):2377–2379.
7. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011;11(5):355–362.
8. Tängdén T, Cars O, Melhus A, Löwdin E. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* 2010;54(9):3564–3568.
9. Murray CK. Epidemiology of infections associated with combat-related injuries in Iraq and Afghanistan. *J Trauma* 2008;64(suppl 3):S232–S238.
10. Murray CK, Hinkle MK, Yun HC. History of infections associated with combat-related injuries. *J Trauma* 2008;64(suppl 3): S221–S231.
11. Scott P, Deye G, Srinivasan A, et al. An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. *Clin Infect Dis* 2007;44(12):1577–1584.
12. Ake J, Scott P, Wortmann G, et al. Gram-negative multidrug-resistant organism colonization in a US military healthcare facility in Iraq. *Infect Control Hosp Epidemiol* 2011;32(6):545–552.
13. Sutter DE, Bradshaw LU, Simkins LH, et al. High incidence of multidrug-resistant gram-negative bacteria recovered from Afghan patients at a deployed US military hospital. *Infect Control Hosp Epidemiol* 2011;32(9):854–860.
14. Griffith ME, Lazarus DR, Mann PB, Boger JA, Hospenthal DR, Murray CK. *Acinetobacter* skin carriage among US Army soldiers deployed in Iraq. *Infect Control Hosp Epidemiol* 2007;28(6): 720–722.
15. Kaspar RL, Griffith ME, Mann PB, et al. 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. *Mil Med* 2009;174(9):899–903.
16. Mende K, Sutter DE, Beckius ML, Murray CK, Hospenthal DR. Recovery of multidrug-resistant bacteria from swabs stored for 1 and 4 weeks to mimic long-distance shipping conditions. Paper presented at: 49th annual meeting of the Infectious Diseases Society of America, October 20–23, 2011, Boston, MA.
17. The National Healthcare Safety Network (NHSN) Manual. Patient safety component: protocol: multidrug-resistant organism (MDRO) and *Clostridium difficile*–associated disease (CDAC) module. Division of Healthcare Quality Promotion, National Center for Preparedness, Detection and Control of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA. 2008. http://www.cdc.gov/nhsn/PDFs/MDRO_CDZDprotocolv41Dec08final.pdf.
18. Centers for Disease Control and Prevention (CDC). *Acinetobacter baumannii* infections among patients at military medical facilities treating injured U.S. service members, 2002–2004. *MMWR Morb Mortal Wkly Rep* 2004;53(45):1063–1066.
19. Murray CK, Horvath LL. An approach to prevention of infectious diseases during military deployments. *Clin Infect Dis* 2007; 44(3):424–430.
20. Griffith ME, Gonzalez RS, Holcomb JB, Hospenthal DR, Wortmann GW, Murray CK. Factors associated with recovery of *Acinetobacter baumannii* in a combat support hospital. *Infect Control Hosp Epidemiol* 2008;29(7):664–666.
21. Yun HC, Murray CK, Roop SA, Hospenthal DR, Gourdine E, Dooley DP. Bacteria recovered from patients admitted to a de-

ployed U.S. military hospital in Baghdad, Iraq. *Mil Med* 2006; 171(9):821–825.
22. Riesenfeld CS, Goodman RM, Handelsman J. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environ Microbiol* 2004;6(9):981–989.
23. Donato JJ, Moe LA, Converse BJ, et al. Metagenomic analysis of apple orchard soil reveals antibiotic resistance genes encoding predicted bifunctional proteins. *Appl Environ Microbiol* 2010; 76(13):4396–4401.
24. Vane EAP, Drost E, Elder D, Heib Y. Behind the scenes: patient safety in the operating room and central materiel service during deployments. In: Henriksen K, Battles JB, Marks ES, Lewin DI, eds. *Advances in Patient Safety: From Research to Implementation.* Vol 3. *Implementation Issues.* Rockville, MD: Agency for Healthcare Research and Quality (US), 2005:469–482.
25. Dansby W, Purdue G, Hunt J, et al. Aerosolization of methicillin-resistant *Staphylococcus aureus* during an epidemic in a burn intensive care unit. *J Burn Care Res* 2008;29(2):331–337.
26. Bernard HR, Speers R Jr, O'Grady FW, Shooter RA. Airborne bacterial contamination: investigation of human sources. *Arch Surg* 1965;91(3):530–533.
27. Dalstrom DJ, Venkatarayappa I, Manternach AL, Palcic MS, Heyse BA, Prayson MJ. Time-dependent contamination of opened sterile operating-room trays. *J Bone Joint Surg Am* 2008; 90(5):1022–1025.
28. D'Avignon LC, Chung KK, Saffle JR, Renz EM, Cancio LC, Prevention of Combat-Related Infections Guidelines Panel. Prevention of infections associated with combat-related burn injuries. *J Trauma* 2011;71(suppl 2):S282–S289.
29. Keen EF III, Robinson BJ, Hospenthal DR, et al. Prevalence of multidrug-resistant organisms recovered at a military burn center. *Burns* 2010;36(6):819–825.
30. Aldous WK, Co E-MA. Factors associated with recovery of multidrug-resistant bacteria in a combat support hospital in Iraq. *Infect Control Hosp Epidemiol* 2010;31(4):425–427.
31. Keen EF III, Murray CK, Robinson BJ, Hospenthal DR, Co E-MA, Aldous WK. Changes in the incidences of multidrug-resistant and extensively drug-resistant organisms isolated in a military medical center. *Infect Control Hosp Epidemiol* 2010; 31(7):728–732.
32. Hospenthal DR, Green AD, Crouch HK, et al. Infection prevention and control in deployed military medical treatment facilities. *J Trauma* 2011;71(suppl 2):S290–S298.
33. Hospenthal DR, Murray CK, Andersen RC, et al. 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. *J Trauma* 2011; 71(suppl 2):S210–S234.