The emergence of antibiotic resistant organisms pose a significant public health concern. The prevalence of antibiotic resistance especially in Gram-negative bacteria continues to increase globally. Since the beginning of Operation Iraqi Freedom and Operation Enduring Freedom, the U.S. military healthcare system has experienced a notable increase in the number of antibiotic-resistant organisms being isolated from wounded Service Members. Although U.S. military involvement in the Middle East is decreasing, Service Members deploy to areas all around the globe where unique antibiotic resistance exists. Exposures may not necessarily lead to infection, but military personnel could carry these organisms home and potentially spread to others. Rapid, simple, cost-effective, and accurate resistance detection tests are critical for antibiotic stewardship and combating the development of antimicrobial resistance, ultimately improving patient care and maintaining the health and wellness of military members and their families.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is used in a variety of microbiology applications. In clinical microbiology, MALDI-TOF MS has revolutionized the identification of bacteria from a matter of hours or days to just minutes. The instrument is coupled to software which compares generated spectra to profiles stored in a reference library and uses an algorithm to produce an organism determination with a score / confidence level. MALDI-TOF MS is routinely employed by many diagnostic laboratories including military treatment facilities (MTFs). The use of MALDI-TOF MS as a cost-effective and rapid technology to determine antibiotic resistance and mechanisms of resistance within a matter of hours has started to gain attention, and a number of promising approaches have been documented in studies to date. Currently, most diagnostic laboratories employ traditional microbiological methods or automated instruments that can take up to 24 hours to provide antibiotic susceptibility information. The development and standardization of rapid and accurate methods to provide faster results, lower costs, and reduce labor requirements are much needed.

With TATRC’s Advanced Medical Technology Initiative (AMTI) funding and support of this project, investigators at Tripler Army Medical Center (TAMC) evaluated two unique antibiotic resistance determination assays using the VITEK MS (bioMérieux, Durham, NC) MALDI-TOF instrument shown in Figure 1 for prediction of bacterial antibiotic susceptibility. The Principal Investigator, CPT Tim Horseman explained that “this study is the first to demonstrate antibiotic susceptibility determination using the VITEK MS manufacturer recommended clinical FDA-approved instrument run settings.” Ultimately, the suggested methodological strategy eases the implementation, validation, and routine use of these assays in diagnostic laboratories.

This study employed VITEK MS to evaluate a total of 140 isolates, with equal numbers of antibiotic sensitive and resistant strains for the following four clinically significant bacterial pathogens: Methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum beta lactamase (ESBL) E. coli and carbapenemase resistant Klebsiella (KPC). These bacteria were selected due to their high resistance rates and their role in nosocomial infections. With TATRC’s funding and support, investigators at Tripler Army Medical Center (TAMC) evaluated two unique antibiotic resistance determination assays using the VITEK MS (bioMérieux, Durham, NC) MALDI-TOF instrument shown in Figure 1 for prediction of bacterial antibiotic susceptibility. The Principal Investigator, CPT Tim Horseman explained that “this study is the first to demonstrate antibiotic susceptibility determination using the VITEK MS manufacturer recommended clinical FDA-approved instrument run settings.” Ultimately, the suggested methodological strategy eases the implementation, validation, and routine use of these assays in diagnostic laboratories.

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used to predict antibiotic non-susceptibility. The extraction method required a number of centrifugation steps and washes before the final solution was applied to the VITEK MS target slide. For the DOT-MGA, a microdroplet of each prepared suspension was pipetted to a VITEK MS target slide (Figure 2). The microdroplets were incubated directly on the target slide in a box with water added to the bottom of the box to avoid evaporation and to provide a humid environment for incubation. After incubation, the liquid broth was carefully ‘wicked’ away from the bacterial cells by touching a wipe to the backside of the microdroplet (Figure 2). Sensitive or non-susceptible interpretations were based on the standard clinical VITEK MS software confidence levels intended for identification of organisms. For bacteria treated with antibiotic, non-susceptible interpretation was a score of ≥ 90%. Conversely, a non-identification on the VITEK MS was interpreted as a susceptible result. Growth controls with no antibiotic were tested for each bacterial strain and required a successful identification, ≥ 90%, for the tests to be deemed valid (Figure 3).

The liquid extraction method and DOT-MGA proved to be reliable assays for K. pneumoniae, E. coli, and S. aureus isolates providing consistent differentiation between non-susceptible and susceptible strains. The liquid extraction method for the Gram-positive and Gram-negative bacteria tested allows for a shorter incubation time, 2-3 hours, than the DOT-MGA, 4-5 hours. The extraction method is advantageous because it seems to be more consistent over a wider range of organisms with inclusion of concentration and cell lysis steps. Extracts can also be stored at -80°C for reference or retest if applicable. The DOT-MGA offers a less laborious, and more cost-effective method for antibiotic resistance determination on VITEK MS than the extraction assay. It also minimizes the opportunity for human error since there are less steps and technician hands-on time. Both methods are equally as sensitive and specific, the promise of each lies in their adaption for clinical diagnostics.

Overall, the extraction assay and DOT-MGA can provide necessary information to clinicians prior to the release of an organism’s full susceptibility profile and within the same day from isolated colonies. Further investigation and standardization of assays with the VITEK MS are important moving into the future. Testing regionally and genetically diverse isolates should be performed to challenge the reliability of the profiling methods suggested in this study. Future enhancements to the assays most notably include assay automation allowing more consistent and rapid sample processing. Results from this study support VITEK MS and these assays as rapid and accurate tools to augment traditional susceptibility testing. Furthermore, these results lay the foundation for a larger, multi-site study to determine feasibility of implementation across MTFs. If implemented clinically, these assays can reduce the cost of patient care and the time to deliver critically needed treatment.