Antibiotic resistance is a significant hindrance to the preservation of life and limb in the military healthcare setting and is a significant threat to U.S. military personnel. Indeed, during the height of the conflicts in Iraq and Afghanistan, there was an increase in wound infections caused by multi-drug resistant organisms. Multi-drug resistant organisms are linked to an increase in the morbidity and mortality associated with wound infection and a concomitant increase in the economic burden of wound care treatment. The effect of multi-drug resistant organisms on the military healthcare system was dramatic between 2002 and 2004 when 102 patients being treated in the military health care system were found to have multi-drug resistant Acinetobacter baumannii blood stream infections. Further investigation revealed that the majority of these patients (85%) were injured in association with combat operations in Afghanistan, Iraq, or Kuwait. Infections with this organism were reported at two major military medical centers and onboard a U.S. Navy hospital ship. At one point, it was reported that 15 to 20% of wounded servicemembers returning from Iraq and Afghanistan were infected with an antimicrobial resistant organism.

To counter the threats posed by antimicrobial resistant organisms to U.S. servicemembers, it is necessary to determine which organisms are currently resistant to antimicrobial therapy, which organisms could become resistant, and where reservoirs of resistant organisms are located. Currently, most clinical laboratories identify resistant organisms by phenotype. In other words, they isolate bacteria from the patient and cultivate the isolate in the presence of various antibiotics. In this way, antimicrobial agents capable of inhibiting the growth of the isolate can be identified. Conversely, this method also reveals those agents that do not inhibit the growth of the isolate which gives an indication of the inherent level of resistance.

One problem of relying on phenotypic methods for identifying antimicrobial resistance is the fact that this method only identifies organisms that are resistant in the laboratory setting. It is well known that bacteria tend to adapt to the host and will display altered properties when compared with laboratory-grown strains. Another problem with utilizing phenotypic methods is the fact that these methods only identify the current state of the isolate and cannot be used to predict the emergence of resistance. To mitigate these shortcomings, the team utilized funding from TATRC’s AAMTI Program to leverage past Department of Defense funding (initially by the Defense Medical Research Program and Defense Threat Reduction Agency) into the development of an antimicrobial resistance microarray or ARDM at the Naval Research Laboratory by evaluating ARDM version 2 (ARDMv.2) in the clinical microbiology laboratory at Tripler Army Medical Center (TAMC). The ARDMv.2 is a small nucleic acid array printed onto a glass slide containing probes for over 200 antimicrobial resistance genes. Nucleic acids extracted from an isolate is incubated with the array allowing resistance genes to bind to the probes. After a brief detection step, the results are read using a portable electrochemical detector and analyzed using a commercial off-the-shelf laptop.

100 isolates of Escherichia coli were examined using this method and found that the majority of antibiotic resistant strains in this study, harbored a gene known as tem-1 that encodes for an enzyme called a beta-lactamase. Beta-lactamases are enzymes capable of degrading antibiotics derived from penicillin. The presence of this gene indicates that whether or not these isolates were able to grow in the presence of penicillin in the laboratory, they are capable of degrading penicillin and thus may be resistant in the patient. This information was not previously available in the clinical laboratory and could potentially help guide treatment decisions. This information can also help track the emergence and spread of antibiotic resistant organisms since a shift in the dominant genes can indicate the introduction of new strains, or a decline in the numbers of previously detected strains. Results with this system can be obtained in as little as 24 hours after the isolation of the organism, and with minimal laboratory staff and a simplified data analysis procedure.

In 2015, TATRC’s AAMTI Program funded the evaluation of the ARDMv.2 at TAMC. This project has the potential to significantly reduce the human and financial costs associated with antimicrobial resistance in the military health system. Future research will involve evaluating this system at DoD overseas laboratories and exploring the possibility of integrating it into the clinical laboratory workflow. “This project has the potential to significantly reduce the human and financial costs associated with antimicrobial resistance in the military health system,” said Principal Investigator, MAJ Michael Washington, PhD from TAMC.