Review Article

The worker microbiome and envirobiomes in the agriculture industry: a narrative review of current applications and understanding for worker health

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Background and Objective: The human microbiome is recognized as essential for the maintenance of human health, but research suggests that chemical and biological [e.g., viruses, such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)] exposures can elicit perturbations in the human microbiome communities. As demonstrated with the coronavirus disease 2019 (COVID-19) pandemic, the occupational environment impacts worker health in numerous ways, one of which may be through changes to the worker microbiome. Particularly, the agricultural environment presents both chemical and biological hazards (i.e., workplace environmental microbiome or envirobiome) that may affect the worker microbiome and ultimately worker health. The state of the science regarding the potential for the agricultural environment to impact worker microbiomes is presented herein.

Methods: A literature search using the PubMed database and the WorldWideScience and Google Scholar search engines was performed to identify English-language, peer-reviewed literature published on or before August 3, 2022 that evaluated microbiomes in the agricultural occupational setting.

Key Content and Findings: A total of 24 relevant publications meeting the inclusion criteria were identified in which the agricultural worker microbiome and/or envirobiome were assessed. The state of the science in this area was evaluated based on the methods and findings of these studies. Several themes emerged regarding the nature of the available of data. Most identified studies focused on animal exposures (especially swine) with very limited data regarding chemical exposures in the agricultural environment. The microbial identification methods employed were largely dominated by 16S sequencing, and the most common worker microbial communities examined were the nasal and gastrointestinal microbiomes. Nearly all studies examined the bacterial microbiome, with few studies examining the fungal or viral envirobiomes or worker microbiomes.

Conclusions: The impact of exposure to chemical and biological agents on the worker microbiome is becoming a central focus of research, including workers in agricultural operations. Based on this review, numerous studies evaluated the worker microbiome or envirobiome among agricultural workers; however, few studies have evaluated both simultaneously. The relationship of the agricultural worker microbiome and envirobiome with worker health outcomes remains understudied. Moving forward, research needs to

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assess the totality of workplace exposures while simultaneously assessing agricultural worker microbiomes holistically. Further, research should consider exposures, lifestyles, and diets outside of the workplace to fully understand short-term and chronic microbiome impacts and subsequent health effects.

Keywords: Microbiome; agriculture; occupational exposure; worker; envirobiome

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Introduction

Background

The human body contains a myriad of microbial communities whose individual functions impart the regulation and convergence of human biology, health, development, and disease resistance (1). Studies have estimated that the human body contains 3.0×10^{13} microbes, approximately the same as the number of human cells (2,3). These distinct microbiomes exist on or in body regions including the skin, sex organs, lungs, stomach, mouth, and nose. Each region has a unique microbial profile existing symbiotically with localized human cell lines and fulfilling a functional niche (3,4). These symbiotic relationships have evolved to provide essential functions that maintain human health and include provision of essential nutrients, regulation of the immune system, protection against infection, contributions to mental health, and regulation of metabolism, among others (4-7). Perturbations in microbial communities can alter their composition and related functions; however, not all changes in microbial communities result in physiologically meaningful changes for the host (8). Thus, a major challenge in the study of human microbiomes is the difficulty in defining adversity or dysbiosis. One must consider whether changes in microbial diversity or communities are beneficial, detrimental, or neutral to the host; however, the complexity of microbial communities, their multidirectional relationship with the host, and the relationships between microbes within the community complicate such evaluation, especially when also factoring temporal impacts (3,4,9,10). The large interindividual variability in microbial community diversity and structure that is considered "healthy" adds additional challenges (4,11,12). However, the community composition of microbiomes within each individual remains relatively constant over time, as a "healthy" community generally demonstrates resilience (4), highlighting the importance of collecting baseline microbiome data in studies.

Altered human microbiomes are associated with various diseases, including inflammatory bowel disease, Parkinson's disease, asthma, obesity, and cancers, among others (3,5,10). The coronavirus disease 2019 (COVID-19) pandemic increased awareness that pathogenic infections, such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), are associated with altered microbial diversity, given that a healthy microbiome contributes to immune function and acts as a viral barrier (7,13,14). The oral, intestinal, and respiratory microbiomes of patients with COVID-19 have been associated with an increase in pathogenic bacteria and decreases in beneficial bacteria, some changes of which were associated with increased severity of disease and susceptibility to secondary infections (15,16). Further, a healthy microbiome supplemented with postbiotics is hypothesized to be associated with less severe COVID-19 disease (7). Finally, the increased use of antimicrobial agents and cleaning products as a result of COVID-19 has raised awareness for the potential impacts of such products on human microbial communities (17).

Rationale and knowledge gap

Recent advancements in clinical microbiology and evolutionary biology demonstrated the necessity of the human microbiome in healthy living and human survival. Investigations to evaluate the impact of everyday and situationally specific exposures from the environment on the human microbiome are underway, but the burden is great considering the wide variety of exposures, including the outdoor, indoor, or occupational environment, diet, and various consumer products, that may have a positive, negative, or neutral impact on all or a part of the human microbiome.

The use of pre/post/pro-biotics, dietary regimens, personal care products, cleaning products, and antimicrobial products that may impact the human microbiome is under personal control; however, there are a variety of factors outside of an individual's control that may affect the diversity and function of the microbiome. Specifically, occupational exposures remain largely outside of an individual's control, yet few studies have evaluated their potential to alter worker microbiomes and worker health (10). Therefore, microbial health remains an understudied and inadequately characterized endpoint and potential biomarker for industrial hygiene and occupational safety and health practitioners.

The occupational environment can influence the health of workers in numerous ways. Although certain injuries or illnesses are required to be reported in various countries, not all are realized as such and thus are underreported across the globe. Further, alterations to worker microbiomes are not considered reportable illnesses, and the potential role of the microbiome to contribute to worker illnesses is understudied. Emerging areas of study regarding worker health include "One Health," which focuses on the interdisciplinary opportunities to improve health across the shared environments including humans, animals, plants, and the environment, which encompasses the occupational environment, as well as Total Worker Health® (TWH), an area of focus which holistically incorporates a multitude of health hazards that could impact workers not directly related to the work tasks, but rather from other factors such as sick leave, shift work, etc. (18,19). Combined with the scientific research examining the connection between the microbiome and human health via holistic and comprehensive approaches, it is within these emerging areas where findings of microbiome fluctuations or trends in workers could positively benefit the ongoing efforts to improve worker health.

The impacts that microorganisms may have in influencing occupational health has been widely discussed, with growing attention among those in the agricultural sector (10,20,21). Animal exposure is likely a source of microorganisms that colonize workers (10). High bacterial loads at livestock farms have been reported as a potential concern to workers and the general population, raising biosecurity concerns and the looming threat of another pandemic (10,21,22). The nature of agricultural operations presents opportunities for human and animal exposure to a wide range of microorganisms and their constituents (e.g., toxins, lipopolysaccharides) and may foster a microenvironment for virulent microorganisms to evolve. Existing research in this area has largely focused on the characterization of environmental microbes (bacteria, fungi) and organic constituents (proteins, toxins) to which workers are exposed rather than alterations of the human microbiome as a direct result of exposure (10,23,24). However, studies of children raised on farms with animal exposure have reported a protective effect on development of asthma and allergies (i.e., the hygiene hypothesis), as well as increased microbial diversity that may protect from pathogenic bacteria (25). Therefore, additional studies are needed to evaluate the nature of the effects of animal exposure on the microbiome and health of agricultural workers, who may not have grown up in with such exposures. The occupational environment among agricultural operations also includes various chemicals and antimicrobial agents, such as fertilizers, pesticides, and antibiotics, to which a worker may be exposed via liquid and aerosols. In fact, several recent scientific publications evaluated the association between pesticide exposure and changes in human gastrointestinal microbiomes (9,26). However, data gaps persist surrounding the impact of both the biological and chemical exposures in the agricultural occupational environment on the human microbiome and the development of short-term or chronic health effects.

Objective

The purpose of this review is to evaluate and present the state of the science regarding the relationship between agricultural work, environmental microbiomes (herein called envirobiomes), and the worker microbiome to identify future challenges and needs in order to protect worker health. We present this article in accordance with the Narrative Review reporting checklist (https://jphe.amegroups.com/article/view/10.21037/jphe-23-11/rc).

Methods

A literature search of peer-reviewed, English-language literature was conducted on August 3, 2022 in the PubMed database and the WorldWideScience and Google Scholar search engine in order to identify studies that examined agricultural worker microbiomes. Three sets of search terms were utilized in each database/search engine in order to capture all relevant studies relating to various sectors of agricultural work: ("agriculture" OR "agricultural") AND ("microbiome" OR "microbiota") AND ("occupation*" OR "worker"); ("agriculture" OR "agricultural" OR "farmer*") AND ("microbiome" OR "microbiota") AND ("occupation*" OR "worker"); ("microbiome" OR "microbiota") AND ("occupation*" OR "worker"). The search strategy is outlined in *Table 1*.

Table 1 The search strategy summary

Items	Specification
Date of search	August 3, 2022
Databases and other sources searched	PubMed, WorldWideScience, Google Scholar
Search terms used	(“agriculture” OR “agricultural”) AND (“microbiome” OR “microbiota”) AND (“occupation*” OR “worker”); (“agriculture” OR “agricultural” OR “farmer*”) AND (“microbiome” OR “microbiota”) AND (“occupation*” OR “worker”); (“microbiome” OR “microbiota”) AND (“occupation*” OR “worker”)
Timeframe	Published on or before August 3, 2022
Inclusion and exclusion criteria	Only English language peer-reviewed original research or review articles published on or before August 3, 2022 were included. Articles that did not evaluate the worker microbiome and/or envirobiome in an agricultural setting were excluded. No additional restrictions were placed
Selection process	Papers that met the inclusion and exclusion criteria were screened first by title and then abstract to exclude literature that did not pertain to agricultural worker microbiomes. Relevant studies were de-duplicated across database/search engine results prior to a full review of each selected study
Any additional considerations	Due to the large number of studies returned by Google Scholar, the first 100 results were screened and the most general search string, (“microbiome” OR “microbiota”) AND (“occupation*” OR “worker”), was not used. Additionally, the Google Scholar search included (“agriculture” OR “agricultural” OR “farmer*”) AND (“microbiome” OR “microbiota”) -bee in order to screen out studies related to the microbiome of honeybees

Discussion

There is growing understanding and awareness of the occupational environment as a contributor to worker health and the importance of the microbiome in human health, particularly in light of the COVID-19 pandemic. Recent research has evaluated how occupational exposures (e.g., chemical and biological) that can affect worker microbiomes (10,20). Across workplaces, agricultural workers represent an important sector in which there are both chemical (e.g., pesticides, antibiotics) and biological (e.g., livestock and associated vector-borne diseases) exposures that may impact worker microbiome communities, which subsequently may impact worker health. In this review, we examined the current state of the science regarding how chemical and biological factors may affect agricultural worker microbiomes, and any associations between such exposures and microbial changes with worker health outcomes (*Table 2*).

Characterizing the microbiome

The interpretation and comparison of microbiome studies is complex, given that the various diversity metrics (e.g., types of alpha and beta diversity), identification processes [e.g., operational taxonomic units (OTUs) and amplicon sequence variants (ASVs)], and methods of sequencing often influence the results and prevent direct comparison of studies (51). Studies identified in this review employed a variety of methods to evaluate the microbiomes of agricultural workers: culture, quantitative polymerase chain reaction (qPCR), 16S/ITS1 sequencing, metagenomic sequencing, and metatranscriptomics sequencing. Culture and qPCR methods identify a specific organism or groups of organisms and are often influenced by an *a priori* hypothesis regarding specific, and sometimes rare, microbes expected to be present or changed (37,38). High throughput sequencing (HTS) methods capture a more holistic and comprehensive view of the microbial diversity (16S), the microbial diversity and the potential functions (metagenomics), and the microbial diversity and the function based on expressed messenger RNA (mRNA) (metatranscriptomics) (52). However, rarer taxa may be more difficult to identify with HTS methods (37,38,53,54). Further, standard HTS does not distinguish viable from nonviable microbes, which may be problematic given that one study reported only 10% of the total bacteria sequenced were viable (55-57). The use of different methods can therefore yield different results. For

Table 2 Summary of study design for identified studies that examined the agriculture worker and/or environmental microbiomes

Reference	Study design	Location	Location/worker population (sample size)	Worker microbiome(s) sampling site and type	Environmental microbiome(s) sampling site and type	Microbial methods	Other environmental factors examined
Boissy *et al.* 2014 (27)	Cross-sectional	U.S.	Swine confinement facilities (N=2) Grain elevators (N=2) Pet-free homes (N=2)	N/A	Site: swine facility surface dust, grain elevator dust, and pet-free home dust Type: bacterial, archaea, fungal, and viral	Shotgun metagenomic sequencing	Exposure to swine and grain dust
Duarte *et al.* 2020 (28)	Cross-sectional	Europe (unspecified country)	Swine farmers, family members, employees (N=54) Broiler farmers, family members, employees (N=24) Swine slaughterhouse workers (N=70)	Site: gastrointestinal Type: bacterial and resistome	Site: gastrointestinal samples of swine, broiler chicken, turkey, and cow Type: bacterial and resistome	Shotgun metagenomic sequencing	Exposure to livestock (swine, broiler, turkey, cow) and antimicrobial use
Ingham *et al.* 2021 (29)	Longitudinal cross-sectional	Denmark	Swine truck drivers (N=47) MRSA + swine truck driver spouses (N=6) Controls from Dutch Lifelines Biobank (N=89)	Site: nasal Type: bacterial	Site: swine truck debris Type: bacterial	16S sequencing, shotgun metagenomic sequencing of culture, and qPCR	Exposure to swine and MRSA prevalence
Islam *et al.* 2020 (30)	Longitudinal	Denmark	Swine farm workers (N=16) Swine farm volunteers (N=8)[†]	Site: nasal Type: bacterial	Site: swine nasal and dermal Type: bacterial	16S sequencing, culture, and PCR	Time of day and duration of exposure
Kates *et al.* 2019 (31)	Cross-sectional	U.S.	Livestock workers (N=26) Non-livestock workers (N=33)	Site: nasal and oral Type: bacterial	N/A	16S sequencing	Exposure to livestock (swine, cattle, poultry, sheep, horses, goats) and presence of pathogenic organisms
Kraemer *et al.* 2018 (32)	Cross-sectional	Switzerland	Swine farmers (N=43) Cow farmers (N=17); considered a control Controls of non-animal exposed individuals (N=26)	Site: nasal Type: bacterial	Site: swine nasal and environmental aerosol Type: bacterial	16S sequencing	Exposure to livestock (swine, cows)
Kraemer *et al.* 2019 (33)	Longitudinal cross-sectional	Switzerland	Swine farmers (N unclear)[‡] Cow farmers (N unclear) Non-exposed controls (N unclear)	Site: nasal Type: bacterial	Site: swine nasal and environmental aerosol Type: bacterial	16S sequencing and qPCR	Seasonality and exposure to livestock (swine, cows)
Kraemer *et al.* 2021 (34)	Longitudinal	Switzerland	Swine farm employees/ swine farmers undergoing change of occupation (N=7) Cow farmers (N=12) Officer workers as controls (N=19)	Site: nasal Type: bacterial	N/A	16S sequencing	Exposure to livestock (swine, cow) and change of occupation
Li *et al.* 2018 (35)	Cross-sectional	China	Herdsmen (N=24)	Site: gastrointestinal Type: bacterial	N/A	16S sequencing	Urbanization and diet
Luiken *et al.* 2020 (36)	Cross-sectional	Europe (9 countries)	Swine farmers, partners, family members, and workers (N=54) Broiler farmers, partners, family members, and workers (N=25) Controls from Dutch Lifelines Biobank (N=46)	Site: gastrointestinal Type: bacterial and resistome	Site: swine farm dust, broiler chicken farm dust, and gastrointestinal samples of swine and broiler chicken Type: bacterial and resistome	Shotgun metagenomic sequencing	Antimicrobial usage
Mbareche *et al.* 2019 (37)	Cross-sectional	Canada	Dairy farms (N=5)	N/A	Site: environmental aerosol Type: fungal	ITS1 sequencing, qPCR, and culture	Type of milking, animal confinement space, cattle feed type, ventilation, temperature, seasonality, and time of day

Table 2 (*continued*)

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Reference	Study Design	Location	Location/worker population (sample size)	Worker microbiome(s) sampling site and type	Environmental microbiome(s) sampling site and type	Microbial methods	Other environmental factors examined
Mbareche *et al.* 2019 (38)	Cross-sectional	Canada	Swine workers (N=25) WWTP workers (N=12) Control sample of university students and workers (N=29)	Site: nasal Type: bacterial	Site: swine barn air and WWTP air Type: bacterial	16S sequencing and qPCR	Exposure to swine
Moor *et al.* 2021 (39)	Cross-sectional	Switzerland	Swine farm workers (N=59) Cow farmer controls (N=22)	Site: gastrointestinal Type: bacterial	Site: swine barn air and swine Type: bacterial	16S sequencing	Antimicrobial treatment of swine and exposure to livestock (swine, cows)
Peng *et al.* 2020 (40)	Cross-sectional	U.S.	Dairy and integrated farm workers (N=20) College students as controls (N=10)	Site: skin Type: bacterial	N/A	16S sequencing	Exposure to livestock (cow and various animals)
Shale *et al.* 2006 (41)	Cross-sectional	South Africa	Red-meat abattoirs (N=4)	N/A	Site: environmental aerosol Type: bacterial	Culture	Temperature
Shukla *et al.* 2017 (42)	Cross-sectional	U.S.	Dairy farmers (N=21) Office workers as controls (N=18)	Site: nasal and oral Type: bacterial	N/A	16S sequencing	Exposure to cows, pet ownership, and hygiene
Stanaway *et al.* 2017 (43)	Longitudinal	U.S.	Farmworkers (N=65) Non-farmworkers (N=52)	Site: oral Type: bacterial	N/A	16S sequencing	Pesticide concentration in blood and seasonality
Sun *et al.* 2017 (44)	Cross-sectional	China	Swine farm workers (N=6) Local villagers (N=6)	Site: gastrointestinal Type: bacterial and resistome	Site: swine gastrointestinal Type: bacterial and resistome	16S sequencing and RT-PCR	Exposure to swine
Sun *et al.* 2020 (45)	Longitudinal	China	Veterinary students conducting internships at swine farms (N=14) Swine farm workers (N=4 or 5/farm)	Site: gastrointestinal Type: bacterial and resistome	Site: swine gastrointestinal, soil, sewage, and ventilation dust Type: bacterial and resistome	16S sequencing, shotgun metagenomic sequencing, and culture	Duration of swine farm work, diet, and work stress
Tan *et al.* 2020 (46)	Cross-sectional	Malaysia	Swine farmers (N=17) Non-farmer human controls (N=16)	Site: gastrointestinal Type: bacterial	Site: swine gastrointestinal Type: bacterial	16S sequencing	Exposure to swine
Van Gompel *et al.* 2020 (47)	Cross-sectional	Netherlands	Swine farmers, family members, employees (N=54) Broiler farmers, family members, employees (N=24) Swine slaughterhouse workers (N=483) Controls from Dutch Lifelines Biobank (N=46)	Site: gastrointestinal Type: bacterial and resistome	N/A	Shotgun metagenomic sequencing	Exposure to livestock (swine, broiler)
Vestergaard *et al.* 2018 (48)	Longitudinal cross-sectional	Denmark	Swine stables, farmer homes, and suburban homes (N unclear)[§]	N/A	Site: environmental aerosol of swine stables, swine farmer homes, and suburban homes Type: bacterial	16S sequencing	Exposure to swine, temperature, and seasonality
Wang *et al.* 2021 (49)	Cross-sectional	China	Live broiler market workers (N=18) Controls (N=18)	Site: gastrointestinal Type: bacterial and resistome	Site: broiler gastrointestinal from farm and broiler market, and pooled environmental samples (feces, cages, soils, sediment, wastewater, chopping boards) Type: bacterial and resistome	Shotgun metagenomic sequencing	Exposure to broilers and genetic factors
Wu *et al.* 2020 (50)	Cross-sectional	China	Swine slaughterhouse workers (N=6) Swine farmers (N=6)	Site: nasal Type: bacterial	Site: environmental aerosol from swine farms and slaughterhouses Type: bacterial	16S sequencing	Exposure to swine

[†], control volunteers included from a separate study; [‡], study reported a total number of samples (N=609) between workers, controls, and environmental, but did not provide breakdown; [§], study included 267 samples in total, with sample sizes varying with location and season. The number of individual swine stables, farmer homes, and suburban homes was not clear. N/A, not applicable; qPCR, quantitative polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; MRSA, methicillin-resistant *Staphylococcus aureus*; WWTP, wastewater treatment plant.

example, no significant differences in the fungal diversity of the air between dairy farms were observed through culture and qPCR, but HTS revealed differences in beta diversity of the air between dairy farms with different animal space and cattle feed practices (37). Therefore, study purpose and methods are essential in contextualizing the results and comparing them to other studies.

The understanding of what makes a microbiome "healthy" is still a topic of debate. Some researchers propose that there are several discrete community compositions or structures that demonstrate common traits, called enterotypes, and all result in a "healthy" microbiome functioning (11,12). Others proposed that the Anna Karenina Principle applies, which asserts that all "healthy" microbiomes are alike and their dynamics are driven by dominant species, whereas each "unhealthy" microbiome is uniquely "unhealthy" and that all species, not just dominant ones, assert effects, which further emphasizes that disruptions of an individual's microbiome are the result of their unique exposure profile (58). Additionally, it is proposed that microbiome health exists on a continuum, whereby discrete enterotypes do not exist (59). Without agreement on the composition and diversity of a "healthy" microbiome, defining and identifying an "unhealthy" microbiome is difficult (60).

Overview of peer-reviewed publications

Currently, we identified studies that evaluated (I) the worker microbiome(s), (II) the envirobiome(s), or (III) both worker microbiomes and envirobiomes across the agricultural sectors. Of the identified 24 studies, 13 examined both an environmental and worker microbiome (28-30,32,34,36,38,39,44-46,49,50).

The studies were generally limited to characterization of the agricultural envirobiome or worker microbiome and were largely cross-sectional in nature, which are not designed to capture temporal changes in the microbiomes and may be confounded by non-representative samples (61). Only seven studies were identified that sampled the microbiome across timepoints (29,30,33,34,43,45,48). Two of these studies evaluated the temporal dynamics in a short-term manner (i.e., hours to days) (29,30) and only one study evaluated the change in microbiomes over months (45). Additionally, three studies focused on determining the effect of season on agricultural workers' microbiomes (33,43,48). Further, only one study evaluated the effects of cessation of work on the microbiome, specifically nasal (34). However, no studies were identified that evaluated the effect of chronic agricultural work on worker microbiomes, particularly those that captured baseline microbial community data. Lastly, no studies examined any correlations in microbial diversity shifts to worker health outcomes, with only one study surveying workers for health outcomes at all (31). Therefore, the current state of the science lacks data to establish causal correlations from exposure to biological and/or chemical exposures to changes in worker microbiomes, let alone to changes in worker health outcomes, such as changes in susceptibility to infections (e.g., those caused by coronaviruses) or development of chronic diseases.

Much of the existing literature regarding agricultural work and changes to the worker microbiome evaluated biological (i.e., livestock) exposures. The most studied agricultural worker population was those that work with swine, with 17 of 24 studies identified examining swine workers and/or swine farm or transportation environments (*Table 2*) (27-34,36,38,39,44-48,50). Fewer studies examined cows/dairy (9 of 24) (31-34,37,39-42) and broilers (4 of 24) (28,36,47,49), both of which were often used control groups relative to swine exposed farmers. Across the studies that examined biological exposures, six looked at the potential for the worker microbiome to be affected by the animal and/or environmental resistome (28,36,44,45,47,49). Only one study evaluated the potential for pesticide and other chemical exposures to affect the worker microbiome (43).

Certain worker microbiomes have been studied more extensively than others. The gastrointestinal and nasal microbiomes were the most well studied, with nine studies examining gastrointestinal microbiomes (28,35,36,39,44-47,49) and nine examining nasal microbiomes (29-34,38,42,50). Three studies examined the oral microbiome of agricultural workers (31,42,43). Only one study examined the skin microbiome (40), and four did not examine any worker microbiomes (only envirobiomes) (27,37,41,48). The ecology of the human microbiome is complex, owing to interactions between microbes in a particular community, between different microbial communities in the human body, and between these communities and the environmental microbial communities (1,62). Two studies examined two different worker microbial communities, but the potential interactions between these communities were not explored (31,42). Despite knowledge that human microbiomes do not exist in isolation and interact with one another and the human body, the current understanding of how such interactions occur is relatively understudied, particularly in agricultural workers. These uncertainties are further compounded by the potential role and impact of environmental exposures to chemical and biological agents inside and outside of the workplace, emphasizing the need to simultaneously evaluate microbiome alterations and environmental exposures.

Agricultural envirobiome & worker microbiomes

The envirobiome consists of the microbes that exist outside of the human body. Humans constantly interface with envirobiomes, at home, at work, and in their communities (63). The agricultural worker may be affected by envirobiomes through interacting with livestock, breathing the ambient air, or contacting surfaces. In total, 17 of the 24 identified studies examined some aspect of the envirobiome in the agricultural setting (27-30,32,33,36-39,41,44-46,48-50). Furthermore, 20 of the 24 studies identified examined some aspect of the agricultural worker microbiome across livestock and non-livestock operations (28-36,38-40,42-47,49,50). However, most of these studies did not evaluate short-term or chronic health effects following alterations in the workers microbiome.

Of seventeen studies that assessed the envirobiome, fifteen examined only the bacterial envirobiomes. The bacteria, archaea, fungal, and viral envirobiome was examined in the dust of swine containment facilities and grain elevators, and although the communities from both were dominated by bacteria, it was reported that there were 30-times lower fungal species from the swine envirobiome than the grain envirobiome (27). Another study was identified in which only the fungal components of the envirobiome of agricultural workers, specifically dairy farmers, were analyzed; known pathogenic fungi were identified in aerosol samples and associated with particular work tasks (37). Previous research has demonstrated that the fungal community in home dust samples is more affected by environmental factors (e.g., season) than the bacterial community (64). However, given the substantial difference in the indoor environment between a residence and agricultural operations, direct comparisons and parallels should not be made. The paucity of studies that examine the fungal and viral envirobiomes in agricultural settings represents a large gap in the current breadth of knowledge. Particularly, the COVID-19 pandemic and spread in workplaces raises the concern for spread of emerging pathogens from and within workplaces.

The agricultural envirobiome samples were obtained from livestock gastrointestinal, nasal, and dermal samples, aerosols, and various debris (*Table 2*). The most well-studied of the agricultural envirobiomes were those related to swine farming. Swine confinement area samples were dominated by different bacterial taxa, had more archaea, and less eukaryotic DNA than grain elevator air samples (27). Although swine microbiomes were demonstrated to be farm-specific, air microbiomes at swine farms were not (39). Kraemer *et al.* (32) identified 82 ASVs associated with swine farming among samples of swine nares, swine farm air, swine farmers, cow farmers, and non-exposed controls. The authors reported that aerosol samples from the swine farms (i.e., envirobiomes) were dominated by Firmicutes and Bacteroidetes, as were the nasal microbiomes of farmers (38,39); non-exposed controls had a lower relative abundance of Firmicutes and were dominated by Actinobacteria and Proteobacteria (38). Further, swine nasal and skin microbiomes were found to be largely dominated by the order Pseudomonadales (genera *Pseudomonas* and *Acinetobacter*), establishing a link between worker exposure to the envirobiome and their individual microbiome (30). Worker behavior and tasks may impact their envirobiome exposure. Therefore, it is necessary for researchers to conduct a comprehensive assessment of the envirobiome simultaneously with the worker microbiome.

Several studies found that the worker nasal microbiome overlapped with that of the swine microbiome and envirobiome of the air sampled at swine farms, although it was often reported that the community composition was heterogenous between workers (30,32,38). Additionally, Kraemer *et al.* (32) reported greater diversity among swine worker nasal microbiomes than non-farmers, whereas the nasal microbiomes of cow farmers were found to be more similar to non-exposed individuals, which further demonstrates that different agricultural operations may result in different impacts on the workers microbiomes (32). Kraemer *et al.* (32) and Moor *et al.* (39) have both suggested that the envirobiomes on swine farms may have a larger effect on the worker microbiomes than the microbiomes from other types of agricultural practices. This suggests an association between agricultural operation and impact of exposure. As such, it is a necessity to evaluate the potential impact associated with the unique exposure profile among each type of agricultural operation.

For the gastrointestinal microbiomes, the beta diversity of both cow and swine workers overlapped but were clustered apart from the air and swine gastrointestinal samples; yet, the swine worker microbiomes did share some microbial components with swine samples (39). Workers exposed to swine for at least eight hours per day had significantly different gastrointestinal bacterial community beta diversity than those who worked with cows, while swine workers performing work for two or fewer hours per day did not (39). Further, in a study of interns on a swine farm for three months, both the swine and soil were contributors to the intern gastrointestinal microbiomes, and the interns' gastrointestinal microbiome became more aligned with that of the swine farm workers compared to the beginning of the study (45). While the effect of temporal variation seems obvious based on the identified studies, the number of studies and sample sizes are still too limiting to draw conclusions. Further, complications that arise when assessing temporal variations include resource limitations and the absence of comprehensive baseline data. However, it is imperative that temporal variation be incorporated into assessment of the worker microbiome, which aligns with fundamental principles in assessment of worker exposure and associated health outcomes.

In addition to biological agents, exposure to chemical agents can also lead to adverse health outcomes and impact the worker microbiome and envirobiome. Pesticide use has been associated with the development of various diseases (65), but the impact of agricultural chemical use on the worker microbiome and agricultural envirobiome remains relatively understudied. In a study of agricultural workers and controls, working status was not correlated with changes in oral microbial diversity; however, blood level of azinophos-methyl did correlate with a reduction in microbial diversity across seasons (66). Additional research is needed to understand the role of agricultural chemical exposure in affecting worker microbiomes and health effects.

Additional challenges in this area of research include assessment of the role of various environmental factors, including seasonal changes, temperature, unique job tasks, and workplace controls, among others. Season was found to impact the spread of swine farm envirobiomes to workers (33). Specifically, it was reported that air samples had higher bacterial loads in the winter compared to summer, which was not only associated with an increase in diversity of swine worker nasal microbiomes in the winter, but also an increase in the proportion of shared bacteria between the swine and swine workers (33). However, dust samples from swine stables and swine farmer homes were not reported to have seasonal variation in microbial composition, although increased bacterial abundance was observed in the summer (48). It was hypothesized that such seasonality is more likely to affect biological factors (e.g., animals) than chemical factors (e.g., pesticides) (20).

Regarding workplace controls, few studies have specifically evaluated the potential for personal protective equipment (PPE), behavior and practices, equipment material, and engineering controls to affect the worker microbiome or envirobiome, especially that of the air (38,41,56). Such controls were implemented during the COVID-19 pandemic to protect from spread of SARS-CoV-2, and thus likely impact the spread of microbes from the envirobiome to the agricultural workers' microbiomes, which may have impacts on their microbial diversity and health. Additionally, personal factors (e.g., diet, residency) may affect the worker microbiomes (35). Despite these challenges, measuring the envirobiome as an indicator of worker microbiomes should be considered, as challenges related to human variability (e.g., genetics) can be ameliorated.

Antibiotic resistance & pathogenic microbes

The role of transmission of antibiotic resistance genes (ARGs) from the agricultural environment to the worker is becoming better understood, particularly the role that swine, and to a lesser extent broilers, may play in the worker resistome, which is the collection of AGRs in an organism (e.g., worker, swine) or among the environment available for uptake (61). In fact, Duarte *et al.* (28) reported that swine farmer gastrointestinal resistomes were more similar to that of swine than the swine slaughterhouse worker resistome, and the proportion of worker resistomes across broiler and swine farmers reportedly shared more characteristics of the swine and cow resistomes than the broilers and turkey resistomes. Likewise, a higher abundance of ARGs was found in swine farmers and swine slaughterhouse workers than in broiler farmers and non-exposed controls (47). In a study of broilers, broiler farmers, and live broiler market workers, increased gastrointestinal microbial diversity was associated with increased ARG diversity, and the microbial and ARG diversity between the broiler farms was different from that of the broiler market workers, highlighting differences among those exposed to the same species, but performing different jobs (49). Further, transfer between the broiler and worker ARGs occurred, and some of the identified ARG determinants were harbored by known clinical pathogenic bacteria (49). Luiken *et al.* (36) reported that the swine and broiler farm worker gastrointestinal resistomes were less abundant and did not overlap with identified farm dust or swine and broiler fecal resistomes. The diversity of the farm dust resistome was greater than the diversity of the swine gastrointestinal resistome (36). In another study, the relative abundance of ARGs in the gastrointestinal resistomes of swine farmers and local villagers was reportedly similar to each other but different than that of the swine (44). The abundance of ARGs of veterinary interns on a swine farm did not change over the three-month duration of the internship; however, the transmission of microbiota and ARGs from environmental sources, including swine and sewage, was observed in the interns' resistomes (45). Challenges to research in this area include that resistomes were found to have geographic variability, which may present limitations in generalizability and comparability of findings between studies (28). Additionally, no studies were identified that evaluated the association between resistomes and adverse health events in workers, and thus the biological meaningfulness of such changes in ARGs remains unknown.

The presence of pathogenic bacteria in agricultural environments and in the microbiomes of agricultural workers was examined in several studies (10). Regarding the nasal microbiome, swine farmers had nasal colonization of potentially pathogenic *Clostridium spp*., which was also detected in the air at swine farms (38). Further, there was decreased colonization of *Staphylococcus* in nasal microbiomes among dairy farm workers compared to non-dairy farmers, which was attributed to potential out competition of potentially pathogenic bacteria in communities with increased diversity (42). Additionally, in a study of livestock workers, it was reported that those working with swine had greater alpha diversity and were more likely to have pathogenic bacteria in their oropharynx than those working with other types of livestock (e.g., cattle, poultry) (31). Sun *et al.* (44) reported that both swine and swine farm worker gastrointestinal microbiomes also had an increased abundance of Clostridiaceae compared to local villagers. In addition to nasal and gastrointestinal microbiomes, Peng *et al.* (40) reported that dairy and integrated farm workers with frequent animal contact had decreased abundance of both *Staphylococcus* and *Streptococcus* on their skin, along with shifts in the skin microbial community structure. Similar results were noticed with envirobiome assessments. For example, Vestergaard *et al.* (48) reported that the dust in swine stables and at swine farmers' homes had a higher abundance of Clostiridales and Clostridiaceae than suburban homes, and Shale *et al.* (41) reported twelve species of *Staphylococcus* were detected in air samples from red meat abattoirs. Collectively, these studies point to the presence of pathogenic microorganisms in both the agricultural worker microbiome and envirobiome.

The potential risk of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in workers exposed to swine is another area of current focus. It was reported that 60% of swine farm workers' nasal microbiota contained MRSA compared to just 10% of controls (38). The nasal alpha diversity of swine truck drivers was reportedly less than that of non-exposed controls, and swine truck drivers acquired MRSA throughout work shifts, which was also associated with changes in the nasal community structure throughout the work shift (29). Although the presence of MRSA in the nasal microbiome was lost and not transmitted to spouses, several swine truck drivers were persistent carriers of MRSA (29). In another study, swine farm workers had nasal colonization of MRSA when sampled both at the farm and at home for up to several days, while short-term farm visitors had a more readily reversible colonization of MRSA and other swine-associated bacteria (30). No studies were identified in which the carriage MRSA in other types of livestock workers was investigated.

Further research is needed to evaluate the role of increased microbial diversity on colonization with specific types of pathogenic bacteria, as increased colonization with various pathogenic bacteria has been associated both with increased and decreased alpha diversity across studies. Additionally, none of these studies evaluated the prevalence of infections associated with altered microbial community structure or abundances of potentially pathogenic bacteria, which represents a critical gap in the literature and an important step in evaluating and protecting worker health. Therefore, additional research is needed to evaluate not only the presence of pathogens in the agricultural envirobiome and worker microbiomes, but for their potential to spread within and outside of the agricultural workplace and to elicit disease.

Holistic approach

Variability and limitations of the studies identified indicate both insights and challenges in measuring and interpreting the human microbiome and the envirobiome collectively. The human microbiome and the envirobiome have interplay and are impacted by the quantifiable internal and external environmental factors and from other possible variables at work (e.g., genetics, tenure, work practices, etc.). Acknowledging and understanding the depth of variability that potentially impacts the relationship is important when attempting to glean meaningful interpretation. Of the occupational microbiome studies that were identified in the Mucci *et al.* (20) review, most did not examine environmental factors. However, several other studies identified focused on both the microbiome of agricultural workers and the envirobiome within their respective agricultural working environment. Although each study has limitations, collectively the studies point towards the likelihood of interactions between the human microbiome and the envirobiome of the working environment. This was demonstrated through the data presented for the most studied agricultural sector – swine agriculture. It was commonly reported that the swine worker nasal microbiome community overlapped with that found in the swine microbiome and aerosol samples (30,32,38). Swine worker microbiomes also exhibited greater diversity than those of non-agricultural workers (32). Studies of both cow and swine farmers suggest that the envirobiomes of swine farms may have a more pronounced effect on the worker microbiomes than the envirobiomes from other types of agricultural practices (32,39). These studies point towards a confluence of variables impacting both the human microbiome and the envirobiome. More research is needed to better elucidate the dominant influences and interactions between the worker and their work environment.

Since Hippocrates' work on lead toxicity around the fourth century B.C.E., there has been an ever-evolving awareness that the work environment can impact worker health, either positively or negatively. A more recent evolution in the science and study of worker health is the concept of TWH, which is the comprehensive approach and holistic integration of all aspects of work that can impact worker safety, health, and well-being (18). This approach extends beyond traditional interventions to protect workers and expands hazard interventions to apply to concepts such as new and emerging technologies by integrating a more holistic view of working conditions and incorporating emerging topics regarding the changing workforce (e.g., temporary workers, gig workers, aging workforce, etc.). Leveraging this approach also allows for the investigation of relationships between non-work-related human health conditions that may become impacted by working conditions as well as an expansion of work-related hazards as they may apply to not just workers, but employers, and communities. The COVID-19 pandemic highlighted the convergence of occupational health and community health in TWH. As we saw with COVID-19, the spread of SARS-CoV-2 was not unique to any one workplace, but included all workplaces as it was a community-spread disease. As such, control measures included all locations in the community, including the workplace, to reduce the spread of SARS-CoV-2, thus implying the importance of TWH. While the extent of control measures such as those put in place during the pandemic may not be required for all biological exposures, lessons learned from the pandemic will inform the selection of control measures that worked and apply those moving forward. Leveraging and extending the concept of TWH can inform community exposure to pathogens, such as SARS-CoV-2. The study of the agricultural sector specifically demonstrates the importance of the integration of TWH and One Health, given the potential for exposure to environmental and biological pathogens, which may affect worker health and disease.

The human microbiome has potential applications as a biomarker of exposure and early indicators of disease, including those that are work-related. For example, one study proposed the use of the sputum microbiome as a biomarker for coal worker's pneumoconiosis (67). Additionally, the evaluation of microbiota in the working environment in relation to the worker microbiome may offer further insights into the understanding of the relationship and impacts between worker and workplace. Care should be taken when extrapolating this potential insight as these new data sources introduce challenges including complex measurement error, exposure correlations, temporal variation, and new biases (68). Future research into these relationships may serve to improve workplace controls to better protect and predict worker health. The envirobiome and its interplay with the human microbiome may one day serve as a leading indicator of disease and illness risk for workers and contribute to the TWH tapestry of worker health and well-being.

Limitations and future research

Our review of the state of the science regarding the understanding of the envirobiome, the worker microbiome, and associated worker health effects among agricultural operations is limited by several factors. As discussed, the direct comparison between studies, and subsequent synthesis of knowledge, is impeded by differences in experimental design, detection, and identification methods (e.g., sequencing, culture), taxonomic identification, and diversity metrics, among others. The types of data reported are primarily driven by the questions posed by the investigators and range from broad characterization across different phylogenic levels to specific abundance of pathogenic bacteria and ARGs. Due to the nature and scope of the studies, few studies evaluated the potential biological and/or chemical exposures, the worker microbiome, and associated worker health effects. The populations evaluated represent only a fraction of agricultural workers and larger scale epidemiological studies are needed to evaluate substantial portions of the population and to consider factors such as sex, culture, age, and race.

Collectively, while the field has done well in the initial investigations of the worker microbiome, including the agricultural worker microbiome as explored herein, a paradigm shift to a more comprehensive approach is required in order to identify causal interactions between workplace exposures (e.g., biological, chemical) and worker microbiome diversity and dynamics, thus illuminating the complexities of these bidirectional effects. This will require looking beyond the characterization of a worker and/or envirobiomes to examine how they interact, and the resulting implications for the health of the worker. Some studies have begun to fill this gap by examining the colonization of pathogenic bacteria in agricultural worker microbiomes (10,29-31,38,40,42,44). However, because most studies are cross-sectional in nature, the farmers have largely not been followed for periods to evaluate whether colonization with such pathogenic microbes leads to adverse health effects or disease. The study of colonization by pathogenic fungi and viruses to the worker microbiome remains relatively unstudied in agricultural workers, but the spread of SARS-CoV-2 within workplaces highlights the importance of evaluating colonization and spread of all microbes in the workplace.

Further, there is an overall lack of studies and investigations into the cumulative impact of a worker's lifestyle and the interaction between impacts from workplace exposure. Although several studies established links between lifestyle and diet outside of work on a person's microbiome, few studies have incorporated impacts from workplace exposure and subsequent changes in human health. Further, few studies have evaluated the potential for one's occupation to not only affect their microbiomes, but those who share the same household (28,47). These gaps can be bridged through the application of existing paradigms and frameworks such as TWH, which is designed to holistically evaluate a worker's exposure in and out of the workplace, as well as potential impacts related to lifestyle and diet.

Conclusions

The microbiome is increasingly recognized for its vital role in human health and disease and could become a critical endpoint in assessing worker populations, including agricultural workers. The use of the worker microbiome as a potential biomarker of exposure to occupational chemicals and subsequently associated diseases, particularly diseases which may not culminate in symptoms until after exposure as lapsed, will require increased use of 16S, metagenomic, and metatranscriptomics sequencing methods to evaluate both diversity and functional microbial changes that may impact the worker. Increased application of these technologies will make them more accessible (e.g., affordability and expertise), which will allow broader use and interpretation of results in the context of worker health. Microbial health may emerge as a key contributor to worker health, along with workplace conditions, workplace policies and practices, and individual worker health (e.g., risk factors, existing conditions). The study of the microbiome in this context lies at the convergence of public health, occupational safety and health, and microbial biology, and will require multidisciplinary coordination to sample, analyze, interpret, and provide necessary protection and intervention when needed. Despite the substantial study on the worker microbiome and envirobiome among agricultural operations, clear gaps in the understanding and knowledge remain, which also likely persist across other industrial sectors. Because agricultural workers are in contact with envirobiomes that contain potentially novel pathogens, attention to agricultural worker health and the use of proper PPE can help prevent the spread novel zoonotic diseases with potential to cause pandemics and prove just as destructive as COVID-19. Thus, ongoing monitoring of the envirobiome of agricultural operations and worker microbiomes may allow for use of leading and lagging indicators of not only chemical exposures, but also exposures from novel microorganisms. Therefore, across all sectors, greater effort is required to ensure that holistic and comprehensive approaches are applied to account for all potential sources of microbial perturbations in a worker's life, both in and out of the workplace. Application of such approaches will provide greater insight into the interactions between and within occupational exposures and their impact on the worker and potential changes in worker health.

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