

COMMENTARY

Research Agenda for Microbiome Based Research for Multidrug-resistant Organism Prevention in the Veterans Health Administration System

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(See introductory commentary by Livorsi et al, pages 186–188.)

Nearly 100 trillion microbes occupying the skin, genitourinary tract, and pulmonary system constitute the human microbiome.¹ This diverse microbiome consisting of bacteria, fungi, parasites and protozoa plays a significant role in health and disease. Impairment of the gut microbiome is implicated in a diverse array of conditions, both noninfectious and infectious, such as cardiovascular disease,² Crohn's disease,^{3,4} and *Clostridium difficile* infection (CDI).^{5,6}

Improvements in sequencing technology and decreases in sequencing costs have facilitated growth in microbiome research. Projects like the Human Microbiome Project⁷ and the European Metagenomics of the Human Intestinal Tract (MetaHIT)⁸ have contributed enormously to our understanding of microbiome complexity and its role in health and disease. Despite this welcome progress, microbiome research is in its infancy, particularly in the context of how the microbiomes influences infection with multidrug resistant organisms (MDRO), including CDI in healthcare settings. Further research aimed at understanding and manipulating the structure and function of the microbiome in human physiology, disease pathogenesis, and infection prevention is necessary.

As the largest integrated healthcare system in the United States, serving 8.9 million veterans, the Veterans Health Administration (VHA) system is poised to lead innovative microbiome research in healthcare-associated infections and MDROs. Here, we discuss the proceedings of a multidisciplinary conference on microbiome research in this area across the VHA. This conference highlighted the role of the human microbiome in colonization with MDROs and infection prevention and developed recommendations to guide

VHA microbiome research in healthcare-associated infection (HAI) and MDROs (Table 1). The importance of a standardized approach to microbiome research, the role of the gut microbiome with special focus on biotherapeutics, and the role of microbiota at extraintestinal sites are the central focus of these recommendations. While the VHA is uniquely positioned to lead such microbiome research efforts, these proceedings have application beyond the VHA as well.

RECOMMENDATIONS

A Framework for Microbiome Research

A VHA-wide standardized research framework will ensure rigorous, reproducible work and foster collaborations across VHA sites. The need for a set of standards in microbiome research is not unique to the VHA.^{1,9} Several methodologies have been applied at all stages of microbiome analysis; however, a gold standard has not emerged. The International Human Microbiome Standards (IHMS) project¹⁰ was established to create a set of standard operating procedures (SOPs), which are crucial to enhancing reproducibility and comparability across microbiome research. Despite the importance of the proposed standards, many labs have not adopted these SOPs.⁹ As the VHA advances microbiome research, a standardized research framework must be developed. The recommended components of this framework are shown in Figure 1.

To ensure high-quality, reproducible microbiome research, a set of standardized methods should be implemented, such as the IHMS SOPs, which offer clinical definitions, sample collection best practices, and laboratory processes. Novel indices, such as a Microbiome Disruption Index being jointly designed

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TABLE 1. Proposed Veterans Health Administration Health Services Research and Development Agenda for Microbiome Research

Topic	What is Known	Next Steps
Microbiota framework within the VA	Currently, there is no comprehensive microbiota research framework within the VA. Others, such as HMP, MetaHIT, and the IHMS project have developed openly-shared standards and protocols.	Adopt a set of standard protocols and bioinformatics tools across multiple applications Implement a framework to disseminate protocols, tools, and data sharing/repository Provide computational and bioinformatics resources to perform analyses Identify core facilities and academic affiliates to assist in sample processing and sequencing
FMT and CDI	Currently ongoing studies on oral FMT and CDI recurrence Largely positive effect	Conduct efficacy randomized controlled trials for prevention of both primary and recurrent CDI Assess long-term impact of FMT and transmission of disease from donor to recipient Assess oral vancomycin prophylaxis vs FMT for prevention of CDI recurrence Conduct qualitative studies on the patient experience, physician and patient perceptions, diet, and familial transmission of <i>C. difficile</i>
FMT and MDRO colonization	Case reports and case series for both VRE and gram-negative organism carriage Reports of VRE decolonization with recurrent CDI Ongoing RCT on FMT and gram-negative organisms in Europe	Identify existing VA practices Conduct efficacy studies and double-blind RCT for different routes of FMT administration and FMT compared to standard care Assess long-term benefits of decolonization, co-colonization, re-colonization, familial transmission, surgical prophylaxis, and geographical and environmental variations Assess differences in effectiveness and efficacy of FMT stratified by colonizing organism and patient populations
Prebiotics and probiotics	Multiple studies on probiotics for CDI and antibiotic-associated diarrhea Widespread use for unclear indications	Assess prebiotics as a form of primary prevention for CDI and antibiotic-associated diarrhea Assess mechanism of action Assess strain choice, dosing, and setting dependencies
Non-GI microbiota research	A more diverse nasal microbiome is associated with less disease burden and better post-surgical outcomes Limited research, especially on skin microbiota	Assess MDRO decolonization and re-colonization in the nares and the impact on skin health Development of probiotics and microbiota manipulation in MRSA decolonization of the nares Determine the role of microbiota in wound and surgical site infections Determine the effect of antiseptics, such as chlorhexidine gluconate bathing, on the skin microbiome

NOTE. VA, Veterans Affairs; HMP, Human Microbiome Project; IHMS, International Human Microbiome Standards; CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; MDRO, multidrug-resistant organism; VRE, vancomycin-resistant *Enterococcus*; RCT, randomized controlled trial; GI, gastrointestinal; MRSA, methicillin-resistant *Staphylococcus aureus*.

by uBiome and the Centers for Disease Control and Prevention (CDC),¹¹ may offer promise in tracking the impact of interventions and biotherapeutics on the gut microbiome, can provide early identification of MDRO colonization or infection, and can serve as a method to standardize classification based on set definitions. While a greater understanding of microbiome compositions is required to create standardized classifications, some metrics do currently exist, such as the community states used to classify the vaginal microbiome.¹²

Presently, sample collection procedures vary widely and are dependent on the research question, the anatomic site, the expected biomass, and preferences of the research participant. The cost of the technique and the necessary sample size

also influence the sampling procedure. While it is not possible to identify a set of sampling techniques appropriate for all studies, it would be beneficial to curate a set of guidelines and best practices.

Uniformity in laboratory processing methods will optimize the ability to compare results across studies. The method of genome extraction shapes the observed microbial composition due to varying cell lysis techniques.¹³ Standardizing methods of genome extraction will require access to core facilities or academic affiliates to carry out sequencing. For VA medical centers without these capabilities, collaboration with a core facility equipped to handle genome extraction is prudent and economical.

Resources for data analysis must be provided to microbiome researchers. Advanced computational and bioinformatics tools

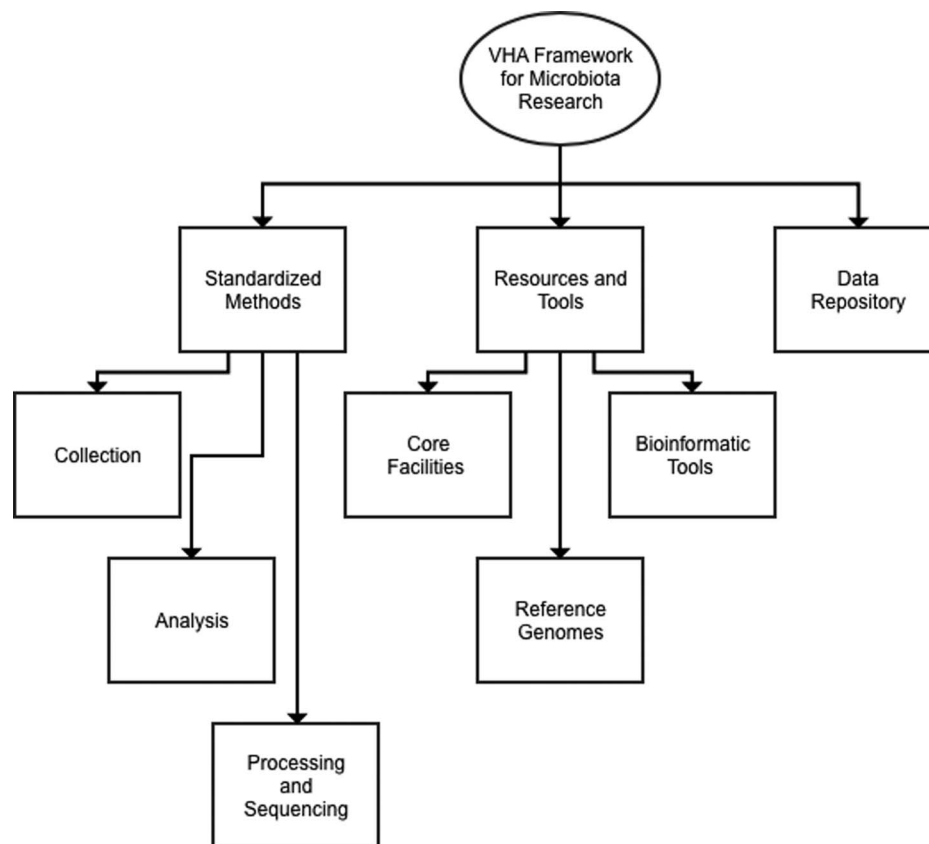


FIGURE 1. Key components of the proposed framework for microbiota research within the Veterans Health Administration (VHA).

are needed to analyze the volume of data produced through next-generation sequencing and shotgun metagenomics studies. Many tools currently exist for analyzing sequencing data, such as Quantitative Insights Into Microbial Ecology (QIIME),¹⁴ mothur,¹⁵ and UPARSE¹⁶ for targeted amplicon sequencing. However, gaps remain in our understanding of the relative advantages and disadvantages of these methods. After sequencing data are processed, statistical software (R is one such example) must be available for data analysis.¹⁷ In addition to providing access to these programs, the VHA could create a central infrastructure of new and innovative analytic methods to be at the forefront of microbiome research and big data analysis in this context.

Data release and sharing into a repository is essential for microbiome research to ensure reproducibility and validation and allow leveraging of existing or planned studies. Sequencing and metadata should be uploaded to the repository frequently and following normal standards¹⁸ so it can be available to the scientific community. Repository data should be reasonably deidentified, consistent with institutional review board policies.

Study Design Considerations

Much of the published literature on the human microbiome and interventions to treat perturbations of the human

microbiome are case studies or uncontrolled case series.¹⁹ The VHA can advance microbiome research by pursuing longitudinal studies, conducting randomized controlled trials (RCTs) of biotherapeutic interventions, investigating geographic differences in the microbiome, examining patient and provider acceptability of biotherapeutics through qualitative methods, and leveraging the Million Veterans Program as a considerable potential biobank for microbiome data.

Longitudinal studies can elucidate the natural history of changes in the microbiome across time, across the healthcare continuum, and in response to various exposures. Changes in the microbiome should be studied across transitions into and out of the hospital, intensive care units, long-term care facilities, community living centers, and pre- and postoperatively.²⁰ By comparing groups of patients taking certain medications of interest (eg, antibiotics, metformin), changes to the microbiome related to these exposures can be identified. The influence of disease processes such as diabetes on the microbiome can also be studied in this way.²¹ The VHA can evaluate the influence of deployment on the microbiome by following the microbiome of military personnel who travel to and from other countries. These longitudinal studies must compare individuals exposed to the variable of interest to those who are not and take care to collect data on other variables that may affect the composition of the microbiome, such as diet, use

of antibiotics, and occupation. These variables should be evaluated as risk factors for microbiome perturbations, and adjusted for in analyses.

Randomized controlled trials evaluating the effectiveness of biotherapeutic interventions to limit microbiome perturbation or restore the gut microbiome must be performed. These interventions include, but are not limited to, fecal microbiota transplantation (FMT), probiotics, and nontoxicogenic *C. difficile*. Randomized controlled trials evaluating the use of these biotherapeutics in primary and recurrent CDI and other MDROs should be pursued.¹⁹ Because these biotherapeutics are organic, studies should account for potential transmission between study subjects, such as nontoxicogenic spores transmitted from intervention and control patients.

Both longitudinal studies and RCTs need to be sufficiently powered to detect clinically relevant differences. A recent review by Hanson and Weinstock²² provides a discussion of methods that can be used for these power calculations.

The VHA should use its strong qualitative and implementation research infrastructure to assess patient and provider views regarding biotherapeutic interventions to alter the microbiome. Patients along the spectrum of health and disease should be surveyed or interviewed to determine attitudes and perceptions toward various types of biotherapeutics and delivery methods.¹⁹ These studies could reveal differences in risk perception and aversion to biotherapeutics among individuals based on prior disease or carrier status. Similarly, provider perceptions and how they approach conversations regarding these biotherapeutic interventions should be explored.

The VHA system is uniquely poised to perform many of the studies described above. Deployment to developing countries and the ability to connect TriCare data with the VHA Corporate Data Warehouse can provide much of the information needed to perform decades-long longitudinal studies with little added burden to the study subjects. Research to determine geographic variation of microbiomes will be necessary to define a “healthy microbiome” and whether interventions need to be geographically specific, which is important for veterans deployed internationally. The shared electronic medical record across inpatient, outpatient, and community living center care within the VHA provides the opportunity to follow patients during transitions of care. The VHA research funding mechanisms can also encourage investigators to add microbiome analyses to studies of antibiotics, decolonization interventions and new devices.

The Million Veteran Program (MVP) could provide a wealth of information to advance microbiome research. As of August 2016, the MVP had collected blood samples for genetic analysis and has performed baseline and lifestyle (eg, dietary habits, environmental exposures) surveys on more than 500,000 veterans.^{23,24} These data are linked with the electronic medical record to create a mega-biobank to improve understanding of how health is affected by genetics, behaviors, and environmental factors.²³ The MVP could also collect stool samples from these Veterans and store them in the mega-biobank, allowing researchers to evaluate how health is affected by the microbiome

and how behaviors and environmental factors may be associated with perturbations of the microbiome. By conducting longitudinal observational studies, RCTs and leveraging currently existing resources, the VHA can lead innovative research aimed to fully understand the gut microbiome so it can be leveraged for veteran and civilian health.

INTERVENTIONS TARGETED TOWARD THE MICROBIOME

Fecal Microbiota Transplantation

Gut dysbiosis is the hallmark of both initial and recurrent CDI. Conventional treatments include antibiotics with activity against *C. difficile*,^{25,26} but these antibiotics have activity against other gut bacteria, limiting the ability of the microbiota to fully recover following CDI and predisposing patients to recurrence.²⁷ Although other factors may also explain why patients have recurrence (eg, low serum antibody response to *C. difficile* toxins,²⁸ use of medications such as proton pump inhibitors,²⁹ and the specific strain of *C. difficile* causing infection^{26,29}), restoration of the gut microbiome through FMT has recently gained acceptance as a safe and effective treatment for CDI. However, the optimal protocol for FMT is unknown; numerous methods of stool preparation, infusion, and recipient and donor preparation have been published.³⁰ Variation exists in diluent selected, site of instillation, method of recipient preparation, and donor screening. Fecal microbiota transplantation has been performed in both inpatient and outpatient settings, and self-administration of fecal enema at home has also demonstrated success.³¹ Although there are numerous variables to consider in protocol design, it is encouraging that FMT appears to be effective regardless of the specific protocol.³² Studies to evaluate the efficacy and the implementation of FMT for CDI are needed to define best practices.

Several key areas were identified as VHA priorities including efficacy RCTs for both primary and recurrent CDI, long-term assessment of the impact of FMT and transmission of disease from donor to recipient, assessment of oral vancomycin prophylaxis versus FMT for prevention of CDI recurrence, and qualitative studies on the patient experience and provider and patient perceptions.

Recurrent CDI. The clinical evidence for FMT is most robust for recurrent CDI, and 3 RCTs in this field have helped to clarify the efficacy of FMT.^{33–35} Each found rates of cure greater than 70%–90% following FMT; however, sample size, methodologic limitations, need for multiple FMT in several patients and limited duration of follow-up in these studies means there remains a need for high-quality prospective trials.

Primary and severe CDI. Few data are available on the use of FMT for primary, non-recurrent CDI aside from a few case reports. A mathematical model of CDI in an ICU assessed the role of FMT on primary CDI³⁶ and predicted a decreased median incidence of recurrent CDI in patients treated with

FMT for primary CDI. The assumptions of the model require validation and prospective testing to address the role of FMT in primary CDI. In a nonrandomized, open-label, before-and-after prospective study comparing conventional antibiotic treatment for CDI versus early FMT via nasogastric infusion, Lagier et al³⁷ found that mortality in the FMT group was significantly less (64.4% vs 18.8%; $P < .01$). This shift in therapy occurred due to clinical need; their hospital in Marseille developed a ribotype 027 outbreak with a dramatic global mortality rate (50.8%). In an epidemic setting with a high mortality rate, early FMT may be beneficial, but the risks and benefits of FMT in primary CDI in endemic situations have not been well described. Similarly, the evidence for use of FMT in severe CDI consists of published case reports, which suggest efficacy and potential for further study.^{38–41}

Patient and provider perceptions of FMT. A commonly cited reason for a limited role of FMT is the aesthetics of treatment. However, few studies exist on patients and provider perceptions regarding FMT. In their 2014 review of FMT for CDI, the Evidence-based Synthesis Program (ESP), a unit of the VA Health Services Research and Development Service, identified no study designed to systematically assess acceptability of FMT among patients. The ESP highlighted these investigations as important next steps in advancing the FMT research agenda.⁴²

Zipursky et al⁴³ surveyed 192 outpatients (only 1 had a history of CDI) on their attitudes toward FMT using hypothetical case scenarios. The results were largely positive, with 81% agreeing to FMT for CDI. The need to handle stool and the nasogastric route of administration were the most unappealing aspects of FMT by participants. More respondents (90%; $P = .002$) agreed to FMT when offered as a pill.

When the same investigators surveyed physician attitudes toward FMT,⁴⁴ they found 83 of 135 physicians (65%) in their sample had not offered or referred a patient for FMT. Frequently cited reasons included institutional barriers, concern patients would find it unappealing, and uncertainty regarding indications. Only 8% of physicians believed patients would choose FMT if given the option. As the role of FMT expands, patient and provider perceptions and attitudes will likely evolve to better align.

FMT for MDROs. The hypothesized mechanism of FMT for CDI is that normal colonic microbiota outcompete and competitively exclude *C. difficile*.³⁵ The same principles may apply to other enteric pathogens, such as vancomycin-resistant *Enterococcus* (VRE). Similar to *C. difficile*, VRE colonization is induced by microbial dysbiosis. Introduction of commensal microbiota may help eliminate VRE colonization through direct effects such as nutrient depletion or immune-mediated factors. In murine models, FMT is effective at decolonizing VRE.^{45,46} In case studies of patients co-colonized with *C. difficile* and VRE, FMT has resulted in the decolonization of both pathogens.^{47,48} However, rigorous evidence of efficacy is lacking, and determining the role of FMT for VRE decolonization requires randomized controlled trials.

Fecal microbiota transplantation may have the potential to reduce colonization with carbapenem-resistant Enterobacteriaceae (CRE). In several small case series, FMT successfully eradicated CRE.^{49,50} In a recent single-center study of 20 patients receiving FMT for recurrent CDI, DNA microarray analysis identified decreased number and diversity of antibiotic resistance genes, which persisted up to a year following FMT.⁵¹ The availability of oral FMT makes it an attractive, relatively low-risk option for eradicating MDRO colonization and warrants further study.

BIO THERAPEUTICS

The role of prebiotics, probiotics, and synbiotics (mixtures of pre- and probiotics) in the human gastrointestinal (GI) microbiome requires further examination. Prebiotics can be harnessed to enhance community structure and immune responses that confer protection against CDI. For example, fructooligosaccharide and lactulose prebiotics increase resistance to colonization with *C. difficile* via several mechanisms. The dairy protein glycomacropeptide (GMP) participates in activities related to GI physiology and metabolic disease and beneficially modulates GI microbiota in mice.⁵²

When administered in adequate amounts, probiotics confer a health benefit. Several large systematic reviews have demonstrated a reduction in incidence of primary CDI when probiotics were used.^{51,53,54} Synbiotics, mixtures of probiotics and prebiotics, confer protection against CDI in mice, reducing viable *C. difficile* in cecal contents compared to controls. Current limitations to the probiotic literature are that many over-the-counter probiotics have no live organisms, do not have sufficient quantities of microbes, or have no mechanistic basis for their proposed action. These findings may explain the conflicting results from probiotic trials and the gap in our understanding of which probiotics are likely to confer benefit. A recent advance in this area is the use of nontoxigenic *C. difficile* spores. In a phase 2 RCT, patients who received non-toxigenic *C. difficile* spores following a resolved episode of CDI had a significant reduction in the risk of recurrence.⁵⁵

The field of biotherapeutics is in its infancy and fundamental biomedical research is needed to understand the potential of these products before widespread implementation and dissemination. Trials of biotherapeutics offer an opportunity to examine GI microbiome changes as a consequence and better understand mechanisms of action.

MICROBIOTA RESEARCH BEYOND THE GASTROINTESTINAL TRACT

The gastrointestinal tract is the most studied human microbiome; however, many other body sites are of clinical relevance to HAI research, particularly the nasal and skin microbiomes.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been the focus of nasal microbiome research. MRSA colonization is associated with decreased nasal microbiome diversity and several bacterial species (*Streptococcus mitis*⁵⁶ and

Corynebacterium spp) influence MRSA colonization and virulence.^{57,58} Studying the effect of therapeutics applied in the nose and longitudinal studies of nasal microbiome changes during hospitalization, antibiotic use and after decolonization are necessary.

Studying the skin microbiome is challenging, but a better understanding of its role is necessary. Constant contact with the environment makes it difficult to distinguish between commensal and transient organisms, and obtaining adequate microbial DNA is challenging given the low biomass of the skin. While skin decolonization with daily or presurgical use of chlorhexidine to reduce infection is becoming more widespread, the impact of antiseptics on long-term skin health and microbial composition is unknown. In addition, the role of the skin microbiome in wound and surgical site infections and the impact of the hand microbiome of healthcare workers in MDRO transmission warrants further study.

CONCLUSIONS

The meeting of a multidisciplinary group of researchers focused on infection prevention and microbiota research identified several key recommendations for the future of microbiota research within the VHA. First, a unified framework including standardized methodologies, access to bioinformatics and computational resources, and provision of a data repository will allow high-quality, reproducible work and collaboration across multiple VHA sites. Incorporating microbiome data into data collection, particularly in infection prevention, will provide the ability to evaluate relationships of the microbiome to lifestyle and clinical factors. Longitudinal studies will elucidate changes in the human microbiome over time and across transitions of care. High-quality RCTs examining the efficacy of various approaches to FMT and qualitative studies exploring patient and provider preferences will clarify the role of FMT in restoration of health and treatment of disease. Additional biotherapeutics should be examined, such as prebiotics, probiotics and synbiotics. Finally, the role of extraintestinal microbiomes, particularly the nose and skin, in health and disease should be studied further.

Given the large role the VHA plays in the health of veterans and civilians and its strong research infrastructure, the VHA is well positioned to lead the next wave of discovery in human microbiome research.

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