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The Current and Future State of Department of Defense (DoD) Microbiome Research: a Summary of the Inaugural DoD Tri-Service Microbiome Consortium Informational Meeting

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ABSTRACT The Tri-Service Microbiome Consortium (TSMC) was recently established to enhance collaboration, coordination, and communication of microbiome research among Department of Defense (DoD) organizations. The TSMC aims to serve as a forum for sharing information related to DoD microbiome research, policy, and applications, to monitor global advances relevant to human health and performance, to identify priority objectives, and to facilitate Tri-Service (Army, Navy, and Air Force) collaborative research. The inaugural TSMC workshop held on 10 to 11 May 2017 brought together almost 100 attendees from across the DoD and several key DoD partners. The meeting outcomes informed attendees of the scope of current DoD microbiome research efforts and identified knowledge gaps, collaborative/leveraging opportunities, research barriers/challenges, and future directions. This report details meeting presentations and discussions with special emphasis on Tri-Service labs' current research activities.

KEYWORDS Department of Defense, TSMC, Tri-Service Microbiome Consortium, Warfighter, bioinformatics, health, microbiome, performance

The Department of Defense (DoD) microbiome research community emerged from a recent workshop (1) with unanimous consent for the development of a consortium to promote coordination across all three Services within the DoD. In December 2016, the Tri-Service Microbiome Consortium (TSMC) was established with the purpose to facilitate collaboration, coordination, and communication of microbiome research among DoD organizations and to serve as a forum for the sharing of microbiome-related resources, materials, and information for the benefit of DoD consortium members. The key activities of the TSMC include monitoring global advances in microbiome research relevant to human health and performance, identifying priority health and performance objectives where microbiome research may provide a solution, communicating potential scientific implications and impacts of DoD microbiome research, and assisting all members of the DoD microbiome research community to identify available resources, including experimental capabilities, bioinformatic tools, and funding opportunities. Toward these activities, a primary goal of the TSMC is to host an annual

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The Tri-Service Microbiome Consortium inaugural meeting report describes current DoD microbiome research efforts and outlines the substantial interest within the DoD for extending knowledge of human and environmental microbiomes to benefit Warfighters

workshop to foster collaboration and share information related to DoD microbiome research, policy, and applications among subject matter experts from federal/state agencies, academic institutions, and industry. After each annual workshop, highlights, results, and collective recommendations will be incorporated into an annual report documenting research activities and strategies of the member agencies. The inaugural workshop was held on 10 to 11 May 2017 and consisted of the following five sessions: (i) Warfighter performance; (ii) biological engineering and synthetic biology; (iii) environmental microbiomes; (iv) bioinformatics, data processing/management and standards; and (v) ethical, legal, policy, and social implications. This report details the presentations and discussions from the inaugural meeting with special emphasis on the Tri-Service laboratory activities. A brief synopsis of the research interests and roles of all speakers and organizational affiliations is presented in Table 1. Below, the compiled summaries provide more specific presentation details.

OPENING REMARKS

Linda Chrisey, Office of Naval Research (ONR), and Blair Dancy, U.S. Army Center for Environmental Health Research (USACEHR), coorganizers of TSMC Inaugural Workshop, gave opening remarks. They highlighted the TSMC mission to enhance DoD collaboration and leverage microbiome activities outside the DoD. Envisioned products include an annual workshop, awareness of the state of the art, identification of research gaps, annual reports, and data call reports monitoring microbiome activities. Lita Proctor, Program Director of the Human Microbiome Project at the National Institutes of Health (NIH), gave a plenary lecture on the Human Microbiome Project, which has catalyzed much of the microbiome work done in the federal government, and described NIH resources for the entire microbiome community, including data sets, computational tools/pipelines, and institutional review board (IRB)/clinical protocols. Discussion topics included the Trans-NIH Microbiome Working Group, a central resource to the external community to improve communication related to NIH microbiome research, the White House Office of Science and Technology Policy Fast-Track Action Committee on Mapping the Microbiome, instituted to monitor microbiome research across the federal government (2), and the May 2016 establishment of the National Microbiome Initiative to develop a federal strategic plan for future microbiome research (3).

SERVICE SPEAKER SUMMARIES

(i) U.S. Navy. Sarah Glaven, Naval Research Laboratory–Washington, DC (NRL-DC), described a multi-omics approach to study electrochemically active biofilms that convert carbon dioxide to organic carbon using energy from a charged electrode surface. The primary autotroph of one model system is uncultivable outside the electrode biofilm, highlighting the need for cultivation-free approaches. Data generated can be used to mine for genetic components of electron transfer pathways in order to design and engineer direct electron transfer between cells and electrodes. Dagmar Leary described the mass spectrometry facilities at NRL-DC, which processes proteomic and metabolomic samples from pure cultures and microbiomes. Experimental designs for targeted and untargeted metabolomic experiments were discussed, as well as an active study exploring the extracellular metabolome of a marine bacterium to increase understanding of bacterial communication and quorum sensing and a study on the intracellular metabolome of melanized fungi to understand the response and mechanism of resistance to radiation. The bottlenecks of current metabolomic approaches and the need for automated and high-throughput data analysis were also discussed.

(ii) U.S. Army. Several speakers discussed Army microbiome research programs as related to environmental exposures. Blair Dancy described efforts aimed at ultimately using human microbiomes as surveillance tools for environmental exposures, highlighting recently completed work that identified gut microbiome signatures of heavy metal exposures in rodents. Jun Hang, Walter Reed Army Institute of Research (WRAIR)–

TABLE 1 TSMC DoD organizations and strategic partners general interests in microbiome research

Organization(s)	Microbiome research interest(s)	Presenting researcher(s)
U.S. Army Center for Environmental Health Research (USACEHR)	Gut microbiome as surveillance for toxicant exposure using systems biology, rodent models, and humanized-mouse model; gut microbiome to enable precision medicine, such as gut-brain axis, and developing computational tools; stress markers in mice associated with changes in microbial community	Rasha Hammamieh, Blair Dancy, Aarti Gautum
U.S. Army Research Institute of Environmental Medicine (USARIEM)	Nutrition-based strategies targeting the gut microbiome for optimizing Warfighter health and performance; microbiome changes related to physiological stressors such as undernutrition and altitude exposure	J. Philip Karl
US Army Natick Soldier Research Development & Engineering Center (NSRDEC)	Employ *in vitro* fermentation models to characterize and understand how specific military-relevant stressors influence the delicate balance within a healthy gut microbiome; understand complex microbial community competitive dynamics	Jason W. Soares
U.S. Air Force Research Laboratory (AFRL)	Pulmonary health biomarker discovery; synthetic biology solutions to microbiome health and Warfighter performance; sensors for DoD-relevant molecules such as trinitrotoluene (TNT); microbiomes related to fouling and corrosion in fuel storage systems	Camilla A. Mauzy, Nancy Kelley-Loughnane, Wendy J. Goodson
Rocky Mountain Mental Illness Research, Education and Clinical Center (MIRECC), Denver Veterans Affairs (VA) Medical Center	Advancing microbiome science and education to benefit military personnel through the Military and Veteran Microbiome Consortium for Research and Education (MVM-CoRE); chronic inflammatory disease and the built environment	Lisa A. Brenner
Johns Hopkins University Applied Physics Laboratory (JHU-APL)	Inter- and intraspatial microbial community relationship within the skin microbiome; skin probiotics for vector protection	David Karig
Walter Reed Army Institute of Research (WRAIR)	Surveying the respiratory microbiome of healthy military personnel	Jun Hang
US Army Medical Research and Materiel Command (MRMC)	Ethical considerations for microbiome analysis; congressionally directed research program support	Donna M. Kimbark, Natalie Klein
Uniformed Services University of the Health Sciences (USUHS)	Multi-body site microbiome analysis (respiratory, skin, and wound) to investigate skin and soft tissue infections in military trainees	D. Scott Merrell
Massachusetts Institute of Technology Lincoln Laboratory (MIT-LL)	*In silico* and *in vitro* gut models for microbiome research, specifically interested in oxygen diffusion in the mucous layer	Catherine R. Cabrera
U.S. Naval Research Lab (NRL)	Synthetic biology for DoD-relevant environmental microbiomes, including energy generation and respiratory microbiomes; proteomics and metabolomics of DoD-relevant environmental microbiomes	Sarah (Strycharz) Glaven, Dagmar H. Leary
U.S. Army Corps of Engineers Environmental Research and Development Center (ERDC)	Molecular markers for toxicants in soil microbiomes; effects of contaminants on skin microbiome using lizard and amphibian models; stochastic models of soil microbiomes coupled to terrain and weather data to evaluate soil health	Karl J. Indest, Robyn A. Barbato
U.S. Air Force Academy	Bacterial and fungal microbiome of the built environment for human health	Andrew J. Hoisington
Office of Naval Research (ONR)	Bioscience program to study human microbiomes and effects on Warfighter resilience and readiness; ethical, legal, and social implication considerations for microbiome research; funding DoD/Navy-relevant research	Linda A. Chrisey, Eric Fried
Army Research Office (ARO)	Microbiology to understand complex community dynamics, engineering of microbial communities and modeling bidirectional gut-brain axis	Robert J. Kokoska
Army Public Health Center (APHC)	Determining the role of microbiome health in risk assessment policy	Laurie E. Roszell
National Institutes of Health (NIH)	Human Microbiome Project to explore role of endogenous microflora in human health; integrative systems biology bioinformatic platform for microbiome discovery studies; microbiota and drug metabolism to combat resistant wound infections	Lita M. Proctor, Linda Duffy, Uma Mudunuri

Viral Disease Branch, discussed a study that characterized the upper respiratory microbiome of military personnel to derive microbiome profiles for association studies during occupational and geographical exposures that may increase risk of lung disease. Karl Indest from Environmental Research and Development Center (ERDC)–Environmental Microbiology Team presented work focused on determining the feasibility of using soil, reptile, and amphibian microbiomes as surveillance tools for environmental contamination. Finally, Robin Barbado, ERDC–Cold Regions Research and Engineering Laboratory, detailed efforts using environmental data to model activity in the soil microbiome, information useful for applications such as bioremediation or energy harvesting using microbial fuel cells.

The remaining Army speakers focused on the gut microbiome as a mediator of Warfighter health and performance. Rasha Hammamieh and Aarti Gautam presented ongoing research within the integrative systems biology program at USACEHR. The program was described as a "disease agnostic" effort utilizing multi-omic and bioinformatic approaches to elucidate interrelationships between military-relevant environments and health outcomes and to identify biomarkers of exposure, illness, and disease. Aarti Gautam elaborated by presenting recent findings demonstrating alterations in the fecal microbiome in a rodent model of post-traumatic stress disorder (PTSD) (4). J. Philip Karl discussed human research efforts within the U.S. Army Research Institute of Environmental Medicine (USARIEM)–Military Nutrition Division that aim to characterize the impact of military stressors on the Warfighter gut microbiome and to identify nutritional strategies for targeting the microbiome to improve performance. He highlighted recently completed studies demonstrating that stressors can have deleterious effects on intestinal barrier integrity, which may be associated with gut microbiota composition and metabolism (5). Jason Soares, Natick Soldier Research Development & Engineering Center (NSRDEC), expanded on that concept, describing how *in vitro* gut fermentation models simulating the human colon can extend results of human studies by efficiently interrogating nutrient-microbiome interactions. Jason Soares concluded by highlighting challenges and considerations for transitioning research findings to food products in the field for the Warfighter.

(iii) U.S. Air Force. Camilla Mauzy, 711th Human Performance Wing (HPW), primarily discussed an effort to discover lung microbiome biomarkers as a function of military-relevant airborne particle exposure and highlighted the potential of a lung probiotic concept. She closed with the future direction of her work on lung tissue imaging, gut organ *in vitro* model development, and alternative animal models of interest. Nancy Kelley-Loughnane, 711th HPW, described DoD interest in synthetic biology to engineer microbiomes relevant for the operational environment, including Tri-Service joint research efforts within the Synthetic Biology for Military Environments (SBME) Applied Research for the Advancement of Science and Technology Priorities (ARAP) program. She further described efforts aimed at developing cell-based stress sensors to couple with an engineered stress relief response by gut microbes. Wendy Goodson presented environmental microbiome research by the Biological Materials and Processing Research Group on microbial communities associated with increased corrosion in storage tanks with biodiesel blends in order to develop and implement fouling mitigation strategies. Andy Hoisington, Air Force Academy, described his joint work with Mental Illness Research, Education and Clinical Center (MIRECC) conducting longitudinal built environment studies performed on cadets' living spaces monitoring human microbiome changes and salivary cortisol levels with results correlated to indoor air quality measurements. Andy Hoisington also highlighted sampling challenges and biases that need oversight to avoid influencing results.

DoD AFFILIATES AND PARTNERS

Linda Duffy, NIH, described using omics to characterize microbiota drug metabolism to combat resistant wound infections and develop diagnostic and therapeutic tools to improve wound treatment for point-of-care diagnostics and to accelerate precision in antibiotic use decisions in field and clinical settings. Uma Mudunuri, National Cancer Institute (NCI), NIH, described the development of SysBioCube, a bioinformatic platform capable of analyzing and visualizing results from microbiome meta-omics experiments. SysBioCube contains data from genomic, proteomic, and metabolomic analyses and currently supports collaborative studies on PTSD, coagulopathy, cancer, and plague. Lisa Brenner, MIRECC and Veterans Affairs (VA), discussed traumatic brain injury and PTSD and correlation with the gut microbiome as an underlying link to build resiliency in psychiatric disorders in veterans (6, 7). She also discussed health-related outcome measurements for translating human microbiome research to clinical practice. Scott Merrell, Uniformed Services University of the Health Sciences (USUHS), presented the role of the microbiome in skin and soft tissue infections (SSTI) at basic training.

Correlative studies tracked development of SSTI and determined risk factors by characterizing skin microbiomes from various body sites (8–10). David Karig, Johns Hopkins University Applied Physics Laboratory (JHU-APL), presented a study examining interpersonal and intrasite variation in skin microbiome. He employed small-scale spatial sampling to explore diversity as a function of skin layer depth to understand microbial dynamics that are needed for maintaining skin health and homeostasis. Catherine R. Cabrera, Massachusetts Institute of Technology Lincoln Laboratory (MIT-LL), discussed an artificial gut to model the mucosal layer oxygen gradient and simulation of mucin secretion.

PROGRAM MANAGER PANEL

Donna Kimbark, Program Manager of the Congressionally Directed Medical Research Program (CDMRP), Medical Research and Materiel Command (MRMC), gave an overview of the CDMRP program cycle and funding opportunities. Robert Kokoska, Program Manager of the Microbiology Program, Army Research Office (ARO), provided an overview of his research thrust area involving analysis and engineering of microbial communities, including two Multidisciplinary University Research Initiatives (MURIs) to understand (i) the skin microbiome and (ii) develop a causative model to understand the gut-brain axis. Linda Chrisey, Program Manager of the Naval Biosciences and Biocentric Technologies Program, ONR, described efforts studying microbiomes and effects on Warfighter resilience and readiness. These efforts included elucidating stress effects on the host or microbiome, the role of the microbiome in the host stress response, and how microbiome engineering can mitigate dysbiosis.

ETHICAL, LEGAL, POLICY, AND SOCIAL IMPLICATIONS

Natalie Klein, MRMC, discussed ethical, legal, and social implications for consideration within microbiome research that may result in personalized medicine, medical countermeasures, performance optimization and enhancement, environmental protection, or implications for use within forensic sciences. Eric Fried, ONR, described practical considerations for recruiting military subjects for microbiome research as well as dealing with their chain of command. Last, Laurie Roszell, Army Public Health Center (APHC), discussed a recent workshop to outline strategies to support integration of microbiome-associated data into health risk assessments.

TAKEAWAYS AND CONCLUSIONS

The TSMC inaugural meeting supported a forum for information exchange to connect DoD scientists and key partners to facilitate collaborative research, share resources, and discuss recent advances in human and environmental microbiome research. The presentations and discussions during the inaugural workshop demonstrated that the DoD community is actively pursuing research uniquely related to defense mission areas such as the following: employing microbiomes as diagnostic, surveillance, and treatment tools; military-relevant stress-mediated microbial/host responses; complex microbial community engineering; developing predictive community population and response models; built environment; integrative multi-omic strategies and translating knowledge to Warfighter solutions. The meeting informed attendees of the breadth and scope of current DoD microbiome research efforts, which will improve research coordination and resource sharing, and identified knowledge gaps across human and environmental microbiomes. Collaborative opportunities and avenues to leverage techniques and capabilities were identified. Resource challenges and technical, programmatic, and translational barriers were discussed, and future directions/priorities were initiated that will balance Service-specific interests while identifying overlapping, critical knowledge for DoD-wide leveraging (e.g., bioinformatic frameworks). Attendees agreed that having an annual TSMC workshop and more targeted topic workshops hosted by specific Tri-Service labs would be beneficial to coordination of DoD microbiome research and facilitate focused technical discussions. Attendees also expressed consensus for expanded academic engagements, including incorpora-

tion of key academic partners into future meetings. In addition, continued/future interaction with overarching programs such as ARAP, CDMRP, MURIs, and NIH, among others were identified as critical for advancing microbiome research to meet the full potential of this rapidly emerging, promising field.

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