Aviation and the Microbiome

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INTRODUCTION: Although the impact of microorganisms on their hosts has been investigated for decades, recent technological advances have permitted high-throughput studies of the collective microbial genomes colonizing a host or habitat, also known as the microbiome. This literature review presents an overview of microbiome research, with an emphasis on topics that have the potential for future applications to aviation safety. In humans, research is beginning to suggest relationships of the microbiome with physical disorders, including type 1 and type 2 diabetes mellitus, cardiovascular disease, and respiratory disease. The microbiome also has been associated with psychological health, including depression, anxiety, and the social complications that arise in autism spectrum disorders. Pharmaceuticals can alter microbiome diversity, and may lead to unintended consequences both short and long-term. As research strengthens understanding of the connections between the microbiota and human health, several potential applications for aerospace medicine and aviation safety emerge. For example, information derived from tests of the microbiota has potential future relevance for medical certification of pilots, accident investigation, and evaluation of fitness for duty in aerospace operations. Moreover, air travel may impact the microbiome of passengers and crew, including potential impacts on the spread of disease nationally and internationally. Construction, maintenance, and cleaning regimens that consider the potential for microbial colonization in airports and cabin environments may promote the health of travelers. Altogether, the mounting knowledge of microbiome effects on health presents several opportunities for future research into how and whether microbiome-based insights could be used to improve aviation safety.

KEYWORDS: Human microbiome, cabin microbiome, aviation, medical certification, dysbiosis.

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Microorganisms have long been known to influence human health, and are frequently associated with negative health outcomes such as pathogenic bacterial infections.[56] Yet, many microbes have a beneficial or even essential function for the life of host organisms.[112,137] In recent years, there has been an increased level of research into commensal human-bacterial relationships in which bacteria benefit from, but have a neutral effect on the host.[45,54] With the advent of high-throughput sequencing, a collection of methods for rapid processing of nucleic acids that enables simultaneous sequencing of multiple fragments in parallel, the desire to investigate the human and microbial genome has increased.[29,47] Sequencing efforts such as the National Institutes of Health Human Microbiome Project have greatly advanced knowledge of the human-microbe relationship, including niche specialization and differences in the microbial community across body sites.[61,85]

The human microbiome is the collection of microbial genomes within a system, while microbiota are the collection of all the microbial organisms (including bacteria, viruses, fungi, etc.) within a certain region, tissue, or organ (**Table I**).[126] In practice, the terms microbiome and microbiota sometimes are used interchangeably, particularly as the use of genomics-based assays to study the microbiota can blur the distinction. The genes in the microbiome are key determinants of what is produced by the microorganisms, including chemical byproducts, metabolites,[10] and proteins.[139] In turn, these metabolites and proteins influence the homeostasis of the human-microbe system. Hence many studies not only use molecular approaches to assess the identity of microbes in a given sample (e.g., by analyzing the highly-conserved prokaryotic 16S ribosomal ribonucleic acid or rRNA region[64]), but also to infer possible

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Table I. Definitions of Common Terms.

TERM	DESCRIPTION
Microbiome	Collection of microbial genomes within a system.[126]
Microbiota	Ecological community of microorganisms within a system.[126]
Commensal	Association of two or more organisms in which one organism derives a direct benefit and the other is unaffected.[54]
Dysbiosis	Disruption of microbiome homeostasis, which may include an increase in microbes harmful to the host, loss of beneficial microbes, or an overall change or reduction in diversity.[31]
16S rRNA Sequencing	Sequencing the conserved 16S ribosomal ribonucleic acid region (16S rRNA) of the genome, often used in high-throughput sequencing studies for identification of bacteria.[64]
Metagenomic Sequencing	Shotgun metagenomics attempts to sequence and analyze the genomes of the microbiome; this approach can be used to assesses the DNA of all the microorganisms in a sample.[98] This is a type of high-throughput sequencing.
Metatranscriptomic Sequencing	Analyzes the RNA transcripts to identify what genes are actively expressed within the sample of the microbiome.[9] This is a type of high-throughput sequencing.
Probiotic	Live cultures of microbes that are ingested or implanted to introduce or increase levels of beneficial microbes.[62]
Prebiotic	Nutrition for the microorganisms currently colonizing a region.[125]
Biomarker	Characteristics that indicate normal processes, pathogenic processes, or responses to treatment.[13]
Thanatomicrobiome	The microbial community associated with the host after death, or postmortem microbiome.[66]
Taxonomic Diversity	The number and abundance of different species or taxa within a region.[91]

functional roles of the microbiota based on their genetic makeup.

The human microbiome develops a natural balance over time,[86] as the microbial flora of a colonized tissue stabilizes. Disruption of the homeostatic balance or equilibrium of the microbial community is known as dysbiosis and can alter the host immune response and susceptibility to disease.[31,110] Changes in the abundance of specific microbiota within the human microbiome have been correlated with many different disease states.[71,84,117] The microbiota of the gut can be altered by the host's diet,[126] antibiotics,[78] probiotics (ingested cultures of beneficial organisms),[62] prebiotics (nutritional support for beneficial bacteria),[125] or intentional inoculation such as fecal transplants.[62]

Although further work will be needed to validate many of the research findings reviewed in this report, components of the microbiome strongly correlated with human health may one day be assayed as biological indicators or biomarkers. Biomarkers can be described as characteristics that indicate normal processes, pathogenic processes, or responses to treatment.[13] Currently, commercially available gut microbiome tests use a stool sample due to ease of collection in comparison to alternative approaches, such as an intestinal mucosa biopsy.[40] These tests do have drawbacks, since people are not equipped to produce a stool sample on command. Meanwhile, researchers are working on correlating metabolites found in blood plasma with taxonomic diversity in the gut.[133] Such studies may one day lead to a blood plasma test for healthy levels of diversity or dysbiosis. Establishing causal linkages between microbial presence or activity and human health would be ideal for development of test kits. However, even strong correlations without knowledge of causality may be informative. For example, if a microbial shift always is associated with a medical condition, a microbiome-based assay that detects the shift may suffice to inform medical diagnosis.

Clinical applications of microbiome tests are still in early stages, and far less attention has been devoted to possible uses for future performance and safety evaluations in specialized fields such as aerospace operations. Approximately 10 years ago, De Voll discussed the relevance of microbial biofilms for the aeromedical field and cabin environments.[28] As scientists develop new insights from the microbiome, it is worth considering applications for future investigation. Many of the diseases correlated with different dysbioses, such as carotid stenosis and diabetes, would be of interest to monitor in pilots, particularly those with special issuances (see Applications section for explanation; 14 CFR § 67.401).

This review begins with a general discussion of associations proposed in research between the human microbiome and health, both physical and psychological. Subsequently, this report speculates on potential future applications of microbiome measurements to aviation, including medical certification of pilots, accident investigations, and assays of fitness for duty. Finally, ways in which microbiota may impact the health of passengers and crew during air travel are reviewed. Rather than serving as a comprehensive review of the very broad field of microbiome research, the purpose of this article is to provide a sample of microbiome topics with potential relevance to aviation safety. Ultimately these ideas are presented to stimulate discussion and consideration of future microbiome research within the aerospace medicine community.

RELATIONSHIPS BETWEEN THE MICROBIOME AND HEALTH

Physical

Researchers are just beginning to understand the reach and severity of conditions that are influenced by the microbiota. Diseases of the metabolic, vascular, neurological, and respiratory systems have been linked to disruption of the microbiota.[20,87,105] The current review discusses some of the research on effects the human microbiome has upon an individual's health, and scientists find more continually. The open source database, Disbiome, has been created by Ghent University to track diseases linked to microbiome dysbiosis.[65]

The microbes of the gut metabolize the food people eat and its digested components, so researchers have looked for correlation with metabolic diseases.[59,114] Recently, Tam et al. identified differences in diversity of the oral microbiota in obese vs. non-obese patients with type 2 diabetes mellitus.[122] Other studies reviewed by Sharma and Tripathi indicate potential mechanisms by which gut microbial activity and dysbiosis influence progression of type I and type II diabetes.[116] Additionally, changes in gut microbiota may influence processes such as lipopolysaccharide secretion and insulin resistance in nonalcoholic fatty liver disease.[87]

Other changes in microbial metabolism have been linked to equally serious conditions in the vascular system. For example, a cross-sectional patient study showed an increased level of *Collinsella* bacteria in patients with carotid stenosis and cerebrovascular events relative to healthy controls.[70] Microbial metabolism may at least partially underlie the association of microbiota and heart disease. Gut microbes are involved in trimethylamine production, which in turn is oxidized by the human host to trimethylamine-N-oxide, a compound linked to atherosclerotic progression.[15,23,75] Trimethylamine-N-oxide is a product of metabolism of phosphatidylcholine and L-carnitine, which are found in meat. Higher concentrations of this metabolite generally are seen in renally compromised patients, as they are unable to clear the phosphatidylcholines adequately.[120] Altogether, this represents a complex assortment of diet, comorbidities, and microbial activity that may influence vascular health.

Further complexity exists in the interactions among the microbiota of diverse tissues, such as the lung and gut. The microbiota of the gut and lung are thought to interact via a pathway named the gut-lung axis (GLA).[16] Research has not yet elucidated the GLA well, or by what means the two systems communicate.[16] There is little evidence to suggest the microbes translocate and interact directly between these two locations, except in disease states that reduce barrier integrity (sepsis, acute respiratory distress, etc.).[16] Instead, they may interact through metabolites or changes in the immune system.[16] Diseases of the lung and respiratory system including asthma and chronic obstructive pulmonary disease (COPD) have been associated with differences in the lung microbiota relative to healthy controls.[1,105,113]

Psychological/Cognitive

Physical wellbeing is often a clear and sometimes visual marker of health, while psychological wellbeing and cognitive ability can be harder to identify. However, assays of the microbiome may provide a novel approach to both understand and improve mental health. Microbial colonization has been shown to impact the neural network for the stress response in mice.[121] Bacterial infection also has been shown to impair memory in mice, while reduced cognitive flexibility coincided with shifts in the gut microbiome of mice fed a high-energy diet.[44,90] Research further suggests a role of the microbiota on host anxiety[25,35,99] and depression,[14] and indicates the potential for improvement with probiotic treatment.[14,60] Probiotics and the microbial metabolite butyrate have been associated with positive effects on cognition.[96] Indeed, Mohajeri and colleagues reviewed several studies of human and animal models in which probiotic and prebiotic intervention was associated with cognitive and behavioral improvements.[96] Very recently, research in Belgium has identified reduced levels of *Coprococcus* and *Dialister* and higher levels of *Bacteroides* enterotype 2 in individuals with depression and lower quality of life survey results.[127]

Similar to the GLA, nervous system function is thought to be influenced by the microbiome through the gut-brain axis (GBA). This is a communication pathway that involves chemical signals and metabolites from the gut microbiota to the neurons.[20] The autonomic nervous system can communicate back to the gut to induce changes in conditions that alter the diversity of the gut flora.[20] Through this crosstalk, many positive and negative effects can arise in the body. For many years, there has been the adage that "stress makes you sick;" this association may in part be mediated by the microbiota. Galley et al. found that a 2-h exposure to a social stressor influenced a change in the proportion of the immunomodulatory species *Lactobacillus reuteri* in the CD-1 mouse strain.[42] Studies indicate that stress alters intestinal mucosa[111] and gut motility,[51] which influence biofilm formation and microbial homeostasis.[89] Experiments in mice advance the idea that microbial colonization influences development of the hypothalamic-pituitary-adrenal stress response.[121] Moreover, knowledge of the human microbiome has relevance for understanding developmental disorders such as autism. Researchers have linked microbial dysbiosis to autism and have even associated the severity of autism with specific changes in the microbiota.[20,119] Several scientists also have shown relief through the use of probiotic[17] and prebiotic[50] approaches as a possible treatment for social symptoms associated with autism.

Additionally, there is the potential for the microbiome to influence sleep, and for sleep in turn to impact the microbiome. Disruption of circadian rhythms may affect microbial taxonomic diversity and gene expression. Specifically, circadian disruption has been shown to alter levels of microbial species and intestinal permeability.[30] Following circadian disruption in mice, Deaver et al. identified a decrease in levels of a gene involved in production of the beneficial metabolite butyrate, as well as an increase in expression of genes associated with lipopolysaccharide production and transport.[30] Butyrate and lipopolysaccharides have multiple impacts on health, with roles in systemic diseases, carcinogenesis, and inflammation.[30] Supplemental support for connections between the microbiome and sleep comes from observations that Verrucomicrobia and Lentisphaerae bacteria are more abundant in patients with better quality of sleep and higher cognitive flexibility.[4] Sleep deprivation has been associated with higher insulin resistance and a shift in the ratio of Firmicutes to Bacteroidetes.[12] Intriguingly, manipulation of the microbiota may help manage the effects of sleep loss. Studies reviewed by Farré et al. suggest probiotic and prebiotic use impacts the microbiota, and ultimately sleep architecture of the host.[39] Research in mice supports the use of probiotic supplementation with *Lactobacillus plantarum* MTCC 9510 to improve the response to sleep deprivation and stress.[33]

In a rat model of the sleep disorder obstructive sleep apnea (OSA), both probiotic treatment with *Clostridium butyricum* and the prebiotic Hylon VII helped to counteract OSA-induced hypertension.[43]

Treatment/Pharmaceuticals

The treatment for physical and psychological conditions may have just as much interplay with the microbiome as the conditions themselves. The intention of antibiotics is to decrease the quantity of bacteria and can influence the health and diversity of the microbiota long after the person has been exposed.[78] Antibiotic use and corresponding shifts in the microbiota also may increase the risk of obesity.[5,78,123]

However, many pharmaceuticals, not just antibiotics,[92] have an effect on bacterial growth. Some prescription drug regimens may induce dysbiosis, which can increase disease susceptibility.[11,78,80] Maier et al. have recently shown that mutations in an *Escherichia coli* gene for antibiotic resistance also impair resistance to human-targeted drugs (nonantibiotic drugs meant to have an effect on the host without an intention for microbiome alterations).[92] Hence one could speculate that hosts harboring antibiotic-resistant bacteria might respond differently to a range of pharmaceuticals. Nonetheless, human-targeted drug resistance does not correlate with antibiotic resistance in all cases.

Because the microbiota of the gut is at least partially responsible for dietary metabolism, it follows that intestinal microbiota may influence the metabolism and efficacy of drugs that are ingested.[82,140] Li et al. review medications such as digoxin, insulin, metronidazole, acetaminophen, and others whose metabolism is affected by the microbiota.[82] For example, methamphetamine is demethylated by Lactobacilli, Enterococci, and Clostridia, potentially causing reduction of the drug's activity.[82] Such findings suggest the potential for changes in metabolism of both illicit and prescription drugs during dysbiosis. Recently, Zimmerman et al. have developed computational strategies for disentangling host and microbiome contributions to drug metabolism.[140] By understanding the confounding variables of drug metabolism, physicians incorporating future insights from microbiome research may one day be able to prescribe with more accuracy an appropriate drug regimen.

POSSIBLE FUTURE APPLICATIONS OF MICROBIOME RESEARCH TO AVIATION SAFETY

Preflight Certification/Disease Analysis

The following paragraphs present several ideas for possible relevance of the microbiome to aerospace medicine and safety. In many areas, general scientific studies are at an early stage, requiring further research into the basic biology and associations between microbiome and health. As findings progress and become accepted by the medical community, future work will still be needed to research use and feasibility of microbiome insights in the specialized field of aviation.

Among the many potential applications to aviation safety, the microbiome may provide insights to physicians who certify pilots. Generally, civilian pilots must possess a current Federal Aviation Administration medical certificate, although a notable exception is the ability to pilot certain noncommercial flights of light sport aircraft (14 CFR § 61.23), or to operate under "BasicMed" (14 CFR § 68). As defined in 14 CFR § 1, a "medical certificate means acceptable evidence of physical fitness on a form prescribed by the Administrator." Requirements for medical certificates can be found in 14 CFR, particularly 14 CFR § 61. Standards for issuance vary with the class of certificate, as described in 14 CFR § 67. Receipt of a medical certificate as held by an aircraft pilot-in-command requires evaluation of visual, mental, neurologic, and cardiovascular standards, as well as general condition. As medical knowledge progresses, the FAA allows pilots with formerly disqualifying conditions to receive certificates in certain cases by issuing either an Authorization for Special Issuance (SI) or a Statement of Demonstrated Ability (SODA) waiver. SODAs are a one-time issuance for nonprogressive conditions, while an SI only remains valid for a defined time interval (14 CFR § 67.401).

Eventually, microbiome research may yield new tools for the certification process by identifying biomarkers for medical conditions[36,106] relevant to pilot certification. As mentioned earlier, there are currently a few commercially available stool sample tests and research correlating blood plasma metabolites and gut microbiota diversity.[133] Based on data from the 2016 Aerospace Medical Certification Statistical Handbook, cardiovascular disease is prevalent among pilots (10.42% of pilots issued a first, second, or third class medical certificate are hypertensive and medical examinations indicate 1.96% of pilots are positive for other heart pathologies).[118] Some cardiovascular conditions require additional monitoring for certification through a SODA or SI. Conditions requiring a SODA include abnormal EKG and static vascular or valve abnormalities while SI conditions include angina pectoris, coronary bypass, and stent insertion. Researchers from several countries have identified changes in the microbiome that correlate with different forms of cardiovascular disease.[67,69] Yin et al. linked an increase in *Enterobacter, Desulfovibrio,* and the phylum Proteobacteria to patients with atherosclerotic stroke and transient ischemic attack.[135] Emoto et al. identified an increase in the Firmicutes:Bacteroidetes ratio in patients with coronary artery disease but could not determine whether the dysbiosis caused the disease.[37] As research findings are validated and microbiome tests are developed, they could provide new sources of information for evaluating eligibility for a medical certificate and one day may serve as biomarkers for impairing conditions. It is also possible that they will provide a new path to eligibility by enhancing differentiation of truly dangerous conditions vs. those that do not pose a risk to pilot or passenger safety, perhaps by indicating the severity of a condition.

Beyond the potential for using the microbiome as a biomarker of conditions relevant to certification, medical advancements targeting the microbiome may need to be considered. One example of potential microbiome therapies for mental health is presented by a recently published clinical trial, in

which the authors report reduced rates of rehospitalization in patients with mania that received probiotic supplementation.[34] Mental health of pilots has received increased attention particularly in light of the 2015 crash of Germanwings flight 9525, with evidence suggesting the copilot suffered from depression.[103] As relevant clinical trials proceed in mental health and other fields, the medical certification process may need future consideration of whether novel treatments targeting the microbiome require modification of standards or requirements for special issuance.

Diabetes is yet another condition relevant to certification that may eventually have microbiome-based therapies. Based on the 2016 Aerospace Medical Certification Statistical Handbook, 1.35% of pilots with a first-, second-, or third-class certificate exhibit diabetes controlled by insulin or hypoglycemic medication.[118] One drug accepted by the FAA for treatment of type II diabetes mellitus is metformin. Metformin may be used to treat a variety of conditions, and research has begun to explore its effects in healthy organisms.[88] Ma et al. suggest use of the drug in healthy mice may result in a beneficial anti-inflammatory effect mediated by the gut microbiota, but also could induce prediabetes.[88] Based on their evaluation of previous work the authors further suggest that metformin may help return the gut microbiome of type 2 diabetes patients to a condition resembling the microbiome of a nondiabetic.[88] As research continues to identify alterations of the microbiome that correspond to disease states, novel findings may lead to improved diagnostic and therapeutic approaches, which in turn may impact medical certification decisions.

Post-Accident Health Analysis

In addition to possible roles in the medical certification process, microbiome analyses have the potential to one day improve aircraft accident investigation. Following a fatal civilian aviation accident in the United States (US), postmortem autopsy specimens of the pilot as collected by a medical examiner or coroner are shipped to the Bioaeronautical Sciences Research Laboratory of the Civil Aerospace Medical Institute (CAMI).[22,73,94] This laboratory is part of the Office of Aerospace Medicine, within the Aviation Safety line of business of the FAA. Over 10 years (2007–2016) the laboratory tested 2,909 individuals from fatal accidents.[101] FAA chemists use specimens for toxicology analyses that test for the presence of combustion gases and drugs (both legal and illicit). Findings may provide clues to assist the National Transportation Safety Board in determining factors, such as pilot impairment or incapacitation, that contributed to an accident. Postmortem analyses of the microbiome may one day augment the toolkit for these determinations. In the criminal justice system, several studies already have proposed roles for the microbiome including microbial fingerprinting, determination of postmortem interval, and use of the skin microbiome as trace evidence.[53,74,95] A new field of work is being developed exploring the postmortem microbiome, also known as the thanatomicrobiome.[19,66,129]

In aviation accident investigations, microbial activity and the microbiome also represent potential contaminants that must be considered. A long-standing challenge in aviation forensic toxicology is determination of whether measurements of alcohol represent fermentation byproducts of the microbial decomposition process, or alcohol ingested by the pilot. Although approaches have existed for over a decade to aid in this determination,[81] functional analysis of microbial metabolism or community composition may provide new ways to distinguish the source of postmortem ethanol findings. Meanwhile, preliminary research has begun to test postmortem human genetic analyses in the presence or absence of bacterial contamination.[18] Based on quantitative Polymerase Chain Reaction (q-PCR) assays, Burian et al. suggest that some human microribonucleic acid (microRNA) gene measurements may be inflated by the presence of bacterial RNA.[18] Hence, tests designed to infer human gene expression must consider sensitivity of the assay for human vs. microbial genetic material.

Perhaps more complicated than the issue of contamination is the potential for microbial metabolism to alter toxicology results or their interpretation. As aforementioned, microbiota may impact metabolism of medications consumed by their host.[82] Thus, microbial metabolism could affect the results of blood or tissue tests for drugs during postaccident analysis. Different metabolites may be present due to microbes degrading the original ingested compound, or the efficacy and/or toxicity of the drug may be altered.[82] As reviewed by Vásquez-Baeza et al., microbes can substantially impact responses to medications ranging from nonsteroidal anti-inflammatory drugs to chemotherapeutics.[128] Consequently, toxicology assays that also incorporate data regarding the presence of certain microbes may help inform determination of whether pharmaceuticals used by a pilot were efficacious in controlling a potentially impairing medical condition.

Despite the challenges presented by microbial activity, microbiome analyses may advance postmortem investigations with new ways to assess cause of death. As reviewed by Ventura Spagnolo et al., temporal shifts in the microbial community after death can guide assessment of postmortem interval, while presence of certain bacteria may indicate the cause of death.[129] For example, presence of bacteria associated with seawater may confirm a finding of death by drowning.[68,129] If validated and incorporated into aviation accident analysis, tests for these bacteria could perhaps assist investigations of aircraft accidents over seawater. Another use of the thanatomicrobiome in predicting cause of death is presented in a study by researchers in Michigan who recently completed a survey of the postmortem microbiota of an underserved, industrial-urban population.[104] Decreased microbial diversity was observed to predict heart disease in the population, based on postmortem sampling correlated with autopsy or antemortem medical history. The taxon *Rothia* appeared in higher abundance for cadavers with heart disease, and was detected 0.48-fold more often in all cases of nonviolent compared to violent death.[104] However, time of sample acquisition can be important; the authors proposed that measurements after 48 h postmortem may be less informative.[104] Proper preservation of cadavers and autopsy specimens may be essential to retain the utility of microbiome data for inferring cause of death.

Fitness for Duty

In addition to monitoring health for certification and informing accident analysis, the human microbiome may become useful in evaluating fitness for duty as part of self-certification or random screening. Currently, random drug testing is one of the key mechanisms of verifying abstinence from illicit or impairing substances. In U.S. civilian aviation, air carriers and safety-sensitive employees are subject to drug testing as described in 14 CFR § 120. Not only may drug screening assays be affected by microbial metabolism[76] as previously discussed, but also the microbiome itself may serve as a novel biomarker for detecting substance abuse.[41,131,134] Fulcher et al. discovered associations between marijuana use and increased levels of *Clostridium* cluster IV, *Ruminococcus, Solobacterium,* and *Fusobacterium*[41] and between methamphetamine use and higher levels of *Porphyromonas* and *Granulicatella.*[41] Volpe et al. identified that cocaine use was related to an increased relative abundance of Bacteroidetes.[131] Pending follow-up work to verify the strength of such correlations, it may be possible to develop tests for drug use based on the fecal microbiota. Whether such tests would prove advantageous in comparison with traditional drug screening techniques is unknown.

Drug testing is an important task for analyzing whether a pilot is fit for duty, but short-term illnesses and medical conditions also may temporarily affect the pilot's decision-making abilities and concomitantly impair that pilot's command of the aircraft. Even common conditions such as a headache may be impairing in some circumstances. For example, the International Classification of Headache Disorders describes migraines as a disabling headache disorder.[55] Migraine headaches have long been associated with high levels of nitric oxide (NO);[102] one method for production of nitric oxide involves bacterial reduction of nitrates (NO_3) and nitrites (NO_2).[124] Gonzalez et al. reported different levels of bacteria that may contain genes for nitrate and nitrite reduction in migraine sufferers vs. individuals without migraines.[48] Tests for these bacteria or their genes one day may improve understanding of the condition. Another potentially incapacitating illness that can alter oropharyngeal microbiota is influenza. A study by Ramos-Sevillano et al. subjected 52 volunteers to influenza by intranasal inoculation and discovered increasing levels in Actinobacteria up to 6 d post infection when compared to the patient's preinfection microbiota levels.[107] Levels of the bacteria returned to baseline preinfection levels by day 28.[107] Such studies ultimately may guide the development of new diagnostic tools for the presence of incapacitating conditions.

Because insufficient sleep is associated with neurobehavioral performance deficits, microbiome-guided tests for impairment following sleep loss could provide additional evidence of fitness for duty. Currently, 14 CFR § 117 specifies rest requirements and reflects the importance of flight crew not being too fatigued for safe operations. Future assays of microbiome shifts that correlate with fatigue could perhaps help in fatigue risk management strategies. Although one recent study suggested that sleep restriction does not substantially impact composition of the human microbiome,[138] overall the field appears to be moving toward acknowledgment of bidirectional interactions between host sleep and the microbiome.[39] As previously stated, a proposed effect of sleep deprivation related to dysbiosis of the fecal microbiome involves a dysregulation of the phyla Firmicutes and Bacteroidetes.[12] The gastro-intestinal tract has its own diurnal fluctuation. Studies reviewed by Asher and Sassone-Corsi reveal a role of the gut microbiota in appropriate function of intestinal circadian rhythm and, in turn, oscillations in levels of gut microbes in response to the gut's circadian cycles.[7] With increased research, in the future there may be a way to analyze shifts in the microbiome as an indicator of impairing levels of sleep loss or circadian disruption.

PASSENGERS AND THE BUILT ENVIRONMENT

Impacts of Travel

Although not unique to travel by air, differences in the microbial community at the source vs. destination environment of the traveler may directly expose passengers and crew to new microorganisms. Factors such as urbanization and climate can influence the local microbial community.[8] In a study by Chase and colleagues, office microbial samples across different cities were sufficiently distinct to allow prediction of the city from which the sample was taken.[21] Gupta et al. collected information on microbiome diversity in many different countries.[52] The population of less industrialized countries had a significant increase in taxonomic diversity, which the authors suggest may be related to certain disease susceptibilities.[52] Not only can there be global differences in the microbial community at different locations, but also differences in the abundance of specific pathogenic or antibiotic-resistant bacteria. Nordahl Petersen et al. analyzed toilet waste for selected pathogens and known antimicrobial resistance genes on long-distance flights arriving in Denmark.[100] Differences were found among flights departing from South Asia, North Asia, and North America, with flights from Asia containing more antibiotic resistance genes. Flights from South Asia had a higher abundance of the human pathogen *Salmonella enterica* and more noroviruses of genotype GII, but a lower abundance of *Clostridium difficile.*[100] Altogether, the relative ease of long-distance transportation afforded by air travel may expose passengers and crew to a new microbial community, including new pathogens.

Whether it be from exposure to new microbes or other mechanisms, microbiome disruption and particularly diarrhea frequently have been associated with travel. As many as 60% of individuals from industrialized countries that travel to developing countries develop diarrhea.[136] It is estimated that 29% of U.S. Department of Defense personnel deployed to a developing country experience diarrhea, and research is being conducted on the gut microbiome to identify a prophylactic treatment.[6] Attempts have been made to associate disease with distinct microbial community changes. In a study of the gut microbiome of healthy travelers and those that developed diarrhea after traveling from the United States to India or Central America, Youmans et al. found a lower Bacteroidetes:Firmicutes ratio in those with traveler's diarrhea.[136] The healthy travelers also

possessed a different ratio relative to a healthy comparison group from the Human Microbiome Project, which the authors interpreted as the potential for even those without diarrhea to experience travel-associated dysbiosis.[136] Yet in another longitudinal study, one traveler who experienced diarrhea while visiting a developing country exhibited the opposite trend, with an increased ratio of *Bacteroides*:Firmicutes.[26] Although these and other conflicting findings shed doubt on the utility of the Bacteroidetes:Firmicutes ratio as an indicator of functional bowel dysbiosis, research continues to progress on tools such as probiotics for treating traveler's diarrhea.[38] Moreover, advances have been made in understanding the mechanisms by which the host microbiome inhibits colonization by pathogens.[130]

The Built Environment: Airplane Cabin and Airport Terminal

The cabin environment may have unique impacts on passengers' microbiota, beyond the more general conditions associated with travel. The microbiota inhabiting indoor structures occupied by humans, and the accompanying microbiome of this "built environment," can be impacted by a variety of factors. In their review, Gilbert and Stephens note that the indoor air microbiota is influenced by the microbiota of the outdoor air, especially with higher levels of ventilation.[46] Higher airflow ventilation has been shown to decrease indoor pathogenic load.[72] Yet in an airplane cabin environment, little is known of the extent to which air exchange could bring onboard new microbes from the upper atmosphere, let alone whether such microbes would be viable or have any impact on passengers and crew. Although airborne dust and associated microbes can travel across continents,[2,8,49] a study of particles with diameters from 0.25 to 1 μm in the upper troposphere reported that only 20% of the particles represented viable bacterial cells.[32] High-energy particulate air (HEPA) filtration of cabin air will remove some microbes.[27]

While few publications exist on the microbiota of airplanes, a recent study of the airplane cabin microbiota assessed over 200 samples from 10 transcontinental U.S. flights.[132] Weiss et al. reported immense variation among individual airplanes, but no systematic pattern of changes in the microbial community before and after the flight.[132] This finding contrasts with an earlier study reporting an increase in microbes from the time of boarding up to midflight, and then a decline starting with the plane's descent.[77] In the work by Weiss and colleagues, most of the microbial community consisted of nonpathogenic microbes or human commensals.[132] Members of the genera *Propionibacterium* and *Burkholderia* were found in all samples, while *Staphylococcus and Streptococcus* occurred in all save one sample; collectively these were characterized as a "core" airplane cabin microbiota.[132] Importantly, the authors concluded that 4–5 h in an airplane cabin did not engender any greater risk to the human occupant than did an equal amount of time in an office environment.[132]

Yet much remains unknown about the microbiome of the airplane built environment and the impact of flight on the human microbiome. Factors such as cruising altitude may affect the cabin microbiome and the microbiome of its human occupants. Indeed, research has suggested potential distinctions in the microbiome among persons living at different altitudes.[83] Further study is needed to determine whether an airplane's brief duration at cabin altitude influences the microbiome of passengers and crew. Moisture levels and features such as material use and ventilation in built environments may be worth consideration with regard to potential impacts on the microbiome.[46,132] Furthermore, Weiss et al. suggest that large-scale differences in cabin microbiota across airplanes may reflect retention of the microbiota from previous passengers, and that improving cleaning regimens could be a preventative measure to address disease transmission.[132] Cleaning regimens could also consider the potential for biofilm formation in cabin environments.[28] Unique aviation environments such as the International Space Station (ISS) and space shuttles also require consideration, and studies relevant to microbes in space have been conducted on topics ranging from detection methods to the core microbiome and biofilms on the ISS.[79,97]

Whereas the cabin microbiome is a relatively new area of research, potential spread of pathogenic microbes among onboard occupants and the cabin air quality have been topics of several investigations. Numerous studies on the cabin air environment have been supported by the U.S. Federal Aviation Administration,[3] including research on infectious disease transmission onboard.[24] Recent work sponsored by Boeing has combined observations of occupant movement on 10 transcontinental flights in the United States with modeling of respiratory disease transmission.[58] Despite the fact that 8 of the 10 flights studied by Hertzberg and colleagues occurred during influenza season, none of their 228 samples of surfaces and cabin air tested positive for 18 common respiratory viruses.[58] Models suggested that droplet-mediated respiratory disease was unlikely to be spread from an ill passenger to those more than one row ahead or behind the individual, but that an infectious flight attendant would have the potential for initiating several infections.[58] Similarly, prior work suggests a small (roughly 2%) risk to passengers seated more than two rows away from an ill individual.[57] Acknowledging that risks vary among diseases with different biological characteristics, other modeling efforts noted the potential for travel by air to facilitate infectious disease transmission nationally and internationally.[115] Ikonen et al. studied deposition of pathogens in the airport and found that, while only 10% of surface samples contained a respiratory virus, the highest rate of samples containing a respiratory virus was on bins in the security line.[63] Although it may be impossible to completely eliminate the spread of infectious microbes onboard an aircraft or in the airport, further investigations drawing upon general methods for influencing the indoor built environment microbiome may guide strategies to enhance aviation travel safety.

CONCLUSION

Research is rapidly uncovering the intricate relationships between microbes and their hosts, with potential for greatly advancing understanding of human health. As scientific advances continue and are validated, it is worth considering the potential

for incorporating such findings in the fields of aerospace medicine and human performance. This review has presented several ways in which microbiota may affect the flying community through potential impacts on health, cognition, and operators' ability to perform their duties. While much of this report has discussed relevance of the microbiome for pilots and passengers, many of the themes can apply to anyone in a safety-critical role. Currently, there is a limited understanding of the specific connections between microbiotic changes and safety-critical health conditions. With increased study medical professionals may better understand how diversity of the microbiotic flora in the body can influence the progression of diseases or conditions relevant to safe operations, and what treatments to pursue. Although beyond the scope of this review, ultimately microbiome research and its application will require careful consideration of ethical, legal, and social implications.[93,108,109]

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