

An Outbreak of Multidrug-Resistant *Acinetobacter baumannii-calcoaceticus* Complex Infection in the US Military Health Care System Associated with Military Operations in Iraq

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Background. We investigated an outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection among US service members injured in Iraq.

Methods. The investigation was conducted in Iraq and Kuwait, in the 2 military hospitals where the majority of injured service members were initially treated. After initially characterizing the outbreak, we evaluated 3 potential sources of infection for the period March 2003 to December 2004. The evaluation included screening samples that were obtained from the skin of patients for the presence of colonization and assessing the soil and health care environments for the presence of *A. baumannii-calcoaceticus* complex organisms. Isolates obtained from samples from patients in US Military treatment facilities, as well as environmental isolates, were genotypically characterized and compared using pulsed-field gel electrophoresis.

Results. *A. baumannii-calcoaceticus* complex organisms were present on the skin in only 1 (0.6%) of 160 patients who were screened and in 1 (2%) of 49 soil samples. *A. baumannii-calcoaceticus* complex isolates were recovered from treatment areas in 7 of the 7 field hospitals sampled. Using pulsed-field gel electrophoresis, we identified 5 cluster groups in which isolates from patients were related to environmental isolates. One cluster included hospitalized patients who had not been deployed to Iraq. Among the clinical isolates, only imipenem, polymyxin B, and colistin demonstrated reliable in vitro antimicrobial activity. Generally, the environmental isolates were more drug susceptible than were the clinical isolates.

Conclusions. Our findings suggest that environmental contamination of field hospitals and infection transmission within health care facilities played a major role in this outbreak. On the basis of these findings, maintaining infection control throughout the military health care system is essential. Novel strategies may be required to prevent the transmission of pathogens in combat field hospitals.

Acinetobacter baumannii-calcoaceticus complex (ABC)

Received 1 December 2006; accepted 28 February 2007; electronically published 8 May 2007.

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Clinical Infectious Diseases 2007;44:1577–84

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1058-4838/2007/4412-0009
DOI: 10.1086/518170

organisms are gram-negative bacteria found in soil and water [1]. They are an emerging cause of health care-associated outbreaks of infection, especially among critically ill and immunocompromised patients [2, 3].

In March 2003, soon after the beginning of the US

This manuscript has been reviewed by the Walter Reed Army Institute of Research and the Centers for Disease Control and Prevention. There is no objection to its publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of Defense, the National Institutes of Health or the Department of Health and Human Services, or other organization listed. This work was prepared as part of their official duties and, as such, there is no copyright to be transferred.

Military's combat operations in Iraq known as Operation Iraqi Freedom (OIF), a marked increase was observed in the number of multidrug-resistant ABC infections among inpatients at Landstuhl Regional Medical Center (LRMC; Landstuhl, Germany) and at Walter Reed Army Medical Center (WRAMC; Washington, DC). Most injured service members were initially treated at these 2 facilities after evacuation from Iraq. Many of these infections were detected at or soon after hospital admission to LRMC or WRAMC, but the source of the infections was not apparent [4]. Here we present the results of our investigation of several possible sources for this outbreak, which include preinjury skin colonization, introduction at the time of injury from environmental soil contamination, and acquisition after injury during treatment in health care facilities.

MATERIALS AND METHODS

Characterizing the Outbreak

From March through October 2003, we used an electronic data collection form to gather demographic, clinical, and microbiological data for the initial 70 inpatients at LRMC and WRAMC who had clinical culture results that were positive for ABC. Data sources included paper medical records, electronic medical records (Clinical Information System; CliniComp), the Composite Healthcare System computerized hospital information system, and the Vitek 1 and 2 (bioMérieux). We also collected information on the specific movements through the military aeromedical evacuation system of 19 (27%) of these 70 patients using the Department of Defense Transportation Command Regulating and Command and Control Evacuation System (TRAC2ES) [5].

Outbreak Source Investigation

Evaluation for ABC skin colonization. We evaluated 2 independent groups. From 23 September through 20 October 2004, we screened 96 ambulatory US casualties who had been evacuated from Iraq to LRMC and who had no known prior hospitalization in Iraq. From 25 October through 23 December 2004, we screened 102 casualties (including both US and Iraqi patients), either at initial presentation to the Emergency Treatment Area or at admission to the intensive care unit in the US Military field hospital in Baghdad (Iraq). We used either a single culture swab (BBL culturette with liquid Amies; BD Diagnostics) to sample the skin in both the axilla and groin areas, or 2 swabs to sample the axilla and groin areas separately.

Environmental sampling (soil and health care facilities). We obtained samples from the environment in 5 field hospitals in Iraq (Baghdad, Dogwood [located southwest of Baghdad], Mosul, Tikrit, and Balad) and 2 field hospitals in Kuwait. At Field Hospital Baghdad, a facility that had a clinical microbiology laboratory, the laboratory officer conducted periodic environmental sampling of selected treatment areas by passing

cotton-tipped transfer swabs over sample surfaces. At the other field hospitals (none of which had a microbiology laboratory), environmental sampling was performed using a standardized sampling protocol developed for this investigation. Sampling kits, containing 25 swabs, were used to sample selected surfaces in and around treatment areas and the soil environment within 25 m of the field hospital. Copan Venturi swabs (Copan Diagnostics) were passed over sample surfaces, labeled, and shipped at room temperature by express courier to WRAMC for processing. A total of 49 soil samples were evaluated; 18 soil samples were collected during environmental sampling using the standard protocol and 31 were archived soil samples. The archived soil samples were collected during the period March 2003 through December 2004 from locations throughout Iraq and Kuwait during routine combat theater environmental assessment. The archived soil samples were stored in screw-top Teflon jars (Savillex) at 2°C–6°C at the US Army Center for Health Promotion and Preventive Medicine (Abdeen Proving Ground, MD).

Laboratory Isolation and Species Identification

Environmental sample swabs obtained from Field Hospital Baghdad were streaked across BBL trypticase with 5% sheep blood and BBL MacConkey agar (Becton Dickinson) and incubated at 35°C for 18–24 h. All other sample swabs were streaked across BBL MacConkey agar and incubated at 35°C for 18–48 h. Cotton-tipped swabs moistened with BBL trypticase soy broth (BD Diagnostics) were placed in soil samples and streaked across BBL trypticase with 5% sheep blood and BBL MacConkey agar and incubated at 35°C for 72 h. Organisms were identified as ABC using standard automated biochemical testing methods [6]. All available isolates were further identified using 16S rDNA sequencing and compared with reference standards [7].

Phenotypic and Genotypic Characterization

All environmental and soil isolates obtained during the field sampling, as described above, and isolates from inpatients treated from March through October 2003 at 4 locations (Field Hospital Baghdad, US Naval Ship Hospital USNS Comfort, LRMC, and WRAMC) were collected for phenotypic and genotypic characterization.

Molecular Typing

Clinical and environmental isolates were strain-typed using PFGE (CHEF Mapper; Bio-Rad Laboratories) according to protocol, with modifications for *Acinetobacter* species [8]. *Acinetobacter* species DNA was digested with *ApaI* (New England BioLabs). PFGE was performed with a 6.0 V/cm gradient, at a 120° angle, at 14°C, and with 7–20-s pulse times, for 18.5 h. Gel images were analyzed using BioNumerics software (Applied

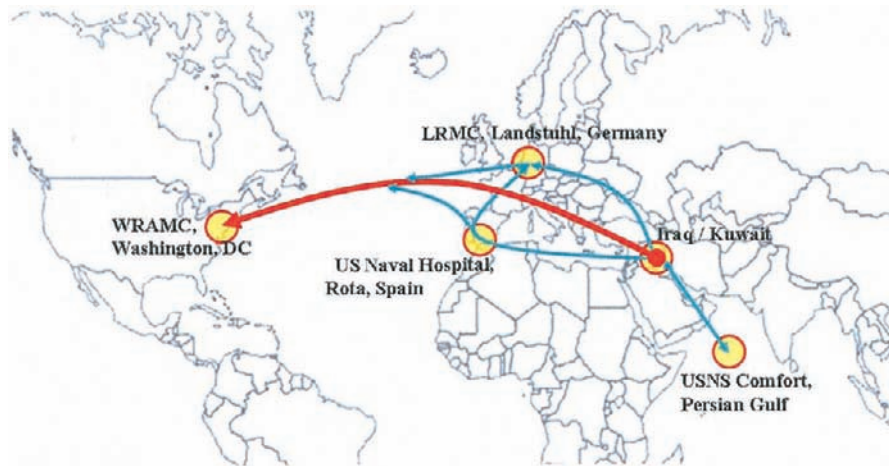


Figure 1. Aeromedical evacuation route from Iraq and Kuwait to US Military hospitals in Europe and the United States. LRM, Landstuhl Regional Medical Center; USNS, US Naval Ship; WRAMC, Walter Reed Army Medical Center.

Maths). ABC strains with >90% similarity on the basis of Dice coefficients were considered to be related.

Antimicrobial Susceptibility Testing

A random sample of the clinical isolates that were collected from inpatients at WRAMC and that were representative of the total genotypic diversity of the investigation and all available health care environment and soil isolates were tested for antimicrobial susceptibility using the Clinical and Laboratory Standards Institute broth microdilution procedure using in-house, prepared frozen MIC panels [9]. Results were interpreted using Clinical and Laboratory Standards Institute breakpoints [10]. Intermediate susceptibility was classified as resistant.

Human Subjects Research Review

This work was conducted as a public health activity other than research and under an Institutional Review Board–approved protocol. Investigators adhered to the policies for protection of human subjects as prescribed in Title 45 Code of Federal Regulations part 46.

Statistical Analysis

The χ^2 test or Fisher's exact test was used to compare proportions. All reported *P* values were 2-sided. Statistical analysis was performed using Stata software, version 8.0 (Stata Corporation).

RESULTS

Characterizing the outbreak. Most of the initial 70 patients who were identified were young (age, <35 years), male Army soldiers who were wounded in combat operations in Iraq. They required ≥ 1 surgical intervention, including fracture fixation (50%), laparotomy (25%), and amputation (20%); 54% re-

quired receipt of mechanical ventilation immediately following injury or during the subsequent hospital stay. ABC isolates ($n = 131$) were obtained from samples taken from extremities (from wounds) (42 patients [32%]), from the airway (sputum, tracheal aspirates, or bronchoalveolar lavage fluid; 32 patients [24%]), of blood (14 patients [11%]), of urine (3 patients [2%]), and from other/unknown sources (40 patients [31%]). We could not determine from the available data whether isolates collected from nonsterile sites were causing infection or colonization

Patients were treated at several facilities during aeromedical evacuation from Iraq. These included field medical facilities in Iraq; the USNS Comfort, which was positioned in the Persian Gulf; US Naval Hospital in Rota, Spain; LRM; and WRAMC (figure 1). For the subset of 19 patients for whom detailed evacuation information was available, the mean number of facilities where patients were treated prior to admission to either LRM or WRAMC was 3.7 (range, 2–5 facilities). On average, patients reached one of these facilities within 5.7 days after injury.

Evaluation for ABC skin colonization. No ABC skin colonization was detected in the group of 96 evacuated ambulatory US patients. Skin colonization by ABC was detected in 1 (2%) of 64 US patients and in 4 (11%) of 38 Iraqi patients who were screened at the time of presentation to Field Hospital Baghdad.

Environmental sampling (soil and health care facilities). A total of 37 ABC isolates were obtained during the period of sampling in and around 7 field hospitals in Iraq and Kuwait. Thirty-six were isolated from samples of the field hospital environment, and 1 was isolated from soil surrounding the field hospital. Most (68%) were collected in critical care treatment areas (11 from intensive care units, 7 from operating rooms, and 7 from emergency departments). In patient care areas, ABC

isolates were recovered from operating room equipment (anesthesia machine [$n = 2$], operating room table [$n = 1$] and light [$n = 1$], unspecified locations in the operating room [$n = 2$], environmental control units [i.e., heaters and air conditioners; $n = 2$], patient beds [$n = 12$], sinks [$n = 8$], and tent walls [$n = 2$]). ABC isolates were also recovered from other locations, including an environmental control unit ($n = 1$), environmental control unit drip lines or soil bags ($n = 4$), a drinking water source ($n = 1$), and soil outside a field hospital nutrition care section ($n = 1$). No ABC organisms were recovered from the 31 archived soil samples.

Twenty-five (20%) of the 125 environmental samples collected from the Dogwood, Mosul, Tikrit, and Balad hospital locations yielded ABC isolates. Detailed records of the sampling conducted were only available from Dogwood and Balad (table 1). Among positive samples (8 total) from these 2 field hospitals, 6 (17%) of 36 were from patient care areas and 2 (8%) of 36 were from other locations.

Phenotypic and genotypic characterization. Thirty-seven environmental and soil isolates and 170 isolates from 145 inpatients (121 US and 24 non-US inpatients) who were treated between March and October 2003 at 4 locations (Field Hospital Baghdad, US Naval Ship Hospital USNS Comfort, LRMC, and WRAMC) were collected for phenotypic and genotypic characterization.

Species identification. A total of 201 of the 207 isolates (37 of 37 environmental isolates and 164 of 170 clinical isolates) were identified using 16S rDNA sequencing. One hundred ninety-seven isolates (98%) were ABC organisms. Among the clinical isolates, 86 (52%) were *A. baumannii* and 78 (48%) were other ABC organisms (unnamed *Acinetobacter* species). Among the environmental isolates, 7 (19%) were *A. baumannii*, 26 (70%) were ABC excluding *A. baumannii*, and 4 (11%) were *Acinetobacter* species not including ABC species. The 1 ABC isolate identified in a soil sample was an unnamed other *Acinetobacter* species.

Molecular typing. A total of 204 of the 207 isolates (34 of 37 environmental isolates and 170 of 170 clinical isolates) were characterized using PFGE. There were 66 different PFGE strains identified among the 170 clinical isolates and 25 different PFGE strains identified among the 34 environmental isolates. There were 5 cluster groups (in which ≥ 1 environmental isolate was genetically related to patient isolates) (figure 2 and table 2). The largest cluster group (cluster group A) included 45 isolates from 43 patients treated at 4 different US Military hospitals and a genetically related environmental isolate obtained from an operating room at Field Hospital Baghdad. This group included isolates from both US and non-US patients and from both OIF casualties and inpatients in US Military hospitals with no link to OIF. In the other 4 cluster groups, environmental isolates from critical care treatment areas in 4 other field hos-

Table 1. Summary of sampling scheme and *Acinetobacter baumannii-calcoaceticus* complex (ABC) isolates recovered during environmental sampling of field hospitals in Iraq (Dogwood and Balad locations).

Field hospital location, sample sites	No. of samples collected	Surfaces or sites where ABC isolates were recovered (no. of isolates)
Dogwood		
Tent wall	14	ICU tent wall (2)
ECU	22	Operating room ECU (1)
Sink	2	ICU ECU (1)
Other	4	Sink (2)
Balad		
Sink	12	ICU bed or litter (2)
Patient bed or litter	6	...
Other	12	...

NOTE. Samples were taken (2 swabs per surface or site) from tent walls located in the intensive care units (ICUs; $n = 2$), the emergency medical treatment (EMT) area, the hospital logistics areas ($n = 2$), the dining facility, and the shower facility; from environmental control units (ECUs; heaters or air conditioners) located in the operating rooms ($n = 2$), the preoperative area, the operating room clean medical supply area, the operating room break area, the ICUs ($n = 2$) and EMT area, the radiography department, the laboratory, and the Patient Administration Division area; from sinks in the laboratory, the blood bank, the radiography department, the EMT area, the specialty clinic, and the ICUs; from the laundry drainage bin, the preoperative area (not otherwise specified [NOS]); from a patient bed or litter in the EMT area, the ICUs, and the specialty clinic; and from the dental clinic (NOS), the pharmacy (NOS), the laboratory ECU's dripline, shower drainage, and the hand wash station at the latrine.

pitals were related to isolates from 13 OIF patients who were hospitalized at WRAMC and LRMC (table 2).

Antimicrobial susceptibility testing. The clinical isolates from WRAMC ($n = 98$) showed broad resistance to fluoroquinolones (33% susceptible), cephalosporins (22% susceptible), and piperacillin/tazobactam (11% susceptible). Only imipenem (90% susceptible), polymixin B (99% susceptible), and colistin (99% susceptible) yielded high susceptibility rates. Environmental isolates ($n = 35$ available) were generally more susceptible than clinical isolates (table 3).

DISCUSSION

ABC organisms have been identified as causes of infection among critically ill patients with traumatic injuries, both in war and in situations of natural disaster [11–13]. Some prior reports have attributed the source to environmental exposure, whereas others have associated these infections with hospital acquisition [11–13]. Soon after the initiation of OIF, there was a dramatic increase observed in the number of ABC infections among patients in the US Military health care system. Most of the infections were caused by highly antimicrobial-resistant organisms and occurred in patients who were critically ill and who had severe traumatic injuries. Our investigation was undertaken

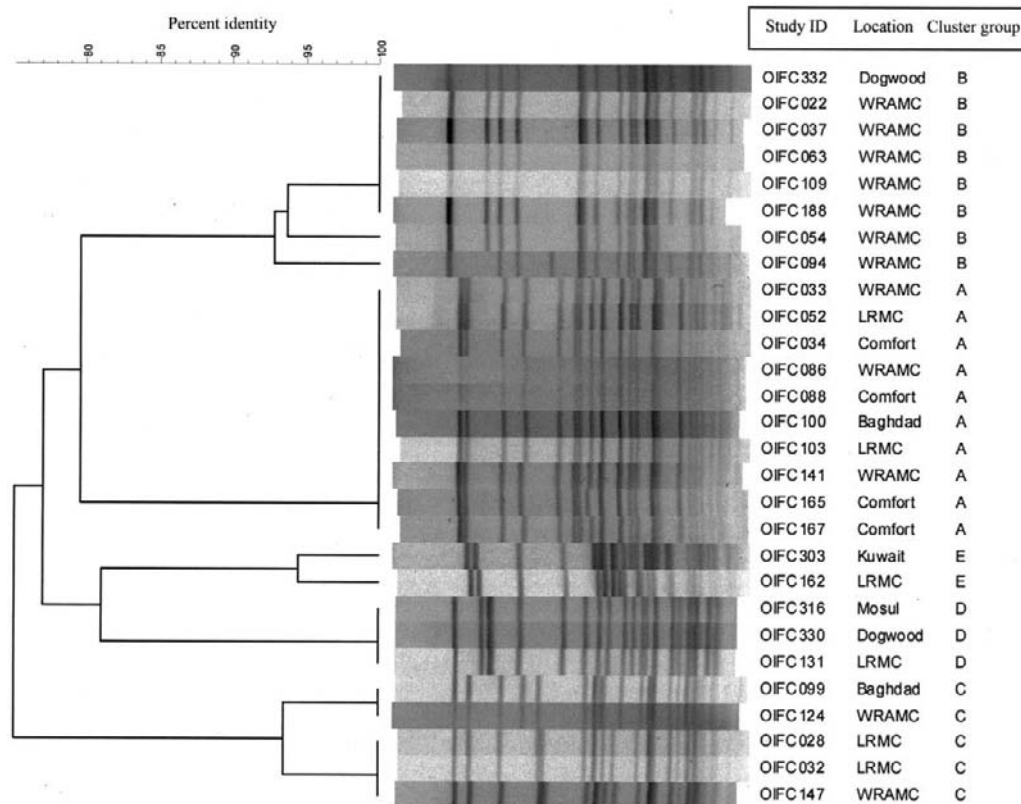


Figure 2. PFGE dendrogram of *Acinetobacter baumannii-calcoaceticus* complex outbreak cluster groups, by location of isolate collection. A representative sample of 10 of the 46 cluster group A isolates were included, given space considerations. LRMC, Landstuhl Regional Medical Center (Landstuhl, Germany); WRAMC, Walter Reed Army Medical Center (Washington, DC).

to determine the source of the infections, to help guide the implementation of preventive and infection-control measures. We hypothesized that infection may have resulted from ≥ 1 of 3 sources: preinjury skin colonization, introduction of the organism at the time of injury, and acquisition after injury during treatment in health care facilities. Our findings suggest that the source of this outbreak of multidrug-resistant ABC infection is likely multifactorial, and that the primary source is nosocomial transmission of organisms occurring in field hospitals.

Because only 1 of 160 US soldiers who were screened was colonized with ABC, we concluded that it was unlikely that preinjury skin colonization was a major contributing factor in this outbreak. We isolated only 1 ABC organism from 49 soil samples evaluated, and it was not genetically related to any of the clinical isolates. Had soil inoculation at the time of injury been the major source of infection, we would have expected to see a preponderance of isolates from samples taken from wounds; rather, we collected a significant number of isolates from nonwound sources. Thus, we concluded that introduction of the organism from environmental contamination at the time of injury was a possible, but unlikely, source of the outbreak. These findings are consistent with findings by Murray et al. [14] that *Acinetobacter* species were not isolated from wounds

immediately after or soon after injury from casualties who were treated at a US Military field hospital in Iraq.

Our data do support a role for environmental contamination and transmission of organisms within health care facilities. ABC organisms were isolated from patient treatment areas in all 7 field hospitals sampled. Using PFGE, we identified 5 ABC cluster groups, each of which included isolates from ≥ 1 hospitalized patient that were genetically related to an environmental isolate recovered from a field hospital. Furthermore, strain typing demonstrated that clinical isolates recovered from 3 patients hospitalized at a military medical facility in the United States who had not been deployed to OIF were genetically related to an environmental isolate recovered from a field hospital in Iraq. Additionally, our findings that the ABC isolates obtained from environmental samples were generally more antimicrobial susceptible than those isolates obtained from clinical specimens suggest that selective antibiotic pressure caused by treatment of patients with broad-spectrum antimicrobial agents may have contributed to the degree and complex nature of the multidrug resistance that we observed [15]. Last, the finding that non-US patients who presented to Field Hospital Baghdad had higher skin colonization rates than did US patients suggests the possibility that these patients, who typically experienced longer

Table 2. Summary of *Acinetobacter baumannii-calcoaceticus* complex isolates recovered from environmental samples of field hospitals that were genetically related to clinical isolates recovered from patients in multiple medical treatment facilities ("cluster groups").

Location (field hospital)	Site where environmental strain was isolated	Cluster group ^a	Total no. of patients with the strain (no. in each hospital)
Baghdad	Old OR ^b	A	43 (2 in Baghdad, 18 in Comfort, 6 in LPMC, and 19 in WRAMC) ^c
Baghdad	New OR ^b	B	4 (2 in LPMC and 2 in WRAMC)
Dogwood	ECU in OR	C	7 (7 in WRAMC)
Dogwood and Mosul ^d	ICU tent wall, ICU sink	D	1 (1 in LPMC)
Kuwait	ICU bed	E	1 (1 in LPMC)

NOTE. ECU, environmental control unit (heater/air conditioner); EMT, emergency treatment area; ICU, intensive care unit; LPMC, Landstuhl Regional Medical Center (Landstuhl, Germany); OR, operating room; WRAMC, Walter Reed Army Medical Center (Washington, DC).

^a PFGE strain "cluster" containing both patient clinical isolates and environmental isolates.

^b Specific location in OR was not specified.

^c Includes 2 patients with isolates recovered at both LPMC and WRAMC.

^d PFGE strain cluster D isolated from 1 patient at LPMC and from the environment at field hospitals at Mosul and Dogwood.

hospital stays in field hospitals and who were often transferred between US and Iraqi hospitals, may have served both to introduce the organism to US field hospitals and as a transmission reservoir. The higher rates of infection with ABC organisms observed among non-US inpatients, compared with US inpatients at Field Hospital Baghdad by Yun et al. [16], supports the hypothesis that non-US patients with prolonged stays in US field hospitals may have served as potential reservoirs of health care-associated infection.

Our findings highlight the emerging challenges that *Acinetobacter* species pose to both military and civilian health care settings. First, *Acinetobacter* species can persist in the environment for long periods, and environmental contamination has been implicated as the source of many health care-associated outbreaks [17–20]. The challenge posed by an environmental reservoir is greatly enhanced in the combat situations in Iraq. The temporary nature of field hospitals amidst desert environmental field conditions make environmental cleaning more difficult, while the frequent influxes of large numbers of casualties over short periods of time increases the risk of environmental contamination with *Acinetobacter* species and challenges the implementation of standard infection-control practices. Second, multidrug resistance frequently limits treatment options. Only imipenem and the polymyxins had in vitro activity against >90% of the organisms tested. Consequently, clinicians relied on colistin, an older antimicrobial agent with a reported history of considerable toxicity [21], to treat a number of infections in this outbreak.

Several important limitations of this investigation must be

considered. First, detailed information regarding injury date, location in Iraq where injury was sustained, initial treatment facilities, and evacuation route was available for only a small group of patients. Secondly, all isolates were not available for study; this may have limited our ability to identify additional strains that were common to patients and the environment. Third, the storage duration and conditions of the archived soil samples may have resulted in limited recovery of soil isolates. In addition, we are unable to determine how representative these soil samples are of all the possible different soil inocula that could have been introduced in wounds at the time of injury. Thus, our findings may underestimate the significance of soil as a source in this outbreak. Fourth, field hospital sampling was not uniformly performed, and sample records were incomplete. This limits direct comparisons of the extent of contamination among areas within a field hospital and among different field hospitals. Fifth, most patients had extensive exposure to health care facilities before they reached a facility where surveillance and diagnostic cultures were performed. This limits our ability to classify infections using definitions of nosocomial transmission on the basis of the duration of exposure to a hospital environment. Sixth, data that identified isolates as causing infection or colonization were not uniformly collected. This further limits our ability to identify likely sources of acquisition and transmission. Last, the observed difference in colonization rates between non-US and US patients presenting to Field Hospital Baghdad may reflect small sample sizes, rather than true differences.

Staff in military hospitals in the United States have responded

Table 3. Summary of antibiotic susceptibility testing of *Acinetobacter baumannii-calcoaceticus* complex clinical isolates from Walter Reed Army Medical Center ($n = 98$) and environmental isolates ($n = 35$).

Antibiotic	Environmental samples		Clinical samples		<i>P</i>
	Total no. of samples	Susceptible samples, no. (%)	Total no. of samples	Susceptible samples, no. (%)	
Ampicillin-sulbactam	35	32 (91)	98	35 (36)	<.001
Amikacin	35	34 (97)	97	56 (58)	<.001
Gentamicin	35	28 (80)	97	18 (19)	<.001
Tobramycin	35	28 (80)	97	33 (34)	<.001
Ciprofloxacin	35	34 (97)	97	31 (32)	<.001
Levofloxacin	35	34 (97)	97	32 (33)	<.001
Imipenem	35	35 (100)	97	87 (90)	.040
Trimethoprim- sulfamethoxazole	35	27 (77)	96	25 (26)	<.001
Piperacillin-tazobactam	35	10 (29)	97	11 (11)	.020
Cefepime	35	25 (71)	97	21 (22)	<.001
Cefotaxime	35	5 (14)	97	3 (3)	.031
Ceftazidime	35	15 (43)	97	16 (17)	.002
Polymyxin B	35	35 (100)	97	96 (99)	.999
Colistin	35	33 (94)	97	96 (99)	.178

NOTE. Antibiotic susceptibility testing was performed using broth microdilution.

to this outbreak by reemphasizing infection-control procedures, including strict hand hygiene, contact isolation, patient cohorting, judicious antimicrobial use, active surveillance for ABC colonization, and increased staff education. In US field hospitals in Iraq, maintaining dedicated operating rooms for patients who are known to be infected with ABC, intraoperative wound dressing management, cohorting of infected patients when possible, instating antibiotic prescribing guidelines, and pursuing the continual education of staff have been implemented. These measures have limited health care-associated transmission within WRAMC; however, rates of colonization with ABC at admission for patients involved in OIF who were hospitalized at WRAMC have remained stable. The implementation of infection-control measures in field hospitals remains challenging.

Advances in battlefield surgical care have improved combat casualty survival [22–24]. To continue to improve the outcomes of these casualties, limiting transmission of health care-associated infections like those due to multidrug-resistant ABC is necessary. Even under the controlled setting of a permanent, noncombat hospital, management of outbreaks of *Acinetobacter* infection is difficult [25, 26]. Even greater challenges exist in the combat setting. Regardless of how *Acinetobacter* species initially enters the military health care system, novel infection control strategies may be needed to help limit the spread of multidrug-resistant organisms, such as ABC, in field hospitals. These advances will have important implications for both military and civilian hospitals, and may lead to better control of all health care-associated pathogens.

Acknowledgments

We gratefully acknowledge Drs. Fred Tenover and Dan Jernigan at the Centers for Disease Control and Prevention (Atlanta, GA) for the critical editorial assistance that they provided in the development of this manuscript, and Dr. Nathan Keller at the Sheba Medical Center (Tel Hashomer, Israel) for generously sharing his experience with *Acinetobacter* species outbreak investigations. We would also like to gratefully acknowledge the specific contributions made by the individual members of the *Acinetobacter* Outbreak Investigation Team: David Dooley and Duane Hostenenthal (expert consultation; Brooke Army Medical Center, Ft. Sam Houston, TX); Helen Crouch, Paula Gray, and Christopher Florez (data collection; Brooke Army Medical Center); Roberta B. Carey and Kristy Kubota (expert consultation; Centers for Disease Control and Prevention); Laura Jevitt, David Lonsway, and Portia Williams (data acquisition and laboratory support; Centers for Disease Control and Prevention); Joseph Malone and Clara Witt (expert consultation; Department of Defense, Global Emerging Infection Surveillance and Response System, Silver Spring, MD); Jay Mansfield (data acquisition; Department of Defense, Global Emerging Infection Surveillance and Response System); Wade Aldous and Abel Trevino (data acquisition and laboratory support; Landstuhl Regional Medical Center, Landstuhl, Germany); Dallas Hack and Andrew O'Brien (study supervision; Multi-national Forces-Iraq, Baghdad, Iraq); Sydney Lee (data acquisition; Walter Reed Army Institute of Research, Silver Spring, MD); Warren Sateren (graphical support for manuscript preparation; Walter Reed Army Institute of Research); Jane Pool and Andrew Shorr (expert consultation; Walter Reed Army Medical Center, Washington, DC); Fluryanne Leach, Marie Pe, and Amy Summers (data acquisition; Walter Reed Army Medical Center); Kerry Snow (study supervision; Walter Reed Army Medical Center); Jack Heller (study supervision; US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD); and Warren Wortmann and Shaina Zobel (data acquisition; US Army Center for Health Promotion and Preventive Medicine).

Financial support. United States Army Center for Health Promotion and Preventive Medicine (Aberdeen Proving Ground, MD).

Potential conflicts of interest. All authors: no conflicts.

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