Chapter 13

MULTIDRUG-RESISTANT BACTERIAL INFECTIONS AS A THREAT TO THE US MILITARY HEALTH SYSTEM: *ACINETOBACTER* INFECTIONS AS A CASE STUDY

EMIL LESHO, DO*; LUTHER E. LINDLER, PHD†; BRUNO PETRUCCELLI, MD‡; AND GLENN WORTMANN, MD§

*Director, Multidrug-Resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, 503 Walter Grant Avenue, 1W102, Silver Spring, Maryland 20910
†Biosurveillance Liaison, Armed Forces Health Surveillance Center, 11800 Tech Road, Silver Spring, Maryland 20904
‡Colonel (Retired), Medical Corps, US Army; formerly, Director, Epidemiology and Disease Surveillance, US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland; currently, Senior Physician/Epidemiologist, US Military HIV Research Program, Henry M. Jackson Foundation for the Advancement of Military Medicine, 3720-A Rockledge Drive, Suite 400, Bethesda, Maryland 20817
§Colonel (Retired), Medical Corps, US Army; formerly, Chief of Infectious Diseases, Walter Reed National Military Medical Center, Bethesda, Maryland; currently, Assistant Director, Infectious Diseases Section, MedStar Washington Hospital Center, 110 Irving Street, NW, Washington, DC 20010*

INTRODUCTION

Although not new to the civilian critical care community, infections with multidrug-resistant (MDR) *Acinetobacter* species were rare in the US Military Health System (MHS) before the wars in Afghanistan and Iraq. In fact, the marked increase in such infections immediately following these conflicts led some to postulate that MDR *Acinetobacter* species may be a result of human engineering with malicious intent, leading some news reports to refer to it as "Iraqibacter." However, there is no evidence supporting this contention. *Acinetobacter* species are environmentally hardy and difficult to eradicate from inanimate healthcare surfaces, but their relatively low virulence makes them poor candidates for weaponizing. In fact, even in the most severely war-wounded patients, *Acinetobacter* species infections are rarely fatal.[1,2] Nonetheless, MDR *Acinetobacter* species pose an equally concerning risk to global public health as bacteria engineered for weapons use.

Infection has always been a complication of war trauma. Treatment of traumatic wound infections has evolved over time, as a belief in "laudable pus" yielded to surgical debridement, and the emergence of penicillin in 1942 ushered in a period when recovery from serious infections became possible, if not expected. However, the 21st century has witnessed the expansion of bacteria that are resistant to multiple antibiotics, and a dearth in new drug development has resulted in infections from bacteria that are resistant to virtually all available antibiotics. These MDR bacteria have become well recognized in hospitals around the world and are especially problematic in locations where antibiotic use is frequent, such as in intensive-care units and long-term acute care facilities. During Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF), forward-deployed US medical facilities often provided acute care to service members who were rapidly medically evacuated, as well as to local national patients who required sustained care. Antibiotic use following traumatic injury to prevent or treat infection was the standard of care, and thus the stage was set for the selection of MDR bacteria, which subsequently spread through the evacuation chain. Although several different species of MDR bacteria emerged to complicate war trauma care, *Acinetobacter calcoaceticus baumannii* (ACB) complex first heralded the problem, which led to the investigations discussed in this chapter.

Bacteria of the genus *Acinetobacter* are glucose nonfermentative, nonfastidious, catalase-positive, oxidase-negative, strictly aerobic, gram-negative, coccobacilli (or pleomorphic) and commonly occur in diploid formation or in chains of variable length. However, different genospecies cannot be easily identified using traditional methods. Members of the genus have been classified in various ways; therefore it is difficult to understand the true status of the epidemiology and clinical importance of these organisms. Since 1986, the taxonomy of the genus *Acinetobacter* has undergone extensive revision. The original single species named *Acinetobacter calcoaceticus* has been abandoned, and at least 32 genospecies have now been proposed, 17 of which have been correlated with species' names. Identifying the members of the genus *Acinetobacter* to the species level by traditional methods is problematic. *Acinetobacter baumannii* (genospecies 2), *Acinetobacter pittii* (formerly known as *Acinetobacter* genospecies 3), and *Acinetobacter nosocomialis* (formerly known as *Acinetobacter* genospecies 13TU) are genetically and phenotypically similar to *A calcoaceticus* (*Acinetobacter* genospecies 1) and hence are grouped in the so-called ACB complex. Molecular methods are needed to identify members of the complex to the species level because each member has a distinct antimicrobial susceptibility profile and shows different clinical characteristics. In this chapter, we use the term *Acinetobacter* or ACB interchangeably and to indicate the overall phenotype that includes the four most clinically relevant species mentioned above. These have gained notoriety for a predilection to cause nosocomial infections and to develop resistance to multiple antibiotics.[3]

The importance of ACB has been recognized with its inclusion in the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, ACB, *Pseudomonas aeruginosa*, and *Enterobacter* species), a group of bacteria identified by the Infectious Diseases Society of America that risk becoming resistant to all available antibiotics.[4] ACB is predominantly associated with medical technology, as most infections occur in the setting of artificial ventilation (ventilator-associated pneumonia), intravenous access (line-associated bacteremia) or urinary catheterization (urinary tract infections).[5] Although spontaneous, invasive infection of immune-competent patients appears to be uncommon, infections associated with trauma have been reported. Following an earthquake in Turkey in 1999, a local hospital experienced an 18.6% rate of hospital-acquired infection (HAI; predominantly wound infections) with MDR ACB being the most commonly isolated pathogen.[6] In 2002, 62% of trauma victims following a bombing in Bali had ACB infections, and in 2004 MDR ACB was discovered in 18% of cultures among injured tsunami victims evacuated from Thailand to Germany.[7,8]

The presence or relative absence of ACB as a "militarily relevant" pathogen prior to 1970 is unknown. Among casualties of the Korean War, at least one blood culture was reported to grow *Achromobacter* species,[9] and during the Vietnam conflict, the predominant gram-negative pathogen recovered from a series of 30 Marines was in the *Mimeae-Herellea-Bacterium-Alcaligenes* group, which is postulated as being ACB. However, it is unclear if any of these bacteria would be classified as ACB using current taxonomy. Furthermore, the organism was not prevalent in other studies of combat wounds during either war.[10–14] The numbers of US casualties in Operations Just Cause (Panama, 1989–1990), Desert Storm/Shield (Iraq, 1990–1991) and Restore Hope (Somalia, 1992–1993) were relatively low, and ACB infection was not reported.[15]

US forces entered Afghanistan in 2001 in support of OEF and Iraq in 2003 in support of OIF, and military healthcare providers shortly thereafter began noting an increase in the number of patients infected with ACB. A collaborative report from military physicians and the Centers for Disease Control and Prevention was published in 2004, highlighting 102 injured service members whose blood cultures grew ACB. Most of these cases were reported from Landstuhl Regional Medical Center (LRMC) and Walter Reed Army Medical Center (WRAMC), with 32 OIF and 29 OEF bacteremic patients. The number of patients with ACB bloodstream infections in 2003 and 2004 exceeded those reported in previous years (one case during 2000–2002 at LRMC, and two cases during 2001–2002 at WRAMC).[16] A review of 211 trauma casualties evacuated from Iraq to the United States Naval Ship (USNS) *Comfort* during the first month of OIF) in 2003 revealed 44 cultures positive for *Acinetobacter* species, representing 33% of all isolates. Specifically, 36% of wound isolates and 41% of bloodstream isolates were of *Acinetobacter* species.[17] A review of MDR bacteria at WRAMC demonstrated a marked increase in the incidence of ACB infections, peaking in 2004 (Figure 13-1), and a study using multilocus polymerase chain reaction (PCR) and mass spectrometry to genotype isolates demonstrated that some of the strains belonged to an atypical and evolving group of isolates, distinct from those found at nonmilitary hospitals in the United States.[18,19] Additionally, molecular typing of isolates from one of the worst outbreaks of MDR ACB in the MHS occurred at WRAMC and revealed eight major clone types, 60% of which were related to the three International Clonal Complex (ICC) types, suggesting multiple independent origins.[20, 21]

The clinical impact of the ACB outbreak associated with OIF/OEF was acute, with lingering downstream effects. A retrospective study of 93 war-related trauma patients at WRAMC with ACB bacteremia determined

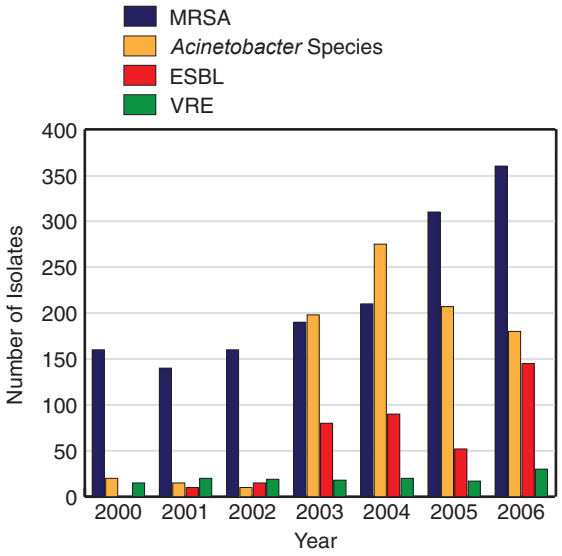

Figure 13-1. Annual incidence of drug-resistant organisms isolated from clinical specimens at Walter Reed Army Medical Center (2000–2006).
ESBL: extended-spectrum β-lactamase-producing organisms
MRSA: methicillin-resistant *Staphylococcus aureus*
VRE: vancomycin-resistant enterococci

that there was a median of 1 day between admission and detection of infection. Of these isolates, 86% were resistant to multiple antibiotics. The 30-day mortality rate in this group of patients was only 2%, and those 2 patients died from massive pulmonary emboli believed to be unrelated to ACB bacteremia. The authors concluded that the absence of severe comorbidities (as measured by the Acute Physiology and Chronic Health Evaluation II score and Charlson comorbidity index), compared to cohorts of patients in other published reports of ACB bacteremia, contributed to the low mortality rate.[1]

Along with bacteremia, military providers began noting the development of ACB wound and burn infections, as well as osteomyelitis. Davis et al reported 23 patients with MDR ACB, 18 of whom had osteomyelitis, 2 with burn infections, and 3 with deep wound infections. Within a mean follow-up period of 9 months, all patients were cured of infection.[22] ACB was reported to be the most prevalent organism recovered from military burn patients injured during operations in Iraq and Afghanistan, with the percentage of ACB resistant to four classes of antimicrobial agents increasing from 17% in 2003 to 2005 to 49% in 2006 to 2008.[23,24] Trauma-related skin and soft-tissue infections were reported by Sebeny et al, who reported their findings

in eight patients with infection due to ACB.[25] Osteomyelitis was noted in several cohorts, often in association with orthopedic fixation devices.[26,27] ACB central nervous system infection associated with trauma was reported in several cases.[28,29]

In addition to causing infectious complications in traumatically injured patients, ACB developed resistance to multiple classes of antibiotics, posing treatment challenges to clinicians. At both WRAMC and Brooke Army Medical Center (BAMC), the prevalence of MDR ACB rose dramatically during the course of OIF/OEF. At BAMC, the percentage of MDR ACB rose from 4% to 55% between 2001 and 2008, while at WRAMC the percentage of ACB that was susceptible to imipenem dropped from 100% in 2002 to 61% in 2006.[19,30] Faced with the loss of traditional antibiotics, physicians turned to other agents, including tigecycline, minocycline, and colistimethate sodium.[31–33] The switch to second-line agents came at a cost, however, as the incidence of acute renal failure (defined as the first three criteria in the RIFLE acronym [risk, injury, failure, loss, and end-stage renal disease]) in 66 patients who received colistimethate sodium was 45%; and 21% of patients stopped therapy because of nephrotoxicity. Fortunately, renal function returned to normal once colistimethate sodium was stopped.

In summary, prior to OIF/OEF, ACB had been recognized as a pathogen among hospitalized patients, and scattered reports had noted an association with civilian trauma. However, its emergence as a pathogen associated with war trauma was unexpected, as was the breadth of its clinical presentation (including bacteremia, skin and soft-tissue infection, meningitis, and osteomyelitis). The organism's ability to develop resistance to multiple antibiotics complicated treatment decisions, leading to the use of more toxic agents. Healthcare on the modern battlefield now extends from the point of injury to US-based, Echelon V (Level V) facilities, separated by a transit time of only a few days. Traumatically wounded patients are cloistered in an intensive-care environment, supported by invasive medical devices (ventilators, chest tubes, urinary catheters and intravenous lines), and administered antibiotics that invariably select for resistant pathogens. In retrospect, the emergence of a nosocomial pathogen was perhaps inevitable, and ACB was well positioned to exploit the opportunity. Trauma-induced disruption of anatomic barriers to infection and antibiotic use appear to select for ACB, and thus the military healthcare system will likely face this challenge again in future conflicts.

EPIDEMIOLOGICAL CONSULTATION

Mission

Lieutenant General James B Peake, who was Surgeon General of the Army and Commander, US Army Medical Command, ordered an epidemiological consultation (EPICON) on August 27, 2004. The charge to the US Army Center for Health Promotion and Preventive Medicine (now the Army Public Health Command) included objectives that had typified military epidemiological investigations for decades and now addressed ACB. Descriptive and risk-factor analysis, identification of sources of infection, and recommendations for control, prevention, and future surveillance were all expected. However, the scope and complexity of the ACB problem prompted an approach that differed substantially from previous outbreak responses. A multicenter effort was organized that included the Walter Reed Army Institute of Research (WRAIR) and the Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS), in addition to four hospitals in the chain of medical evacuation from Southwest and Central Asia: the 31st Combat Support Hospital (31st CSH in Iraq, Level III), LRMC (Level IV), WRAMC (Level V), and BAMC (Level V). Twenty principal personnel, including four civilians, provided data or conducted local studies to support the investigation; these included clinicians, epidemiologists, infection control practitioners, microbiologists, an environmental scientist, and a statistical programmer. Numerous other individuals from various military organizations served as consultants or collaborators, including Navy and Air Force personnel, and advice from medical and laboratory contacts with ACB experience in other countries, particularly those in Southwest Asia, was also obtained.[34]

The three-way networking of public health, research, and clinical assets was not a new endeavor; neither was applying tools like genetic fingerprinting, web-based data collection, and multipoint conferencing. Nevertheless, bringing these together in response to the ACB problem seriously threatening hospitalized military beneficiaries set a new precedent for the MHS; just as the coordinated, international response to severe acute respiratory syndrome, on a broader scale, had set a new precedent for global public health the previous year.

Acinetobacter infections had been rare at LRMC and WRAMC; but cases began to emerge in 2002 during the first year of OEF in Afghanistan and their frequency accelerated immediately after OIF began in March 2003.

Since microbiology and clinical data within military hospitals were archived, confirmation of an outbreak at LRMC and WRAMC was rather straightforward. Even after adjusting for the number of admissions, intensive-care bed days, and the frequency of culture specimen collection, rates of all types of ACB infection exceeded historical counts at these hospitals. At LRMC, 36 bloodstream infections were observed in 2003 and 2004, compared to only 4 in the preceding 3 years. At LRMC and WRAMC combined, the average number of ACB isolates had increased from 1 per month during 2002 to 1 per day during 2003 and 2004.[34]

Among the aims of data analysis and interpretation by EPICON investigators were: (*a*) weighing the relative importance of wound contamination at the time of injury with that of nosocomial infection; (*b*) determining the role of Southwest or Central Asia as a geographic source, versus a primary role of intensive hospital care regardless of geographic location; and (*c*) distinguishing the importance of fomite versus person-to-person transmission as an underlying propagation mechanism. It was not assumed *a priori* that these were mutually exclusive dichotomies; and much was already well known about ACB as a species complex, such as the ability of the organisms to colonize humans in addition to the inanimate, or built environment of the hospital, and soil and water.[35] The newly accumulated evidence was mixed with respect to each of these aims when considered individually; however, taken together, the findings empirically supported a set of practical countermeasures and ongoing surveillance procedures.[34]

First Reservoirs in the Combat Zone

A baumannii strain distribution across international borders has been described, with evidence of drug resistance transfering between globally distant hospitals.[36,37] Evidence of drug-resistant *A baumannii* as a growing problem in Southwest Asia was revealed during the outbreak in US military hospitals, both through investigators' conversations with hospital infections experts in the region and through published reports.[38,39] Also, surveillance at the 31st Combat Support Hospital in Baghdad had shown that non-US patients (coalition and local national) were colonized with ACB at a proportion that was more than five-fold that of US patients. Among hospital staff, 15 pairs of hands had been screened in one series, and none of the specimens grew ACB. During a 2-month period after the EPICON was initiated, 102 screening specimens from the US field hospital in Baghdad were obtained from patients receiving care in the emergency treatment area or being admitted to intensive care. One (2%) of 64 US patients and 4 (11%) of 38 Iraqi patients were found to be colonized. Furthermore, the hospital ship providing Level III care in the Persian Gulf during the early weeks of OIF (USNS *Comfort*, discussed earlier) was receiving primarily Iraqi nationals when *Acinetobacter* isolates had become relatively common.[17,25]

Screening of 96 ambulatory patients evacuated from Iraq to Landstuhl from its usual catchment area revealed no ACB skin colonization. However, among 472 inpatients admitted to LRMC during the same period, 19 (3.9%) had skin cultures that were positive for ACB. Patients admitted to intensive care had a colonization prevalence of 10% (relative risk [RR] = 2.8 compared with regular ward admissions, $P < 0.0001$). Patients with ACB bloodstream infection were found to have the infection within 48 hours of arrival (over 50% on the day of admission), suggesting acquisition before admission to Level IV in the most seriously affected ACB patients.[34]

Analysis of locations in Iraq and Afghanistan from which patients were evacuated to Landstuhl over time revealed that the US hospital at Ibn Sina (Baghdad) contributed patients with the highest proportion colonized or infected with ACB, and that originating from field hospitals in Iraq presented a higher risk than from Afghanistan. When the results of colonization studies at three echelons (Baghdad, LRMC, and WRAMC, 2003 to 2004) were examined together, including stratification between admission and discharge screening at LRMC and WRAMC, the proportions of patients with positive ACB cultures showed a clear, progressive increase with each level of care.[34]

At least one culture of the inanimate hospital environment from each of seven field hospitals (five in Iraq, two in Kuwait) was positive for ACB. Specific sampling locations were documented for 37 isolates. All of these were subjected to 16S rDNA (ribosomal deoxyribonucleic acid) sequencing, and 34 also underwent pulsed field gel electrophoresis (PFGE). In addition, there were 170 isolates available from 145 individuals tested in 2003 while they were inpatients at Baghdad, the USNS *Comfort*, LRMC, or WRAMC. All of these underwent PFGE, and 164 of them underwent 16S rDNA sequencing. The results are in the Molecular Analysis section, but key to the issue of strain importation was the demonstration that 43 patients treated at 4 different military hospitals were infected with related strains from a single cluster group which, in turn, was genetically related to an isolate derived from environmental sampling of an operating room in the Baghdad field hospital. This group included both US and non-US patients, and both those who had and those who had not deployed to the Central Command area of operations. No cultures were positive for ACB organ-

isms when 31 archived soil samples from the general environment of Iraq and Kuwait were tested (collected March 2003 to December 2004 from various locations, not in the vicinity of hospitals).[21] A separate report, also published in 2007 but focusing on Canadian soldiers with *A baumannii* ventilator-associated pneumonia, similarly described genetic linkage between a field hospital environmental isolate (from a ventilator intake filter in Afghanistan) and isolates from four soldiers whose hospital treatment continued in Canada. Linkage to clinical specimens at Level IV (LRMC in these cases as well) was also found for three of the patients.[40]

Observations based on colonization and environmental studies of ACB may be confounded, biased, or diluted in significance when low-yield skin sampling sites are used for surveillance and colonization studies. This can also occur when reliance on routine cultures fails to distinguish *A baumannii*, the species most often causing opportunistic infection, from clinically less-significant species.[41,42] During the EPICON, investigators found poor agreement between different body sites (eg, axilla versus groin, hands, feet, or forehead) at LRMC and BAMC when skin cultures were observed for ACB growth; and most isolates from environmental sampling were ACB other than *A baumannii*. Thus, a separate focus on clinical specimens, and the subjecting of both clinical and environmental isolates to species identification and molecular typing, provided critical data for the investigation.

In addition to the EPICON, separate endeavors by Air Force personnel contributed data regarding ACB. In 2005, 83 environmental samples were taken from two C-141 aircraft used for aeromedical evacuation from Iraq to Germany, and from a deployed hospital of the Expeditionary Medical Dental Group (332nd EMDG, Balad Air Base, Iraq). The source locations included the walls, seats and floors from the front, middle, and back sections of the aircraft; the operating rooms (ORs) and wards of the hospital; and a variety of equipment (litters, litter straps, life pack monitor covers, and outer surface of endotracheal tubes). Also sampled were personnel working directly with patients while receiving, flying with, or transferring them. Three samples were taken from personnel working with patients from both the gloves and hands of caregivers. Finally, 16 of 58 patients who were transported during the observed flights were screened for ACB at LRMC. All of the environmental, equipment, personnel, and patient specimens were negative for ACB, except for one sample taken from a patient air warmer in the Balad hospital, which produced an imipenem-sensitive isolate. Of course, the possibility of colonization or contamination during transport of known, ACB-infected patients could not be ruled out.[34]

At the 332nd EMDG hospital, the surgical staff observed a significant reduction in ACB infections after implementing very aggressive infection control procedures, which indirectly supported the conclusion that the outbreak at higher echelons of care was likely preceded by nosocomial transmission in field hospitals. Countermeasures applied in Balad included strict enforcement of contact precautions as well as standardized intraoperative wound management, imposed conservative use of antimicrobials, and initiated special interventions (ie, plastic draping for all OR entrances, opening an additional OR for the most contaminated wounds, opening an additional ward to reduce patient crowding, greater use of heat and bleaching for linen cleaning, and thorough cleaning of ORs and wards, including weekly filter and duct cleaning in environmental control units).

Nosocomial Infection as the Dominant Problem

When the EPICON report was submitted in mid-2005, nosocomial transmission had been clearly documented in at least 39 *A baumannii* infection cases at WRAMC, and three quarters of these were cross infections in patients who were not evacuated from a deployed setting, including four civilians who died with bloodstream infection as an underlying cause. There had also been a fatal case at LRMC in a non-OEF/OIF patient: a 63-year-old woman who had been admitted to the ward with exacerbation of chronic obstructive lung disease, and who initially improved. Unfortunately the case went on to illustrate the serious impact ACB was having on persons completely unassociated with military operations. On hospital day 11, there was a sudden clinical deterioration, requiring the patient to be transferred to intensive care and mechanically ventilated. She expired the next day. Culture results supported the diagnosis of *A baumannii* pneumonia and bacteremia. During the patient's hospital stay, at least five patients colonized with *A baumannii* had been admitted to the same ward and one of them stayed in the same room, but PFGE analysis distinguished the colonized patient's strain from that of the deceased. However, an isolate completely matching that of the deceased was obtained from a patient staying in a different room on the same ward. This favored transmission by healthcare personnel over fomites in the room as an explanatory mechanism in this case.[34]

An analysis of nosocomial ACB transmission at WRAMC and BAMC revealed that the resulting infections primarily involved the respiratory tract, and that ACB acquisitions were primarily among civilian beneficiaries. Comparing 2004 with 2003 at LRMC and WRAMC combined, ACB wound specimens accounted for a diminishing fraction of positive

cultures, while bloodstream infections increased as a proportion of ACB infections. At LRMC the respiratory tract was considered a likely portal of entry for many of the nosocomial infections and, after excluding specimens taken from the skin and wounds, the respiratory tract was also the most common site of infection. The urinary tract was not a major site of infection, but infection there was more common at WRAMC than at LRMC.

The EPICON report concluded that

> While all stages of the military healthcare system can propagate or sustain the presence of *A baumannii* on patients, the initial source of the current outbreak appears to be the (Level III facilities) in Iraq and Afghanistan. Nosocomial transmission accounts not only for some of the infections at (higher echelons) but also for the initial infection of US troops who acquire the infection before or during strategic MEDEVAC [medical evacuation]. Patients with relatively long inpatient stays in these hospitals (especially non-US patients) represent a likely reservoir for transmission of the organism. Pre-hospital, primary wound infections in theater are not likely to have a significant role in transmission.[34]

With respect to environmental surface contamination versus colonized people, both were linked sufficiently to transmission to warrant both enhanced sanitation (room, equipment) and strict personal hygiene. Despite such measures, control of nosocomial transmission and of further progression toward drug resistance continued to prove extremely challenging for the MHS in the years following recognition of the *A baumannii* outbreak.[43] Nevertheless, it is highly likely that morbidity and mortality would have continued to increase without strict preventive interventions, as now promulgated in national guidelines.[44]

MOLECULAR ANALYSIS

Strain Collection and Sampling

Around April 2003, physicians in the MHS noticed a marked increase in the number of *Acinetobacter* infections within Level IV and Level V medical treatment facilities (MTFs). The formal investigation described in the previous section was launched the following year. A critical part of the EPICON was to, "identify the cause(s) or source(s) of infection." More than 200 clinical and environmental isolates were collected from 148 different patients and 37 environmental isolates collected in and around 7 deployed field hospitals in the Central Command area of responsibility. These isolates were referred to WRAIR for genetic analysis. The results of this EPICON were published in the journal *Clinical Infectious Diseases*.[21]

Genetic Analysis, Characterization, and Identification of the Source

Molecular epidemiology was performed on the clinical and environmental *Acinetobacter* isolates using PFGE and 16S rDNA sequencing in a single-blinded study.[21,45] A total of 201 of the 207 isolates were identified using 16S rDNA sequencing. The clinical isolates were almost evenly split between *A baumannii* and other ACB organisms. In contrast, only 19% of the environmental isolates were *A baumannii*, and 70% were ACB organisms other than *A baumannii*. PFGE was able to establish 66 clinical isolate clusters and 25 different environmental isolate clusters when clusters were defined as greater than 90% identical. Three different PFGE clusters contained isolates from clinical samples and the environmental isolates that were 100% identical. The matching environmental and clinical isolates were obtained from Camp Dogwood and WRAMC; LRMC, WRAMC, USNS *Comfort*, and Field Hospital Baghdad; and Mosul, Camp Dogwood, and LRMC. Additionally, two isolates from LRMC were 100% identical to an ACB isolate from WRAMC.[21] Taken together, these results strongly indicated that the outbreak of MDR *Acinetobacter* infections seen in the larger military medical centers began as nosocomial infection that originated in CSHs in Iraq. In essence, the MHS had become "infected" with *Acinetobacter* because patients who were not involved in Iraqi military operations became infected with the organisms.

Military and civilian casualties from OIF were evacuated to the United States and the United Kingdom. Hospitals in the United Kingdom reported an outbreak of *Acinetobacter* infections soon after OIF commenced. Initially, there was concern that the infections caused by *Acinetobacter* may have been due to an intentional release of the organism. To determine if the isolates obtained in the United Kingdom were similar to those found in US casualties; the two countries initiated a collaboration and analyzed representative strains.[46] The laboratory at WRAIR chose representative isolates from all of the major clusters that had been identified by PFGE and supplied them to the UK investigators. The UK Laboratory of HealthCare Associated Infections compared their *Acinetobacter* isolates to the ones supplied by the WRAIR laboratory. PFGE revealed that three of the US isolates were similar to the UK isolates with greater than 90% similarity. The antibiotic susceptibility profiles among these three isolates were

also similar. DNA sequence analysis of the integron region associated with antibiotic resistance in *Acinetobacter* revealed that two of these common isolates had identical sequences. The third common isolate revealed a duplication of one gene in the US isolate but was otherwise identical to the UK isolate. These results suggested that there was a common origin for the *Acinetobacter* isolates causing wound infections in both the United States and the United Kingdom.

Research, epidemiological investigation, and molecular typing indicated that the *Acinetobacter* infections were nosocomial such that casualties were becoming infected in theater, then the organism was becoming disseminated through the medical evacuation chain. A study utilizing multilocus PCR and mass spectroscopy (MS) was undertaken to analyze how the genotype of the organism might change over time in an MTF. A total of 267 *Acinetobacter* isolates were analyzed; 216 of the isolates were isolated from 2002 through 2004 and were part of the original EPICON previously analyzed by PFGE.[21,47] The additional isolates in this study were obtained from the American Type Culture Collection and from European hospitals. *A baumannii* accounted for 83% of the total isolates and could be divided into 46 unique sequence types (STs). This study also showed a strong correlation between isolates obtained from US OIF casualties and isolates obtained from European hospitals. Although the resolution of the PCR MS technique was lower than PFGE, there was good correlation between the two molecular methods. Additionally, this group analyzed the change in genotype of the *Acinetobacter* over time at WRAMC using PCR MS.[18] This study compared the ST of isolates obtained from 2002 to 2004 with the ST of strains isolated from 2006 to 2007 at WRAMC. The STs were relatively constant; a few minor STs either disappeared or increased with time, yet the major STs remained constant. Comparison of STs with nonmilitary hospital isolates revealed the distribution of STs was markedly different between the two groups. The antibiotic susceptibility profile generally correlated with ST as well. The study suggested that the *Acinetobacter* population in WRAMC had become less diverse and more stable with time. This was possibly due to effective countermeasures, such as sanitation and specific early therapy that resulted in reduction of less fit *Acinetobacter* strains both in patients and in the environment.

Antibiotic Resistance

MDR in *Acinetobacter* increased with time, making it more difficult for the MHS to respond to the threat. The genus is known as an opportunistic pathogen that resides in the environment and is naturally resistant to many antibiotics[35,48]; however, the organism also responds to antibiotic treatment by acquiring antibiotic-resistant genes. Interestingly, isolates obtained at BAMC from deployed service members were generally more resistant than those from nondeployed personnel.[49] Resistance to the drug of choice, imipenem, increased as casualties from OIF continued. Several studies have looked at which genes are involved in the MDR phenotype of *Acinetobacter* involved in military wound infections.

The study by Hujer et al used antibiotic-resistant, gene-specific PCR to analyze selected isolates from WRAMC to determine which MDR-associated genes the strains harbored.[50] Approximately 20% of the 75 isolates they analyzed were resistant to imipenem. Many isolates encoded multiple genes that were responsible for resistance to a class of antibiotic. Almost all of the isolates were resistant to ciprofloxacin through chromosomal housekeeping gene mutation. The strains were also highly resistant to cephalosporins by the production of beta-lactamase belonging to seven different classes of genes. Ninety percent of the imipenem-resistant isolates encoded the *bla*OXA-23 beta-lactamase allele. In another study, based on microarray analysis of antibiotic resistance genes found in 102 *Acinetobacter* isolates obtained from the National Naval Medical Center (NNMC) in 2006, 93% of the imipenem-resistant isolates were found to encode the *bla*OXA-23 beta-lactamase.[51] All of the imipenem-resistant isolates belonged to one of two PFGE clusters. Accordingly, the increase in imipenem resistance in *Acinetobacter*-colonizing OIF casualties was mostly due to acquisition of a single allele of beta-lactamase. The *bla*OXA-23 gene has been shown to be associated with bacterial mobile genetic elements allowing for rapid resistance acquisition and spread.

MILITARY HEALTH SYSTEM RESPONSE TO *ACINETOBACTER* AND OTHER "ESKAPE" PATHOGENS

Following the EPICON described above, many clinicians, scientists, and microbiologists recognized the use and value of establishing a centralized laboratory for receiving and archiving multidrug-resistant organisms (MDROs). Some of the major referral centers, such as NNMC, BAMC, and WRAMC, had already been preserving some MDR isolates, especially *Acinetobacter*, recognizing their inherent scientific and epidemiologic value. However, there was no central and standardized repository. Additionally, the institutions lacked the necessary human and financial resources to fully characterize these isolates. Centralized collection,

comprehensive characterization, and long-term storage of MDROs is essential to understanding the healthcare challenges and informing future approaches. The Agency for Healthcare Research and Quality estimates that 5% to 10% of all inpatients acquire one or more HAIs during their stay, at an annual cost of $28 to $33 billion. Twenty percent of HAIs are considered preventable through surveillance programs; however, as of late 2008, no agency in the Department of Defense was performing what would become the mission of the MDR Organism Repository and Surveillance Network (MRSN): to conduct enterprise-wide epidemiologic surveillance of MDROs to inform clinical practice and healthcare policy and enhance infection control.

The idea for a repository ("Joint Bacterial Repository") was presented to the leadership of the US Army Medical Research and Materiel Command in 2008; however, given the heightened sensitivity to biosurety amid the ongoing anthrax investigation involving scientists in the command, senior leaders were reluctant to authorize and establish what might be misperceived as another "freezer farm." A few months before, DoD-GEIS (now part of the Armed Forces Health Surveillance Center) had funded two small surveillance studies in Eastern Iraq during the troop surge of 2007.[52,53] These became the proof of concepts for a less static repository and more dynamic surveillance network. These studies demonstrated that regardless of location or environment, a distant facility could submit samples to a central laboratory and could receive useful and actionable information relating to infection control. The concept of a central repository laboratory at the nexus of a multifacility, bidirectional surveillance network (Figure 13-2) was successfully proffered to the US Army Medical Command (MEDCOM) in early 2009.

In June 2009, the bacterial diseases branch of WRAIR in Silver Spring, Maryland, launched the MRSN. Under a performance improvement mandate from MEDCOM, Army hospitals, including those in Iraq and Afghanistan, submit MDROs isolated from clinical infections and active surveillance efforts. Isolates undergo integrated phenotypic, clonal, and phylogenetic analyses, including high-resolution ordered whole genome restriction optical mapping, followed by archival cryopreservation. The MRSN works closely with the Centers for Disease Control and Prevention to ensure genotyping methods are the same. Repository personnel provide epidemiologic reports and infection control information to hospitals and policy makers, conduct site assistance visits, and post site-specific and global antibiograms on a secure website.[54,55] Other US military services are encouraged, but not required, to participate. Synergy is achieved through interagency collaboration and information sharing with the other military services, especially the Army's Pharmacovigilance Center and the Navy and Marine Corps Public Health EpiData center, and with international military and civilian colleagues.

Substantial evidence supports the MRSN's role in enhancing infection prevention and control efforts and reducing associated healthcare costs.[56–59] Authors of one outbreak investigation concluded that if an aggressive surveillance program had not been in place, the source of an outbreak of severe ventilator-associated pneumonia in Canadian soldiers caused by MDR *A baumannii* would have been missed.[60] The Association of Professionals in Infection Control and Epidemiology manual lists over 70 references demonstrating the usefulness of surveillance data in reducing infection occurrence and supporting the use of surveillance data to improve the quality of healthcare outcomes and processes.[61] McQuillen et al cited more than 20 studies quantifying the financial impact of HAIs and the cost savings associated with surveillance.[62] Infection control component monitoring and feedback, the approach taken by the MRSN, has been key in reducing the rate of HAIs (see Figure 13-2).[63,64] Choosing the correct therapy requires knowledge of the underlying disease, the previous patient colonization, and the microbial trends in the community, as well as in the specific healthcare facility. Submitting isolates to the MRSN makes the latter two possible by enabling the MRSN to produce facility-specific and regional antibiograms. Using that modus, the MRSN has achieved notable firsts in the MHS and, in 2010, won first place in the Army Surgeon General's Excalibur Award for health innovation practices. In 2012, the MRSN received accreditation by the College of American Pathologists (CAP), and in 2013 it won the Military Health System Award for Healthcare Innovation.

Currently more than 30 hospitals, including some in unstable areas and war zones, request molecular assistance with outbreak investigation or submitted isolates (an average of 375 per month). Isolates are identified and their susceptibility tested with the three most commonly used commercial automated susceptibility testing systems (VITEK 2 [bioMérieux, Inc, Durham, NC]; MicroScan [Siemens Medical Solutions, Malvern, PA]; and Phoenix [Becton, Dickinson, and Company, Franklin Lakes, NJ]). Discordant results of susceptibility testing are resolved by use of microbroth dilution panels. These data enable direct comparison of the three systems across multiple antibiotics and organisms, ranging from 8 drugs for *A baumannii* to 14 drugs for *Escherichia coli*, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus*.

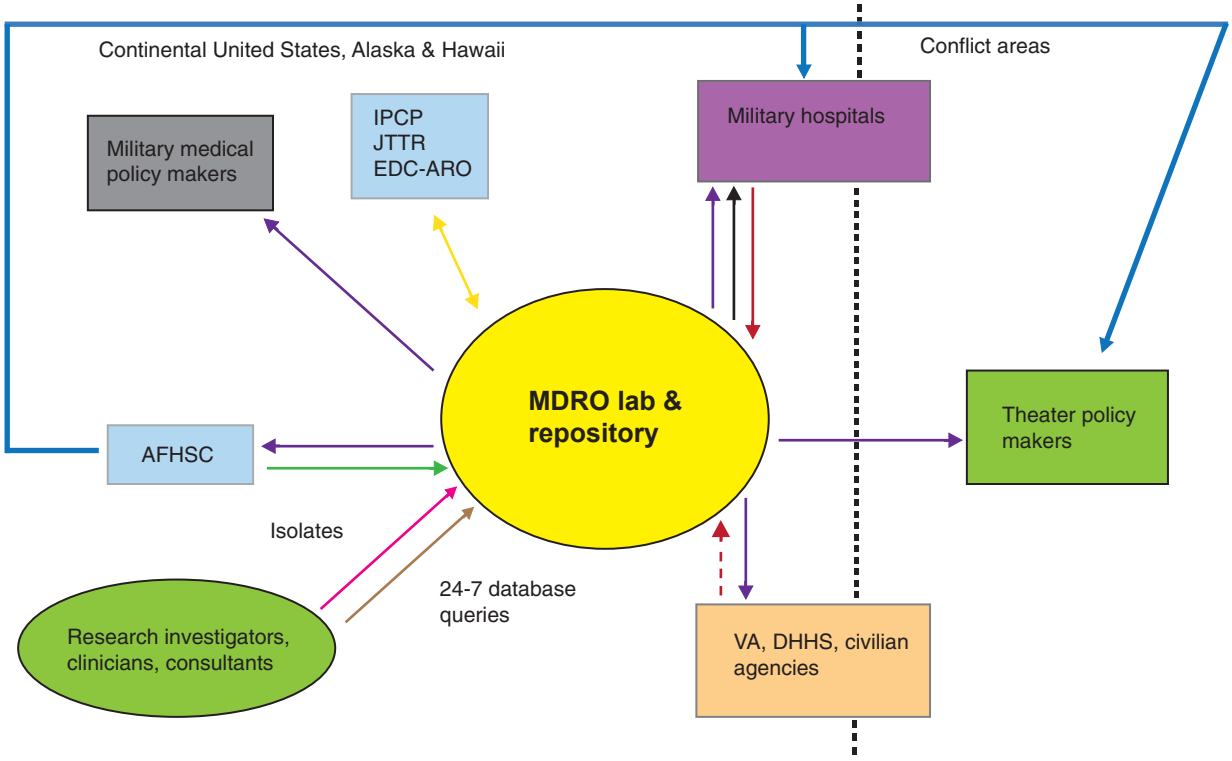

Figure 13-2. The *purple-shaded square* represents medical treatment facilities (Roles 3, 4, and above), which submit isolates and associated clinical data (*red arrow*) to the repository at Walter Reed Army Institute of Research (WRAIR; *yellow oval*) and in turn receive reports and assistance from WRAIR (*black arrow*). Reports and guidance will also be provided to theater medical leaders and combatant commanders (*green box via purple arrow*). Bidirectional information sharing and technical assistance can occur between the repository and other agencies, such as the Infection Prevention and Control Panel, the Joint Theater Trauma Registry, and the Navy's Public Health EpiData Center Antibiotic Resistance Organization (EDC-ARO; *blue box via yellow arrow*) to enhance surveillance and reports. Data and reports from the repository will also be forwarded to the Armed Forces Health Surveillance Center (AFHSC; *purple arrow*) for broad dissemination (*via blue arrows*); the AFHSC can also assist with epidemiologic analysis or generation of the final reports (*green arrow*). Reports and information can also be provided to consultants for microbiology, infectious diseases, surgery, trauma, the surgeons general and others (*gray box via purple and blue arrows*). Consultants, policy makers, clinicians, and research investigators will be able to query certain portions of the database through web-enabled architecture (*olive-tan arrow*). Investigators can request de-identified isolates and specimens for approved research protocols (*pink arrows*).
AFHSC: Armed Forces Health Surveillance Center
DHHS: US Department of Health and Human Services
EDC-ARO: EpiData Center Antibiotic Resistance Organization
IPCP: Infection Prevention and Control Panel
JTTR: Joint Theater Trauma Registry
VA: US Department of Veterans Affairs

Unique to MRSN, associated clinical and demographic information, including securely maintained personal identifiers, is also submitted, helping to avoid isolate duplication when producing antibiograms and enabling MDRO tracking across regions. Isolates without this information are collected, but their ability to inform infection control efforts is severely degraded.

The MRSN developed and validated multiplex real-time PCR platforms to test isolates for the presence of clinically important antimicrobial resistance genes, such as *mupA*, *qac*A/B, *PVL*, *cfr*, and *etA* in *Staphlococcus* species; all variants of *bla*NDM and *bla*KPC, and the most common variants of *bla*VIM and *bla*IMP, and the most relevant alleles of *bla*OXAs in carbapenem-resistant gram-negatives. These are usually plasmid-borne genes that encode for high-level mupirocin resistance (*mupA*), chlorhexidine tolerance or resistance (*qac*A/B), or carbapenemase production (*bla*KPC, *bla*NDM, *bla*VIM, *bla*IMP, and certain *bla*OXAs). They contribute to the dissemination

of biocide or antibiotic resistance. *Bla*NDM1 carried on a novel plasmid was detected in *Providencia stuartii* from Afghanistan.[65] The MRSN was first to report *qac*A/B in methicillin-resistant *Staphylococcus aureus* in the United States, which had higher tolerance of chlorhexidine gluconate than those that did not have the gene.[66] A cluster of colistin-resistant *Acinetobacter* emerged during therapy with colistin. This clone or strain, MLST 94, appeared to have a tendency to develop colistin resistance rapidly upon exposure to colistin, so the MRSN developed an assay capable of detecting the genetic element associated with that strain. It combined optical mapping and sequencing to identify gene copy number changes in sequential *Acinetobacter* isolates from the same patient.[67] The MRSN also improved the speed and reduced the cost of optical genome mapping by successfully mapping multiple genomes on the standard map card.[68]

The MRSN provided the foundation for enhanced *de novo* assembly of high-throughput pyrosequencing data using optical genome mapping, and demonstrated the first clinically relevant application of next-generation sequencing in the MHS.[69] It also allowed identification of a heretofore notoriously difficult-to-characterize species of *Acinetobacter* that is typically misidentified by commercially available identification systems.[70] The intent is that the MRSN become a reference laboratory for DoD-GEIS for high-resolution characterization of the MDR ESKAPE pathogens, with the ultimate goal of becoming the first CAP-accredited laboratory for optical mapping and sequencing in the DoD. Aligned with the One Health philosophy, the MRSN also supports or performs canine and environmental surveillance. Currently the MRSN has collaborators in 32 hospitals from 12 countries in Central and South America, Europe, Asia (including Iraq and Afghanistan), and North America. The demonstrable power of surveillance increases in proportion to the geographic area surveyed and the degree of sharing between those who need to know and those who can act on findings to improve patient safety.[61] To that end, the MRSN continually invites more international civilian and military colleagues to collaborate and submit isolates.

The Way Forward

Current typing technologies have been useful in revealing relationships between isolates of ACB, but they are unable to resolve differences between closely related isolates from small-scale outbreaks, where chains of transmission are often unclear. Increasingly, genome scale epidemiology is required to detect and respond to outbreaks of highly resistant and virulent bacterial "superbugs."[71–73] Recall the recent fatal outbreak at the National Institutes of Health and the amount of sequencing and computing power that was needed to determine the origination and pattern of spread.[72] Another study investigated a polyclonal outbreak of MDR *A baumannii* using whole genome sequencing. Comparison of the complete genome sequences of three dominant outbreak strain types showed that these strains diverged before their arrival at the authors' institution despite all belonging to the same epidemic lineage (International Clonal Complex II).[72] The simultaneous presence of three divergent strains of the same lineage is in accordance with its increasing prevalence in international hospitals, further supporting the ongoing adaption of clonal complex II to the hospital environment.[72,74] Finally, a recent study found that nearly every strain of ACB from one integrated hospital system in the United States was unique despite being indistinguishable by conventional sequence typing methods, and in some cases by core single nucleotide variation typing.[73]

The recent availability of rapid and inexpensive whole genome sequencing permits detailed investigation of genetic differences between bacterial isolates belonging to a single species and gives insight into the nature of genetic changes between isolates under antibiotic selection pressure. This is the approach now taken by the MRSN, and has been used to elucidate the evolutionary origin of an outbreak of colistin-resistant *A baumannii* containing a novel operon, the genomic characterization of a separate clone of *Acinetobacter* responsible for a group of fatal infections, and a new strain type in Honduras.[2,75] Overall, as whole genome sequencing technologies continue to improve with respect to price and speed, these approaches should become the gold standard for rigorous epidemiological analysis in variable circumstances ranging from single-hospital outbreaks to worldwide epidemics, and from retrospective analysis to real-time monitoring.

Applying basic and translational research methods to improve surveillance conducted for quality improvement and infection control resulted in the evolution of the MRSN into the Antimicrobial Resistance Monitoring and Research (ARMoR) Program. It is an enterprise-wide collaboration to aid in infection prevention and control. This approach consists of a network of epidemiologists, bioinformaticists, researchers, policy makers, and hospital-based infection preventionists who collaborate to collect relevant antimicrobial resistance monitoring (ARM) data, conduct centralized molecular characterization, and use ARM characterization feedback to implement ap-

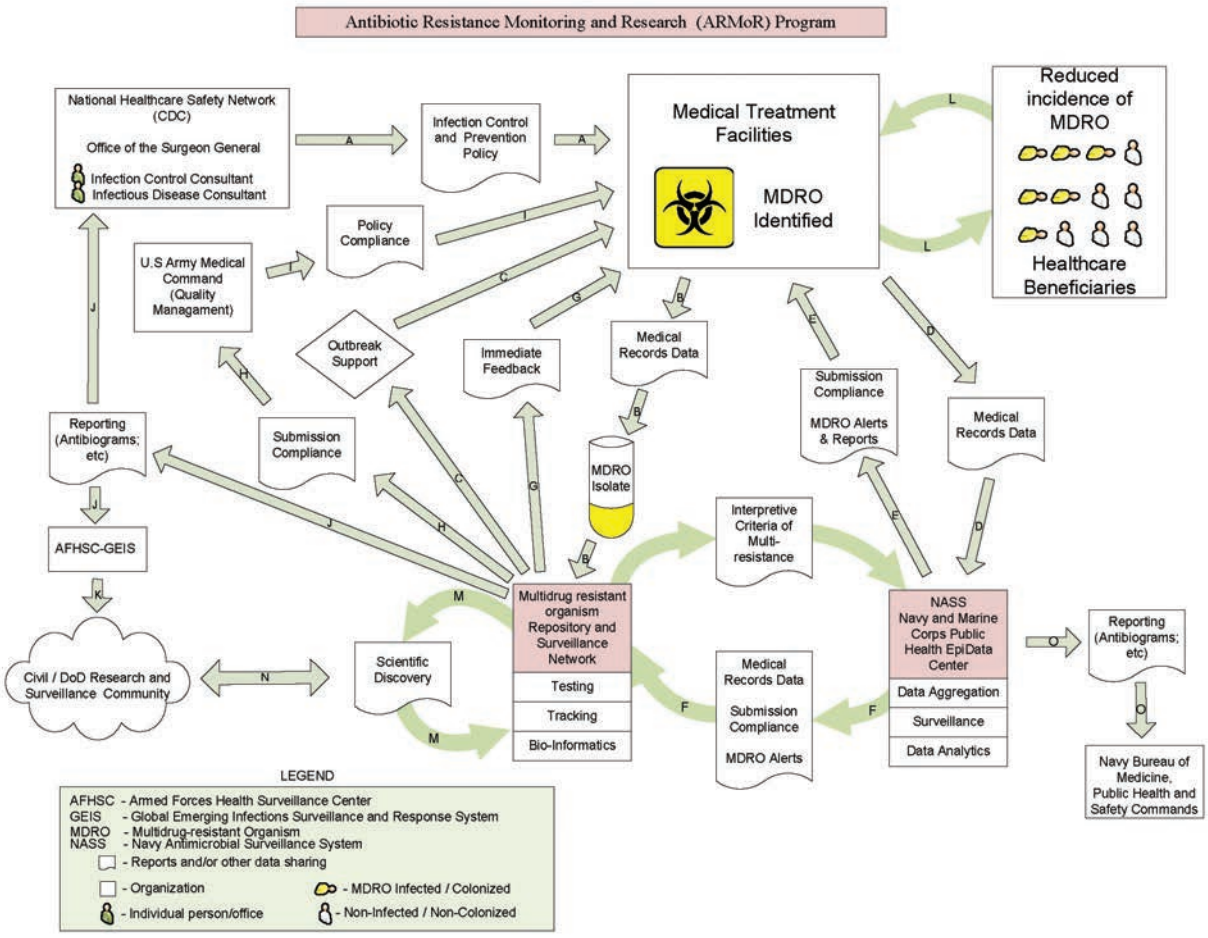

Figure 13-3. The Department of Defense Antibiotic Resistance Monitoring and Research Program (ARMoR) isolate and data flow.

propriate infection prevention and control measures. Policy compliance and enforcement further distinguish the ARMoR Program from the MRSN (Figure 13-3). A particularly concerning type of ARM, carbapenem-resistant *Enterobacteriaceae*, peaked immediately after the program was launched, then significantly declined. Similarly, there have been no further reports or outbreaks of another concerning type of ARM, colistin resistance in *Acinetobacter*, in the DoD since the program was initiated.

SUMMARY

The MHS response to *Acinetobacter* exemplifies how different parts of the MHS coming together to investigate and respond to a major threat to wounded warriors and all MHS beneficiaries. Infectious disease physicians recognized the problem early and engaged DoD medical leadership to task public health professionals with identifying the problem so actions could be taken to stop these costly infections. Public health professionals took advantage of laboratory expertise that had been initially developed for the DoD chemical and biological defense program to perform molecular analysis to determine genetic relationships among the *Acinetobacter* isolates. This fact underscores the need for collaboration between the Chemical Biological Defense Program and that part of the MHS that conducts "routine" infectious disease and public health investigations. Such collaboration supports service members and enhances the MHS's ability to respond during crises.

REFERENCES

1. Kang G, Hartzell JD, Howard R, et al. Mortality associated with *Acinetobacter baumannii* complex bacteremia among patients with war-related trauma. *Infect Control Hosp Epidemiol*. 2010;31(1):92–94.

2. Lesho E, Yoon EJ, McGann P, et al. Emergence of colistin-resistance in extremely drug-resistant *Acinetobacter baumannii* containing a novel pmrCAB operon during colistin therapy of wound infections. *J Infect Dis*. 2013;208(7):1142–1151.

3. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev*. 2008;21(3):538–582.

4. 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.

5. Maragakis LL, Perl TM. *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. *Clin Infect Dis*. 2008;46(8):1254–1263.

6. Heath CH, Orrell TC, Lee RC, Pearman JW, McCullough C, Christiansen KJ. A review of the Royal Perth Hospital Bali experience: an infection control perspective. *Healthcare Infection*. 2003;8(2):43–54.

7. Maegele M, Gregor S, Steinhausen E, et al. The long-distance tertiary air transfer and care of tsunami victims: injury pattern and microbiological and psychological aspects. *Crit Care Med*. 2005;33(5):1136–1140.

8. Lindberg RB, Wetzler TF, Newton A, Howard JM, Davis JH, Strawitz J. The bacterial flora of the blood stream in the Korean battle casualty. *Ann Surg*. 1955;141(3):366–368.

9. Heggers JP, Barnes ST, Robson MC, Ristroph JD, Omer GE Jr. Microbial flora of orthopaedic war wounds. *Mil Med*. 1969;134(8):602–603.

10. Kovaric JJ, Matsumoto T, Dobek AS, Hamit HF. Bacterial flora of one hundred and twelve combat wounds. *Mil Med*. 1968;133(8):622–624.

11. Matsumoto T, Wyte SR, Moseley RV, Hawley RJ, Lackey GR. Combat surgery in communication zone. I. War wound and bacteriology (preliminary report). *Mil Med*. 1969;134(9):655–665.

12. Murray CK, Yun HC, Griffith ME, Hospenthal DR, Tong MJ. *Acinetobacter* infection: what was the true impact during the Vietnam conflict? *Clin Infect Dis*. 2006;43(3):383–384.

13. Tong MJ. Septic complications of war wounds. *JAMA*. 1972;219(8):1044–1047.

14. Murray CK, Hinkle MK, Yun HC. History of infections associated with combat-related injuries. *J Trauma*. 2008;64(3 Suppl):S221–231.

15. *Acinetobacter baumannii* infections among patients at military medical facilities treating injured U.S. service members, 2002–2004. *MMWR*. 2004;53(45):1063–1066.

16. Petersen K, Riddle MS, Danko JR, et al. Trauma-related infections in battlefield casualties from Iraq. *Ann Surg*. 2007;245(5):803–811.

17. Wortmann G, Weintrob A, Barber M, et al. Genotypic evolution of *Acinetobacter baumannii* strains in an outbreak associated with war trauma. *Infect Control Hosp Epidemiol*. Jun 2008;29(6):553–555.

18. Zapor MJ, Erwin D, Erowele G, Wortmann G. Emergence of multidrug resistance in bacteria and impact on antibiotic expenditure at a major army medical center caring for soldiers wounded in Iraq and Afghanistan. *Infect Control Hosp Epidemiol*. 2008;29(7):661–663.

19. Adams MD, Goglin K, Molyneaux N, et al. Comparative genome sequence analysis of MDR *Acinetobacter baumannii*. *J Bacteriol*. Dec 2008;190(24):8053–8064.

20. Scott P, Deye G, Srinivasan A, et al. An outbreak of MDR *Acinetobacter baumannii-calcoaceticus* complex infections in the US military healthcare system associated with military operations in Iraq. *Clin Infect Dis*. 2007;44:1577–1584.

21. Davis KA, Moran KA, McAllister CK, Gray PJ. MDR *Acinetobacter* extremity infections in soldiers. *Emerg Infect Dis*. 2005;11(8):1218–1224.

22. Keen EF 3rd, Robinson BJ, Hospenthal DR, et al. Incidence and bacteriology of burn infections at a military burn center. *Burns*. 2010;36(4):461–468.

23. Keen EF 3rd, Robinson BJ, Hospenthal DR, et al. Prevalence of MDR organisms recovered at a military burn center. *Burns*. 2010;36(6):819-825.

24. Sebeny PJ, Riddle MS, Petersen K. *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma. *Clin Infect Dis*. 2008;47(4):444–449.

25. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. *Clin Infect Dis*. 2007;45(4):409–415.

26. Mody RM, Zapor M, Hartzell JD, et al. Infectious complications of damage control orthopedics in war trauma. *J Trauma*. 2009;67(4):758–761.

27. Gleeson T, Petersen K, Mascola J. Successful treatment of *Acinetobacter* meningitis with meropenem and rifampicin. *J Antimicrobial Chemother*. 2005;56(3):602–603.

28. Kortepeter MG, Lemmon JW, Moran KA. A soldier with traumatic brain injury and meningitis. *Clin Infect Dis*. 2005;41(11):1604–1605, 1675–1676.

29. Keen EF 3rd, Murray CK, Robinson BJ, Hospenthal DR, Co EM, Aldous WK. Changes in the incidences of MDR and extensively drug-resistant organisms isolated in a military medical center. *Infect Control Hosp Epidemiol*. 2010;31(7):728–732.

30. Griffith ME, Yun HC, Horvath LL, Murray CK. Minocycline therapy for traumatic wound infections caused by the MDR *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex. *Infect Dis Clin Pract*. 2008;16(1):16–19.

31. Hartzell JD, Neff R, Ake J, et al. Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. *Clin Infect Dis*. 2009;48(12):1724–1728.

32. Schafer JJ, Mangino JE. Multidrug-resistant *Acinetobacter baumannii* osteomyelitis from Iraq. *Emerg Infect Dis*. 2008;14(3):512–514.

33. Petruccelli BP. Investigating Acinetobacter Baumannii Infections at U.S. Army Military Treatment Facilities 27 August 2004 to 27 May 2005. Epidemiological consultation no. 12-HA-01JK-04. Available from: Commander, US Army Center for Health Promotion and Preventive Medicine, ATTN: MCHB-TS-D, Aberdeen Proving Ground, MD 21010.

34. Allen D, Hartman B. *Acinetobacter* species. In: Mandell, Douglas, Bennett, eds. *Principles and Practice of Infectious Disease*. Philadelphia, PA: Churchill Livingstone; 2000: 2339–2344.

35. Petersen K, Cannegieter SC, van der Reijden TJ, et al. Diversity and clinical impact of *Acinetobacter baumannii* colonization and infection at a military medical center. *J Clin Microbiol*. Jan 2011;49(1):159–166.

36. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities. *J Infect Dev Ctries*. 2009;3(5):335–341.

37. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug-resistant *Acinetobacter baumannii*. *Emerg Infect Dis*. Jan 2005;11(1):22–29.

38. El Shafie SS, Alishaq M, Leni Garcia M. Investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii* in trauma intensive care unit. *J Hosp Infect*. 2004;56(2):101–105.

39. Tien HC, Battad A, Bryce EA, et al. Multi-drug resistant *Acinetobacter* infections in critically injured Canadian forces soldiers. *BMC Infect Dis*. 2007;7:95.

40. Howard A, O'Donoghue M, Feeney A, Sleator RD. *Acinetobacter baumannii*: an emerging opportunistic pathogen. *Virulence*. 2012;3(3):243–250.

41. Peleg AY, de Breij A, Adams MD, et al. The success of *Acinetobacter* species; genetic, metabolic and virulence attributes. *PLoS One*. 2012;7(10):e46984.

42. Murray CK, Yun HC, Griffith ME, et al. Recovery of multidrug-resistant bacteria from combat personnel evacuated from Iraq and Afghanistan at a single military treatment facility. *Mil Med*. 2009;174(6):598–604.

43. Rosenbaum P, Aureden K, Cloughessy M, Goss L, Kassai M, Streed SA. *Guide to the Elimination of Multi-Drug Resistant* Acinetobacter Baumannii *Transmission in Healthcare Settings*. Washington, DC: Association for Professionals in Infection Control and Epidemiology; 2010.

44. Munoz-Price LS, Weinstein RA. *Acinetobacter* infection. *N Engl J Med*. 2008;358(12):1271–1281.

45. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev*. 1996;9(2):148–165.

46. Centers for Disease Control and Prevention. PulseNet: Pathogens and Protocols webpage. http://www.cdc.gov/pulsenet/pathogens/index.html. Accessed June 17, 2014.

47. Turton JF, Kaufmann ME, Gill MJ, et al. Comparison of *Acinetobacter baumannii* isolates from the United Kingdom and the United States that were associated with repatriated casualties of the Iraq conflict. *J Clin Microbiol*. 2006;44(7):2630–2634.

48. Ecker JA, Massire C, Hall TA, et al. Identification of *Acinetobacter* species and genotyping of *Acinetobacter baumannii* by multilocus PCR and mass spectrometry. *J Clin Microbiol*. 2006;44(8):2921–2932.

49. Hawley JS, Murray CK, Griffith ME, et al. Susceptibility of *Acinetobacter* strains isolated from deployed U.S. military personnel. *Antimicrob Agents Chemother*. 2007;51(1):376–378.

50. Hujer KM, Hujer AM, Hulten EA, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrob Agents Chemother*. 2006;50(12):4114–4123.

51. Huang XZ, Frye JG, Chahine MA, et al. Genotypic and phenotypic correlations of multidrug-resistant *Acinetobacter baumannii-A. calcoaceticus* complex strains isolated from patients at the National Naval Medical Center. *J Clin Microbiol*. 2010;48(11):4333–4336.

52. 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.

53. Lesho E, Ake J, Huang X, et al. Amount of usage and involvement in explosions not associated with increased contamination of prehospital vehicles with multi-drug-resistant organisms. *Prehospital and Disaster Medicine*. 2013;28(2):107–109.

54. Lesho E, Kraft D, Kirkup BC, et al. Surveillance, characterisation, and preservation of multidrug-resistant bacteria. *Lancet Infect Dis*. 2011;11(1):8–10.

55. Waterman P, Kwak Y, Clifford R, et al. A multidrug-resistance surveillance network: 1 year on. *Lancet Infect Dis*. 2012;12(8):587–588.

56. Haley RW, Culver DH, White JW, et al. The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. *Am J Epidemiol*. 1985;121(2):182–205.

57. Richards C, Emori TG, Peavy G, Gaynes R. Promoting quality through measurement of performance and response: prevention success stories. *Emerg Infect Dis*. 2001;7(2):299–301.

58. Slater F. Cost-effective infection control success story: a case presentation. *Emerg Infect Dis*. 2001;7(2):293–294.

59. Wenzel RP, Pfaller MA. Infection control: the premier quality assessment program in United States hospitals. *Am J Med*. 1991;91(3B):27S–31S.

60. Jones A, Morgan D, Walsh A, et al. Importation of multi-drug resistant *Acinetobacter* spp infections with casualties from Iraq. *Lancet Infect Dis*. 2006;6(6):317–318.

61. Arias KM. *Surveillance Programs in Healthcare Settings*. 2nd ed. Washington, DC: Association for Professionals in Infection Control and Epidemiology; 2009.

62. McQuillen DP, Petrak RM, Wasserman RB, Nahass RG, Scull JA, Martinelli LP. The value of infectious diseases specialists: non-patient care activities. *Clin Infect Dis*. 2008;47(8):1051–1063.

63. Apisarnthanarak A, Fraser VJ. Feasibility and efficacy of infection-control interventions to reduce the number of nosocomial infections and drug-resistant microorganisms in developing countries: what else do we need? *Clin Infect Dis*. 2009;48(1):22–24.

64. Kaye KS, Engemann JJ, Fulmer EM, Clark CC, Noga EM, Sexton DJ. Favorable impact of an infection control network on nosocomial infection rates in community hospitals. *Infect Control Hosp Epidemiol*. Mar 2006;27(3):228–232.

65. McGann P, Hang J, Clifford RJ, et al. Complete sequence of a novel 178-kilobase plasmid carrying bla(NDM-1) in a *Providencia stuartii* strain isolated in Afghanistan. *Antimicrob Agents Chemother*. Apr 2012;56(4):1673–1679.

66. McGann P, Kwak YI, Summers A, Cummings JF, Waterman PE, Lesho EP. Detection of *qacA/B* in clinical isolates of methicillin-resistant *Staphylococcus aureus* from a regional healthcare network in the eastern United States. *Infect Control Hosp Epidemiol*. Nov 2011;32(11):1116–1119.

67. Waterman P, Riley MC, Kwak YI, et al. Lesho. *Comparative Genomics of Serial Isolates: Can Genome Mapping Provide a Road Map?* Paper presented at: 49th Annual Meeting of the Infectious Disease Society of America, 2011; Boston MA.

68. Riley MC, Lee JE, Lesho E, Kirkup BC Jr. Optically mapping multiple bacterial genomes simultaneously in a single run. *PLoS One*. 2011;6(11):e27085.

69. Onmus-Leone F, Hang J, Clifford R, et al. Enhanced de novo assembly of high throughput pyrosequencing data using optical genome mapping. *Plos One*. 2013;8:e61762.

70. McGann P, Milillo M, Clifford RJ, et al. Detection of blaNDM-1 in *Acinetobacter schindleri* during routine surveillance. *J Clin Microbiol*. 2013;51:1942–1944.

71. Sahl JW, Gillece JD, Schupp JM, et al. Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in *Acinetobacter*. *PLoS One*. 2013;8(1):e54287.

72. Snitkin ES, Zelazny AM, Montero CI, et al. Genome-wide recombination drives diversification of epidemic strains of *Acinetobacter baumannii*. *Proc Natl Acad Sci U S A*. 2011;108(33):13758–13763.

73. Wright MS, Haft DH, Harkins DM, et al. New insights into dissemination and variation of the health care-associated pathogen *Acinetobacter baumannii* from genomic analysis. *MBio*. 2014;5(1):e00963.

74. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS One*. 2012;5(4):e10034.

75. Waterman PE, McGann P, Snesrud E, et al. Bacterial peritonitis due to *Acinetobacter baumannii* sequence type 25 with plasmid-borne new delhi metallo-β-lactamase in Honduras. *Antimicrob Agents Chemother*. 2013;57(9):4584–4586.