

Department of Defense Microbiome Research: A Summary of the Second Annual DOD Tri-Service Microbiome Consortium Informational Meeting

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Abstract

The United States Department of Defense (DOD) Tri-Service Microbiome Consortium (TSMC) was chartered in December 2016 by the U.S. Office of the Assistant Secretary of Defense for Research and Engineering to enhance collaboration, coordination, and communication of microbiome research among 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. This report details the outcomes from the second annual TSMC workshop, held 8-10 May 2018, in Bethesda, MD.

Introduction

The Department of Defense (DOD) microbiome research community emerged from a workshop at the Natick Soldier Research, Development, and Engineering Center in 2015 with a unanimous consensus for the development of a consortium to promote coordination of microbiome research efforts across all three Service Components within the DOD [1]. On 6 December 2016, the Office of the Undersecretary of Defense for Research and Engineering [OUSD(R&E), then Office of Assistant Secretary of Defense (OASD(R&E)); Director, Human Performance, Training and Biosystems Directorate] chartered the Tri-Service Microbiome Consortium (TSMC). The purpose of the TSMC is 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 all DOD members. The inaugural TSMC workshop was held on 10-11 May 2017 and consisted of five sessions, including (1) warfighter performance; (2) biological engineering and synthetic biology; (3) environmental microbiomes; (4) bioinformatics, data processing/management, and standards; and (5) ethical, legal, policy, and social implications [2]. The second TSMC workshop, reported here, was held on 8-10 May 2018, in Bethesda, MD, and consisted of five sessions, including: 1) warfighter performance; 2) warfighter protection; 3) experimental approaches and data processing; 4) environmental microbiome research; and 5) moderated discussions (models systems, “omics,” bioinformatics, and human studies). The overall attendance and number of federal and non-federal organizations participating has increased since the 2015 workshop (Figure 1). A brief synopsis of the research interests of all speakers, including organizational affiliations, is

presented in Table 1. Compiled summaries that provide more specific details on presentations and moderated discussions are given here.

Opening Remarks

Dr. Linda Chrisey, Office of Naval Research (ONR), and Dr. Nancy Kelley-Loughnane, Air Force Research Laboratory (AFRL), provided opening remarks. As Chair of the TSMC, Dr. Chrisey provided a programmatic overview of TSMC activities. She highlighted the TSMC mission to enhance DOD collaboration and leverage microbiome activities outside of the DOD. Of particular interest to the community were the manuscript publications of the first DOD Microbiome Meeting in 2015 [1] and the inaugural TSMC meeting in 2017 [3]. Dr. Chrisey discussed TSMC activities, including its participation in the Microbiome Interagency Working Group [2] and contributions to the Interagency Strategic Plan for Microbiome Research, FY 2018-2022, that was released in April 2018 [4]. She concluded with announcements of the Army Research Office award of a Multidisciplinary University Research Initiative (MURI) project and other collaboration efforts. Dr. Nancy Kelley-Loughnane provided an overview and update on the OUSD(R&E), Biotechnologies for Health and Performance Council (BHPC), which aims to bring together DOD scientists, policymakers, and operational end users interested in identifying and developing biotechnology solutions for the DOD's human health and performance issues.

Service Speaker Summaries

U.S. Army Overview

Dr. Rasha Hammamieh, Program Director, Integrative Systems Biology Program (ISBP), at the U.S. Army Center for Environmental Health Research (USACEHR), gave an overview of Army research activities. She introduced the U.S. Army Engineer Research and Development Center (ERDC) laboratory that focuses on environmental microbiome research and microbiome profiling in reptiles and amphibians after chemical exposures, permafrost microbiome diversity, and the use of the microbiome as a bioremediation tool. Also discussed was the U.S. Army Research Institute of Environmental Medicine (USARIEM), Military Nutrition Division, which focuses on the gut microbiome in response to military-relevant stressors, as well as nutrition-based strategies to enhance physical and cognitive performance. Other efforts described included the profiling of the microbiome during severe sleep restriction, which may influence warfighter energy balance during sustained operations. The U.S. Army Combat Capabilities Development Command - Soldier Center (CCDC-SC) collaborates with USARIEM to investigate the influence of the gut microbiome on warfighter health and performance. The CCDC-SC microbiome research focuses on integrating bacterial ecology, intestinal physiology, and human nutrition research with the intention to a) characterize military-relevant stressors on gut microbiota, b) explore the fundamental mechanisms of symbiosis, c) assess microbiota changes on warfighter performance, and d) recommend scientifically validated nutritional interventions to mitigate dysbiosis.

The USACEHR's efforts focus on extending the characterization of the host microbiome in response to stressors of military relevance, such as psychological stress, chemical exposure, microgravity, and toxicant exposures. USACEHR's efforts also involved studies on the effect of microgravity on the gut microbiome and the gut-brain-axis using mice from the National Aeronautics and Space Administration (NASA) Rodent Research 4 Project [5], where mice samples were retrieved after residing on the International Space Station for 21 days. Understanding the molecular mechanisms involved in the process of bone healing in space will improve treatments for patients suffering from various injuries. In addition, research at USACEHR focuses on studying changes in the microbiome that modulate toxicity and microbiome engineering to improve performance and mitigate adverse health effects from environmental toxicants. The USACEHR team was also developing data analytics platforms for metagenomics sequencing, as well as *in silico* metabolite annotation architecture using a central portal called "SysBioCube," which serves to facilitate data collection, integration, mining, and file sharing [6].

Dr. J. Philip Karl from USARIEM discussed human subject research efforts on gut permeability in operational environments. His earlier published work on Arctic training in young adults has shown metabolism and intestinal microbiota changes corresponded with increased intestinal permeability [7]. He discussed studies conducting controlled-diet experiments at sea level and high altitude. The research addressed unintentional weight loss at very high altitude, historically attributed to loss of appetite and increased physical activity. The hypothesis was that feeding a higher protein diet would preserve muscle mass. The use of a tightly controlled diet with altered protein and similar carbohydrate intake

was investigated. The preliminary data suggest that altitude had a higher influence on gut microbiome than diet, and that diet may induce stressor-dependent compositional changes and enhance Soldier performance.

Scientists from the CCDC-SC gave multiple presentations. Jason Soares focused on exploring the use of *in vitro* batch fermentation reactors and human fecal samples to study functional dynamics of metabolic processing by gut microbiota. The CCDC-SC team is able to generate a multi-dimensional time- and dosage-dependent dataset studying the functions and processes of the bacterial community. Preliminary data of the cranberry A-type proanthocyanidins prebiotic potential generated from *in vitro* studies showed differences in abundance ratios after fermentation. Sarah Pearce discussed the interface in the form of intestinal mucin layers and focused on *ex vivo* approaches using three-dimensional intestinal organoids that mimic intestinal structure and function. These organoids produce cytokines, making them a relevant model and testing bed. Both of these presentations support the hypothesis that nutritional interventions may prove useful as a potential mitigation strategy to improve performance/health. Kenneth Racicot's presentation focused mainly on regulatory guidelines for "microbiota-directed food" and identification of transition points in DOD research. He outlined the FDA categories/classifications and database for food additives where live microbial cultures are Generally Recognized As Safe (GRAS). He emphasized live bio-therapeutics require premarket review, and the intended use of the probiotic will govern the regulatory category or categories that to which the FDA will assign the product, and thus the FDA office responsible for oversight.

Dr. Derese Getnet, Major (MAJ), U.S. Army, and Dr. Aarti Gautam presented ongoing research within the Integrative Systems Biology Program at USACEHR. MAJ Getnet discussed an experiment of temporal, low-dose exposure to the nerve agent soman in a mouse model, how it alters the gut microbiome, and how it can help with diagnosing asymptomatic patients [8]. The microbial health index for clinically relevant bio-specimens and metabolites may be used to determine nerve agent exposure and to design wearable technology in the future. Dr. Gautam's presentation emphasized the impact of Western diets on psychological trauma and traumatic brain injury in animal models. Both of these presentations focused on a multi-assay approach to reveal connections between military-relevant environments and health outcomes, and to identify biomarkers of exposure, illness, and disease [9-11]. Dr. John Lewis of USACEHR spoke on gut microbiome changes during toxic chemical exposures and the possibility of using the microbiome for environmental health surveillance. He described the use of humanized-gut-microbiota mice in a germ-free facility to study the effects of various toxicants. He noted that there are seasonal and individual variations in the donated human microbiome, and that it is important to study multiple human donors and carefully examine the signal-to-noise in the data.

Dr. Robyn Barbato from Cold Regions Research and Engineering Laboratory talked about the application of soil microbiome data to address Army problems with weapons range sustainability and remediation at an Alaska military

installation. She takes a systems-based approach by combining lab and field measurements to understand how climate change impacts soil microbes, an important consideration for ongoing military operations. Her presentation was focused on continuously frozen grounds (permafrost) and replicating the conditions of permafrost thaw to measure the microbiome, as well as microbiota composition. It is important to fill this knowledge gap to enable robust predictions of changes in ecosystem functions by studying the metabolic functions of the microbiome communities and soil stability.

Dr. Chris Sund from U.S. Army Research Laboratory presented the laboratory's efforts aiming to repurpose the gut microbiota for chemical production. Their work focuses on microbial components of the human gut that are already adapted to a food-waste stream and can be exploited for commodity chemical production. Their model analysis predicted that every organism benefited from interactions with another microbe, as revealed by increased biomass fluxes in co-culture versus monoculture. This indicated that microbiome combinations, including bacterial and fungal species, are enriched for mutualistic and commensal interactions. This approach can be an alternative to metabolic engineering.

U.S. Air Force Overview

Dr. Michael Goodson from the AFRL, 711 Human Performance Wing (711 HPW), in collaboration with Dr. Mark Simons, Lieutenant Commander, U.S. Navy, from the Navy Medical Research Center, discussed alterations in the microbiome population and the impact on deployment-related diarrhea. Deployment-associated diarrhea can afflict over 50% of deployed personnel, with the majority experiencing multiple episodes [12]. Dr. Goodson outlined a project

studying gut microbiome before, during, and after deployment, as related to gut microbial composition and diarrheal onset. Stool samples and daily real-time electronic surveys relating to diet, exercise, sleep, and health were collected from personnel deployed to Honduras. Microbial diversity was assessed using 16S rRNA analysis. The ultimate goal of the project is the identification of microbiome components protective against diarrheal infection and possible prophylactic treatment.

Dr. Camilla Mauzy from the AFRL,711 HPW, discussed ongoing efforts in examining the influence of inhalation exposures on lung the microbiome [13]. She described a possible *Lung Microbiome Dysbiosis- Inflammatory Cycle*, modified from Dickson et al. [14], in which inhaled toxins, volatile organic compounds, or particulate matter could initiate host pathogenesis using two possible mechanisms: 1) interaction with the epithelium and/or mucosal layer that modifies the local growth environment and thus increases select bacterial strains, and 2) direct and selective alterations of commensal bacterial growth. In both cases, microbial signal outputs could exacerbate the lung disease by modulating the immune response. Dr. Mauzy discussed data from an *in vivo* inhalation exposure study of sand and/or burn pit emissions in which lung lavage microbiome populations were analyzed using 16S rRNA sequencing. Emissions significantly altered the lung microbiome when compared to sand or controls, and bacterial diversity shifts continued at least 90 days post-exposure.

The development of *in vitro* models of the gut microbiome, as well as its interactions with the brain via the gut-brain axis, was discussed by Dr. Heidi Coia from the AFRL, 711 HPW. She described some of the currently available models and identified some of the advantages and disadvantages of each, which vary in complexity, host/bacteria interactions, and accurate translation to *in vivo* mechanisms. Dr. Coia discussed ongoing efforts, in conjunction with Dr. Tyler Nelson at AFRL, to leverage the Transwell® efforts into an equivalent model on a microfluidics platform for improved cellular differentiation and maintenance by the manipulation of shear stress, tension, and surface parameters. As part of the evaluation pipeline, she described how the *in vitro* gut-brain-axis model data output is being used by Dr. Elaine Merrill (AFRL) to develop *in silico* models to describe, interpret, and understand behavior of the test system.

U.S. Navy Overview

Dr. Brian Eddie and Dr. Anthony Malanoski presented experiments studying an autotrophic marine biocathode community at the Center for Bio/Molecular Science and Engineering (CBMSE) at the Naval Research Laboratory (NRL). Dr. Eddie focused on functional characterization of microbiomes using transcriptomics. He described a very modular pipeline developed at NRL for analyzing microbiome transcriptomes and discussed specific results from this study [15, 16]. Relative mRNA levels indicated which organisms were more active in the community at the time of sampling. In particular, *Cd Tenderia electrophaga* was the most active organism and is assumed to be responsible for the overall function of the biocathode, even though it was not the most abundant member of the community. He also mapped the

transcriptomics data onto known metabolic models to make biological sense of the data. The team is going to apply this pipeline to other microbiome communities, *i.e.*, the respiratory microbiome, in the near future.

Dr. Anthony Malanoski discussed 16S rRNA and shotgun metagenomic sequencing applications to biofilms/microbiomes [17]. He used nine variable regions of the 16S rRNA gene to identify which ones would be best for future studies. Although he observed differences in the intensities among the variable regions, the inter-sample comparison was very reliable if comparing the same region. His results showed that 16S rRNA sampling gave much higher sampling depth than metagenomic sequencing; however, it did not identify *Cd Tenderia electrophaga*, an autotrophic organism responsible for the biocathode function, due to the novelty of its 16S rRNA sequence.

Dr. Judson Hervey, from CBMSE at NRL, presented a bioinformatics workflow for high-performance computing systems, focusing mainly on the proteomics of microbial communities and the Sipros Ensemble application [18]. The increasing data volume was identified as a major bottleneck in metaproteomics. High-performance computing is expected to speed up data processing and analysis, as well as allowing for creation of protein databases from matched metagenomic sequences of the microbiomes. To demonstrate this, he re-analyzed several publicly available datasets from the ProteomeXchange repository. These datasets included human and human-associated microbial communities from oral biofilm [19], infant gut [20], and inflammatory bowel disease models [21]. The Sipros Ensemble returned comparable results with improved confidence and in significantly less time. This demonstrates that Sipros is scalable to high

performance computing systems and eliminates the need for two-step database searches commonly used in the metaproteomics field.

Dr. Christopher Duplessis, Commander, U.S. Navy, from the Naval Medical Research Center (NMRC), discussed their microbiome sample collection methods and assessment of the microbiome in cases of travelers' diarrhea. They compared the use of Whatman® paper cards and swab methods to determine whether extreme temperature conditions have impacts on sample collection as would be applicable to a real world scenario. The preliminary data using standard methods on human fecal samples showed excellent correlations in measurement of microbial diversity and abundance when Whatman® paper was used. Duplessis also emphasized follow-up studies will examine different storage conditions, collection, timeframes, and methods with greater sample numbers.

DOD Affiliates & Partners

Dr. Elaine Hsiao, Assistant Professor from the Integrative Biology and Physiology program at the University of California, Los Angeles, emphasized that there are microbiota effects on neurophysiology by production of neuroactive molecules that modulate the immune system as well as directly activate neurons. Her focus on microbiome-gut-brain circuits for microbial modulation of host cognition in response to diet and stress is part of a new MURI project awarded by the Army Research Office (ARO). Her preliminary data on mice given ketogenic diet showed *Akkermansia muciniphila* as an abundant species by day four of the diet, as well as an increase in *Parabacteriodes*.

Another keynote address was by Dr. Harris Wang, Assistant Professor for Systems Biology at Columbia University Medical Center, who discussed some options for industrializing microbiome research. His team focuses on the key principles that drive the formation, maintenance, and evolution of genomes within and across microbial populations. There is a growing interest in culturomics - culturing and identification of unknown bacteria that inhabit the human gut. He emphasized the value of a Modular Automated Microbial Biobanking and Analysis (MAMBA) system using metabolomics and drug and cellular screening for linking phenotypic information to genomic sequences. Their team is working on a method, DIVERS, that is based on replicate sampling and spike-in sequencing. The team's focus is on studying disease-relevant microbial ecosystems as found in the human body using synthetic biology approaches.

Dr. David Karig from the John Hopkins University Applied Physics Laboratory delivered a presentation on a skin microbiome. He highlighted the importance of spatial sampling for the measurement of inter- and intra-diversity in the skin microbiome. His team has developed data analysis tools that assist with classification, visualization, and quality control of the metagenomics data. Both interpersonal and intra-site variation in the community structure is a significant challenge with data collected from skin samples. They are assembling a collection of skin microbiome isolates for research. Their *in vitro* approach uses co-culture of keratinocytes with isolates to understand microbial-modulated toxicity. The team is also testing carbon source utilization by skin microbes and determining antibiotic resistance gene flow in skin communities. This will eventually inform building of synthetic community, they are interested in expanding their studies to ionizing radiation and organophosphate exposure.

A fourth Keynote lecture was given by Dr. Jack Gilbert, Professor, Department of Surgery, University of Chicago, focusing on microbiome-wide association studies to identify relationships between microbial activity and human health. He stressed the importance of individual exposures to various environments that shape our unique microbiomes. He cited the literature in the fields of asthma, food allergies, and autism that show changes in bacterial species and the involvement of microbial metabolites, emphasizing the magnitude of spatial location in microbial diversity. The number of clinical trials related to the microbiome is steadily increasing. He introduced the Extension of Community Health Outcomes (ECHO) program to study microbiome influences on the immune system that may affect brain development and the potential for school readiness programs. His team is also working with the National Institute of Justice to study microbial forensics for the potential utility of microbial information in criminal cases.

Takeaways and Conclusions

Multiple DOD groups are conducting microbiome studies relevant to the warfighter, including the use of the host microbiomes for diagnosis, prognosis, surveillance, and intervention. These efforts focus on microbiome profiling in response to military-relevant environments and stressors, engineering of complex microbial communities, microbiome in built environments, and integration with the host multi-omic data.

The TSMC workshop consisted of sessions focused on warfighter performance, warfighter protection, experimental approaches and data processing, and environmental microbiome research. The workshop also included separate

moderated discussions on model systems, omics, bioinformatics, and human studies. The groups discussed issues and challenges related to microbiome research in the DOD. These challenges include Cloud access, data standardization and sharing, commonality of data capture, sampling strategies on the battlefield, data analysis, and recommendations for manipulation of the microbiome.

This workshop was an opportunity to discuss the scope of the DOD microbiome efforts and the research gaps relevant to the warfighter. It was also a great venue for collaboration and knowledge sharing, as well as discussion about technical and programmatic challenges.

The workshop participants emphasized the importance of interaction with academia, industry, and other government agencies for the advancement of microbiome research in the DOD.

Disclaimers

The views, opinions, and findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

This research complied with the Animal Welfare Act and implementing Animal Welfare Regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and adhered to the principles noted in The Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Declarations

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Table 1. TSMC DOD Organizations general interests in microbiome research		
Organization	Microbiome Research Interests	Affiliates 2017 & 2018
Office of Assistant Secretary of Defense for Research and Engineering (OASD (R&E))	Identify and integrate research activities to maximize warfighter effectiveness. Focus areas include Human Systems, Medical and Life Sciences, Regulatory Oversight and Environmental Sciences	Dr. James B. Petro

U.S. Army Center for Environmental Health Research (USACEHR)	Gut microbiome as a 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. Temporally altered fecal microbiota and urine metabolome following soman poisoning. Heavy metal effects on diverse microbiota.	Dr. Rasha Hammamieh Dr. Aarti Gautam MAJ Derese Getnet Dr. John Lewis
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. Lead for Army human nutrition intervention research.	Dr. J. Philip Karl
U.S. Army Combat Capabilities Development Command - Soldier Center	Seek to characterize and understand how specific military-relevant stressors influence the delicate balance within a healthy gut microbiome. In vitro gut fermentation models, <i>in vitro/ex vivo</i> intestinal physiology models, food product development and production, regulatory and transition challenges.	Mr. Jason W. Soares Mr. Kenneth Racicot Dr. Sarah Pearce
U.S. Air Force Research Laboratory (AFRL)	Pulmonary health biomarker discovery, the role of intestinal microbiome in deployment-associated diarrhea, 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, in vitro gut-brain models for bacteria-host interactions.	Dr. Camilla A. Mauzy Dr. Nancy Kelley-Loughnane Dr. Michael Goodson Dr. Heidi Coia Dr. Wendy Goodson
Johns Hopkins University Applied Physics Laboratory (JHU-APL)	Inter- and intra-spatial microbial community relationship within the skin microbiome. In vitro models and data analysis tools that assist with classification, visualization, and quality control of the metagenomics data.	Dr. David Karig
US Army Medical Research and Materiel Command (MRMC)	Ethical considerations for microbiome research: specifically, the difference between informational dilemmas (e.g., personal identity, privacy, data security, etc.) and interventional dilemmas. Recommendations for policymakers to incorporate synthetic biology into the ELSI-microbiome discussions.	Dr. Natalie Klein

Navy Medical Research Center (NMRC)	The role of intestinal microbiome in deployment-associated diarrhea. Validation of field-expedient sampling methods to assess microbiome in the setting of travelers' diarrhea.	LCDR Mark Simons CDR Christopher Duplessis
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. Functional microbiome characterization using transcriptomics. Informatics workflow for high-performance computing systems. Shotgun metagenomics sequencing methods for interrogating biofilms and microbiomes	Dr. Sarah (Strycharz) Glaven Dr. Dagmar H. Leary Dr. Brian Eddie Dr. Judson Hervey Mr. Anthony Malanoski
U.S. Army Corps of Engineers Environmental Research and Development Center (ERDC)	Molecular markers for toxicants in soil microbiomes. Effect 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.	Dr. Karl J. Indest Dr. Robyn A. Barbato
Office of Naval Research (ONR)	Biosciences program to study human microbiomes and effects on warfighter resilience and readiness; ELSI considerations for microbiome research; funding DOD/Navy relevant research.	Dr. Linda A. Chrisey
Army Research Office (ARO)	The microbiology program supports basic research in fundamental microbiology that can help advance needs in Soldier protection and performance. The two primary research thrusts within this program include microbial survival mechanisms and analysis and engineering of microbial communities. Using microbiology to understand complex community dynamics, engineering of microbial communities and modeling bidirectional gut-brain axis.	Dr. Robert J. Kokoska
Army Research Laboratories (ARL)	Repurposing gut microbes for commodity chemical production predicts the whole as greater than the sum of the parts. Using microbial consortia versus single isolates to provide unique and efficient routes to remediate waste streams. Genome-scale metabolic models with flux-balance analysis for community dynamics prediction.	Dr. Matthew Perisin

Office of the Air Force Surgeon General (OTSG)	Total exposure health; integrating the science of the microbiome to enhance human performance. Instituting a paradigm shift in military medicine from disease-injury to a more holistic approach that focuses on health and prevention.	Dr. Richard Hartman
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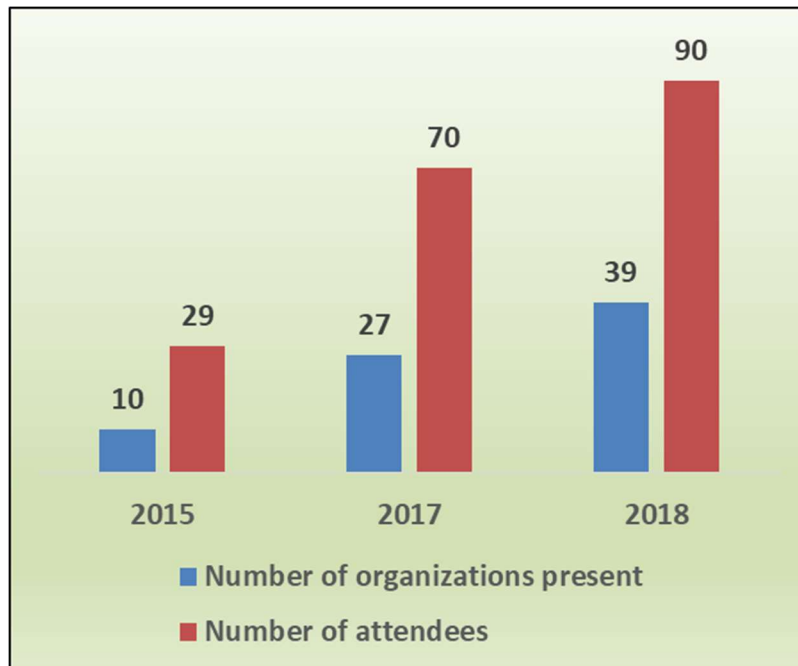


Figure 1. Attendance and number of organizations present at the three DOD microbiome meetings.

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