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The Human Microbiome and Understanding the 16S rRNA Gene in Translational Nursing Science

Nancy J. Ames, RN, PhD,

Clinical Nurse Scientist, Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD

Alexandra Ranucci, BS,

Post-Baccalaureate Intramural Research Award Recipient, Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD

Brad Moriyama, Pharm.D., and

Clinical Pharmacist, Pharmacy Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD

Gwenyth R. Wallen, RN, PhD

Chief Nurse Officer (Acting), Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD

Abstract

Background—As more is understood regarding the human microbiome, it is increasingly important for nurse scientists and health care practitioners to analyze these microbial communities and their role in health and disease. 16S rRNA sequencing is a key methodology in identifying these bacterial populations that has recently transitioned from use primarily in research to having increased utility in clinical settings.

Objectives—The objectives of this review are to: (a) describe 16S rRNA sequencing and its role in answering research questions important to nursing science; (b) provide an overview of the oral, lung and gut microbiomes and relevant research; and (c) identify future implications for microbiome research and 16S sequencing in translational nursing science.

Discussion—Sequencing using the 16S rRNA gene has revolutionized research and allowed scientists to easily and reliably characterize complex bacterial communities. This type of research has recently entered the clinical setting, one of the best examples involving the use of 16S

Corresponding Author: Dr. Nancy J. Ames, National Institutes of Health Clinical Center, Nursing Department, Building 10, Room 2B10, 10 Center Drive, Bethesda, MD 20892 (names@nih.gov).

Nancy J. Ames, RN, PhD, is Clinical Nurse Scientist, Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD.

Alexandra Ranucci, BS, was a Post-Baccalaureate Intramural Research Award Recipient, Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD, at the time this paper was prepared. She is currently a MD/MPH Candidate, Tulane University School of Medicine

Brad Moriyama, Pharm.D., is Clinical Pharmacist, Pharmacy Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD.

Gwenyth R. Wallen, RN, PhD, is Chief Nurse Officer (Acting), Nursing Department, National Institutes of Health (NIH) Clinical Center, Bethesda, MD.

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sequencing to identify resistant pathogens, thereby improving the accuracy of bacterial identification in infection control. Clinical microbiota research and related requisite methods are of particular relevance to nurse scientists—individuals uniquely positioned to utilize these techniques in future studies in clinical settings.

Keywords

16S rRNA sequencing; human microbiome; nursing research; translational research

Research on the human microbiome, once a fairly unknown field, has grown exponentially in the past decade. Human beings are now known to be born with certain bacteria, archaea, and viruses that live as symbionts. All humans are “colonized” at birth and further develop and perturb the microbiome in response to different environmental factors and stimuli. These bacteria also serve important roles in human processes; for example, some bacteria in the human microbiome benefit their human host by assisting in digesting complex molecules (den Besten et al., 2013). Not all bacterial inhabitants are beneficial, however: disease-causing pathogens can proliferate in the microbiome, potentially leading to life-threatening conditions such as metabolic endotoxemia (Patterson et al., 2016). Understanding the many trillion bacterial inhabitants of the human microbiome and how they interact with their host is essential, not only from a research viewpoint, but also from a clinical perspective. Using the 16S rRNA gene to identify bacteria in these multiple biomes is the first step.

In 2008, with the start of the Human Microbiome Project (HMP), a National Institutes of Health (NIH) funded collaboration aimed at characterizing the healthy microbiome, scientists have begun to understand some of the characteristics of core, healthy microbiomes (Huse, Ye, Zhou, & Fodor, 2012; Human Microbiome Consortium, 2012b). Less is known, however, about the dynamic changes that occur in the microbiome, the importance and magnitude of which is becoming clearer as the microbiome has been identified in a multitude of body sites and implicated in an ever-increasing number of diseases (Blaser, 2014). Better characterized microbiomes such as the oral, skin, and gut have evolving implications in health and disease (Clemente, Ursell, Parfrey, & Knight, 2012; Schloss, 2014; Xu et al., 2015). But biomes such as the lung, and vaginal microbiomes are emerging as important clinical research targets (Beck, Young, & Huffnagle, 2012; Ravel et al., 2011). Many diseases and conditions have been correlated with microbiome dysbiosis, including chronic obstructive pulmonary disease (Mammen & Sethi, 2016), inflammatory bowel disease (Bakhtiar et al., 2013), dermatitis and other atopic diseases (Grice et al., 2008), as well as bacterial vaginosis and stillbirth (Han et al., 2010; Ravel et al., 2011).

This paper will provide an overview of the importance of the human microbiome in health and disease and outline a key methodology to interpret these complex communities. Developing an understanding of the microbiome and the methods used to describe it is particularly relevant to nurse scientists. Although many other disciplines merge bench science with symptom science, nurse scientists are uniquely positioned to contribute to the growing translational science of the microbiome because of their strong research history in symptomology. The significance of 16S sequencing in answering research questions important to nursing science will be illustrated by first presenting a review of three clinically

relevant microbiomes—the oral, lung and gut microbiomes. Then, the human microbiome will be explored in the clinical environment, with particular emphasis on the use of 16S rRNA microbiome sequencing in tracking outbreaks of resistant bacteria. Finally, limitations to advancing current microbiome research will be broadly considered.

Background

Table 1 lists useful websites with information about current research on the human microbiome.

The Healthy Microbiome

In order to understand the role of the microbiome in disease, scientists must have a baseline from which to compare. Fortunately, the HMP has undertaken an unprecedented categorization of the healthy human microbiome of several different body sites through 16S sequencing of multiple variable regions (Human Microbiome Project Consortium, 2012a, 2012b). The goal of the HMP was to sequence 3,000 genomes from both cultured and uncultured bacteria (http://www.hmpdacc.org/reference_genomes/reference_genomes.php), contributing not only to the understanding of the healthy biome, but also assembling the largest reference database for future comparison and classification (Jumpstart Consortium HMP Working Group, 2012). Samples were collected from 242 adults resulting in 5,177 taxonomically characterized communities comprising the airway, skin, oral, gut, and vaginal biomes (Human Microbiome Consortium, 2012a). One hundred and thirty-one individuals were sampled at an additional time point in order to examine longitudinal changes. Results were characterized by the number and abundance of each organism in order to describe overall diversity. Oral and stool communities were the most diverse, while vaginal communities showed the lowest diversity (Human Microbiome Project Consortium, 2012b). Diversity was further classified into alpha and beta, with alpha representing the diversity of organisms within each site and beta describing the differences in species composition between subjects. For example, saliva had high alpha diversity and a number of unique organisms, but when examining the beta diversity across individuals, the overall oral microbiome was similar, resulting in a low-beta diversity. The skin microbiome had the highest beta diversity and lower alpha diversity, logically depicting the susceptibility of this microbiome to environmental perturbations. In general, changes in the human microbiome within subjects over time was much smaller than the intersubject variability, implying that the microbiome is relatively stable over time, yet unique to each individual (Human Microbiome Project Consortium, 2012b). Communities were affected by outside factors such as oxygen, moisture, pH, host immunology, and microbial interactions (Human Microbiome Project Consortium, 2012b). With the wealth of data characterizing the healthy microbiome now available as a result of the HMP, researchers must seek to further understand the complexities of the human microbiome and delve deeply into functional metabolomics of these bacteria to advance knowledge of their role in health and disease.

16S rRNA and Next-Generation Sequencing to Characterize the Human Microbiome

Historically, identification of bacteria relied solely upon culture methods that often failed to identify certain bacteria that do not grow in common media (Srinivasan et al., 2015). This

method of bacterial identification is especially challenging when examining a community of organisms with different individual growth characteristics. Many organisms resist growth in culture like *Burkholderia* spp. and *Mycobacterium tuberculosis* (Srinivasan et al., 2015).

The 16S rRNA sequencing method has allowed for a simple and effective alternative to microbial culture. The 16S rRNA gene codes for a ribosomal subunit that is widely conserved among bacteria and contains hypervariable regions interspersed among conserved regions of its sequence (Clarridge, 2004). These hypervariable regions are unique to each bacterial species, allowing for classification or taxonomy. The conserved regions, on the other hand, allow for the development of universal primers that bind to known sequences shared among most bacteria. Short read sequencing of approximately 200–400 base pairs is capable of targeting one or several proximal regions through the use of specific primers that align to the conserved regions on either side of the hypervariable target (Ghyselinck, Pfeiffer, Heylen, Sessitsch, & De Vos, 2013). Of the nine hypervariable regions, some regions better characterize bacteria and the choice of which region, and therefore the appropriate primers to use, is an important step in study design (Ghyselinck et al., 2013; Mizrahi-Man, Davenport, & Gilad, 2013). For example, the V1-V3 region is preferred by some in studies examining the oral microbiome (Zheng et al., 2015). This issue of selection of the hypervariable regions is by no means straightforward and remains controversial. Some scientists believe that sequencing multiple variable regions is the best option, providing a nonbiased, comprehensive view of these complex microbiomes (Barb et al., 2016).

Before next generation sequencing, most research characterizing bacterial communities utilized Sanger sequencing, which allowed for longer reads—often greater than 500 base pairs (Vincent, Derome, Boyle, Culley, & Charette, in press). However, the increased speed, reduced cost, simpler protocols, and comparable read lengths of next-generation sequencers have allowed them to virtually replace Sanger sequencing (Rhoads & Au, 2015). Regardless of sequencing method, final results are represented in operational taxonomic units (OTUs), which is a sequence identifying an organism usually at the genus or species level (Dickson et al., 2016).

One of the major constraints in all microbiome studies is the complexity and amount of data that is obtained (Dudhagara et al., 2015). These “big datasets” require a careful bioinformatics plan for analysis in order to provide clear, concise results capable of furthering scientific discovery in this rapidly changing field. The nurse scientist requires collaboration with experienced bioinformaticists who understand the issues and complexity involved in these analyses. Scientists may also collaborate with a microbiome research center; examples are the University of Maryland’s Institute for Genome Science (<http://www.igs.umaryland.edu/>) and the University of Chicago’s Microbiome Center, which coordinates with Argonne National Laboratory, Argonne, Illinois and Marine Microbiological Labs at Woods Hole, Massachusetts (<https://microbiome.uchicago.edu/>).

Selected Microbiomes

In this section, microbiomes of particular interest to researchers are reviewed. Detailed information about studies cited is available (see Table, Supplemental Digital Content 1).

The Oral Microbiome

The mouth contains many different structures and tissues, each of which hosts a characteristic biotic community. However, in the HMP data, a comparison of the tongue and saliva specimens reveals that they share similar genera (Human Microbiome Project Consortium, 2012b). The type and abundance of organisms in the plaque microbiota vary greatly between samples collected above the gum line (supragingival) or below (subgingival) (Segata et al., 2012). Additionally, saliva bathes the entire cavity, continuously introducing some of its bacteria into other oral samples. More specifically, saliva represents an example of a microbiome with high alpha but low beta diversity (Human Microbiome Project Consortium, 2012b). These distinct environments present multiple challenges to researchers, as all sites are dynamic, microbial communities constantly changing over time in response to different stimuli and diseases. It is difficult to know how to compare the different regions, and it is even more difficult to decide which region, if any, is representative of the entire oral microbiome. In the HMP, nine sites from the oral cavity were sampled from healthy volunteers: the saliva, tongue dorsum, hard palate, buccal mucosa, keratinized gingiva or gums, palatine tonsils, throat and supra- and subgingival plaque (Human Microbiome Project Consortium, 2012b). The results demonstrated that the alpha diversity was influenced by body habitat as all oral samples grouped together in a principal components analysis (Figure 1; Human Microbiome Project Consortium, 2012b.). HMP participants were healthy volunteers who met about 35 major exclusion criteria, including some directly relevant to the oral microbiome: periodontal disease, more than eight missing teeth, halitosis and untreated cavitated carious lesions or oral abscesses (www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000228.v3.p1). Therefore, assumptions cannot be made about diversity among participants with good oral hygiene, for example.

Core oral microbiome—Based on molecular methods, it is not clear whether a “core oral microbiome” exists (Zaura, Keijser, Huse, & Crielaard, 2009), which is a concept redefining the diversity of the oral microbiome. Keijser et al. (2008) identified 3,621 species-level phylotypes (DNA sequences that are compared to a database) in saliva and over 6,888 in plaque. This estimate of diversity in the oral microbiome is much greater than previously reported. K. Li, Bihan, & Methé (2013) studied the HMP database and identified a relatively small and stable core oral microbiome in all nine oral sites. In addition, this study examined a minor core microbiome where bacteria were in low abundance, but present in the majority of the samples. For example, the tongue dorsum revealed three important taxa: *Actinomycetales* unclassified, *Bacilli* unclassified, and *Peptostreptococcaceae peptostreptococcus* (K. Li et al., 2013). Identification of these low abundance microorganisms provided support for the hypothesis of a “minor core oral microbiome” (K. Li et al., 2013). Future studies may benefit from knowledge about the presence or absence of these organisms.

The oral microbiome and pneumonia—In the clinical setting, interest in the oral cavity began with publication of papers linking plaque biofilm, sampled from patients receiving care in medical ICUs and elderly living in institutions, with pathogens later associated with nosocomial infections (El-Solh et al., 2004; Fourrier, Duvivier, Boutigny, Roussel-Delvallez, & Chopin, 1998; Heo, Haase, Lesse, Gill, & Scannapieco, 2008;

Scannapieco, Stewart, & Mylotte, 1992). Results showed an important association between pathogens identified in the mouth and bacteria in the lung. The proposed pathogenic theory included aspiration of pathogen-contaminated saliva trafficked to the lungs where pneumonia developed. The saliva-to-lung hypothesis became the driving force behind randomized control trials in critical care to examine whether oral decontamination would decrease pneumonias in intubated, critically ill patients (Bergmans et al., 2001; Houston et al., 2002; DeRiso, Ladowski, Dillon, Justice, & Peterson, 1996; Segers, Speekenbrink, Ubbink, van Ogtrop, & de Mol, 2006). Three out of four studies used chlorhexidine oral rinses in a cardiac surgery population (DeRiso et al., 1996; Houston et al., 2002; Segers et al., 2006) and one (Bergmans et al., 2001) used a combination of topical antibiotics applied to the oral cavity. The Bergmans et al. (2001) study was performed in three critical care units in the Netherlands in a mixed critical care population. Segers et al. (2006) used chlorhexidine rinses and chlorhexidine ointment in the nasal passages. All four studies demonstrated effectiveness: decrease in nosocomial infections in the treatment group (DeRiso et al., 1996; Segers et al., 2006), decrease in nosocomial pneumonia in a subgroup of patients intubated longer than 24 hours (Houston et al., 2002), and decrease in ventilator-associated pneumonia in the treatment group (Bergmans et al., 2001). Findings from these studies and a recent meta-analysis (Hua et al., 2016) provide strong evidence to support the use of oral hygiene care that includes chlorhexidine mouthwashes or gel in decreasing the risk of ventilator-associated pneumonia (VAP). The Institute for Healthcare Improvement developed other strategies to decrease the risk of VAP and called these strategies that included among others a “sedation vacation” and peptic ulcer prophylaxis, the VAP Bundle (Resar et al., 2005). A recent study provides an extensive cost-benefits analysis of the traditional VAP Bundle and other methods to prevent VAP (Branch-Elliman, Wright, & Howell (2015).

Oral–gut microbiome connection—The oral and gut microbiomes may be linked. Using the HMP database, Ding and Schloss (2014) found that the oral and gut microbiomes were predictive of each other, despite differences in the microbiota; they speculated that, although the gut and oral microbiome have different members, the “oral bacterial populations seed the gut” (p. 358). Dysbiosis in the salivary microbiome of patients with inflammatory bowel disease (IBD) (Crohn’s disease $n = 21$; ulcerative colitis $n = 14$) when compared to healthy controls ($n = 24$) provides additional information about an oral–gut link (Said et al., 2014); the dysbiosis was characterized by a significant increase in *Prevotella* and *Veillonella* and a decrease in two other genera, *Streptococcus* and *Haemophilus* in IBD patients compared to healthy controls (Said et al., 2014). This finding could be important in future studies of the gut and oral microbiomes, potentially helping to define both. This connection could also have important clinical implications. For example, if this change in the oral microbiome communities was consistent across populations and predictive of IBD, a simple, salivary sample could be used for diagnosis. Future studies examining the predictive ability of salivary biomarkers are needed.

Lung Microbiome

Until recently, infectious disease texts have described the lung as sterile (Mandell, Bennett, & Dolin, 2010), a fact now known to be incorrect. Pulmonologists and microbiologists held

the “sterile hypothesis” due to an inability to identify bacteria using conventional culture methods in normal, healthy individuals. A large percentage of the bacteria that compose the oral and lung microbiomes are unculturable or difficult to grow in the clinical laboratory (Bahrani-Mougeot et al., 2007). In addition, sampling of the lower respiratory tract is difficult, invasive, and requires a skilled bronchoscopist in order to obtain samples safely. Identification of members of the lung microbiome has advanced with 16S rRNA sequencing, resulting in a much more detailed understanding of the microbial makeup of the lung biome (Dickson, Erb-Downward, Martinez, & Huffnagle, 2016).

One of the first studies suggesting an association between bacteria and inflammation in the lung was performed to test this relationship in mice (Herbst et al., 2011). Although this study did not use sequencing it provided evidence of a connection. Germ-free mice were found to have airway hyperactivity and alteration in certain immune cell lines compared to controls when stimulated with ovalbumin—a protein and immune stimulant, a process that was reversed when the germ free mice were colonized with the commensal bacteria from the controls. In another study, 16S rRNA was used to identify bacteria from airway microbiota obtained through bronchial lavages and protective specimen brushings in three groups: asthmatics ($n = 11$), patients with chronic obstructive lung disease (COPD) ($n = 5$), and healthy controls ($n = 8$) (Hilty et al., 2010). Not only were none of the airways sterile, the microbiota of patients with asthma or COPD was described as “disturbed.” Despite the small sample size, prior thinking was challenged and the concept of the sterile bronchial tree and lung added to the evidence that disease states and dysbiosis of the microbiome were somehow related. A subsequent study by Charlson et al. (2012) collected samples from multiple upper airway sites in six normal volunteers with “potential heavy lung colonization” and compared oral and lung samples. This study and others provided strong support that the lung has a unique microbiome (Charlson et al., 2011; Dickson et al., 2014; Morris et al., 2013).

Historically, microbiome studies have been limited by small sample size. This is especially true in the lung microbiome studies, perhaps because of the invasive nature of the sampling. However, accumulation of data in large, public-access reference databases may correct this limitation (McDonald, Birmingham, & Knight, 2015).

Recently, researchers have begun to investigate the relationship between the gut and lung microbiomes. A study performed in mice infected with *Streptococcus pneumoniae* found that intact intestinal microbiota conferred pathogenic protection when compared to mice that had their gut microbiota depleted (Schuijt et al., 2016). The alveolar macrophages of the mice with depleted gut microbiomes had decreased ability to engulf and destroy bacteria (Schuijt et al., 2016). Like the germ-free mice studied by Herbst et al. (2011) that were less likely to respond appropriately to airway inflammation than mice with a normal microbiome, the gut–lung axis demonstrated in this study highlights that communication between two different microbiomes may play an important role in disease protection. A recent review highlights this complexity by relating the dysbiosis of the gut to the lung microbiome and the impact of chronic lung disease (O’Dwyer, Dickinson & Moore, 2016).

Gut Microbiome

The gut microbiome is one of the most studied biomes and has been shown to contain the greatest number of organisms, the vast majority of which are located in the colon (Sender, Fuchs, & Milo, 2016). The number of bacteria in the gut microbiome has been estimated to range from 10^{13} to 10^{14} organisms (about 100 trillion) (Gill et al., 2006). (Recently, the number of bacteria in the entire body was estimated to be about 3.9×10^{13} ; Sender et al., 2016). No matter what the final number of bacteria is estimated to be, it is undisputed that the vast majority of the gut bacteria belong to two major bacterial phyla: Bacteroidetes and Firmicutes (Mahowald et al., 2009).

The gut microbiota serve an important role in the gastrointestinal system by breaking down short-chain fatty acids (SCFAs) (Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012). Disruption of the normal bacterial community (gut dysbiosis) has been implicated in irritable bowel syndrome (Kennedy, Cryan, Dinan, & Clarke, 2014), inflammatory bowel disease (Rehman et al., 2016) and gastrointestinal malignancy (Abreu & Peek, 2014). Human and animal studies have also directly linked changes in the gut microbiota to obesity (Graessler et al., 2013; Kasai et al., 2015; Ridaura et al., 2013; Turnbaugh et al., 2009). Antibiotics have been shown to create gut dysbiosis by disrupting the balance of the microbiota (Modi, Collins, & Relman, 2014), a change that can be lasting. For example, macrolide antibiotics changed the gut microbiome for up to two years when fecal samples of 142 children were studied (Korpela et al., 2016). Antibiotic use also has been implicated in changes in gut microbiota and weight gain, especially in children (Saari, Virta, Sankilampi, Dunkel, & Saxen, 2015; Scott et al., 2016). Antibiotic exposure, bacterial resistance genes and the gut microbiome represent a complex area of research that requires further study (Halpin et al., 2016; Raymond et al., 2016)

Gut microbiome across the lifespan—Figure 2 represents a graph of important microbiome changes over time (Borre et al., 2014). The gut microbiome is formed early in life and is influenced at birth by many factors, including maternal health (Mueller, Bakacs, Combellick, Grigoryan, & Dominguez-Bello, 2015). Gut microbiome varies distinctly as a function of vaginal or Cesarean section (C-section) delivery (Rutayisire, Huang, Liu, & Tao, 2016). The skin bacteria of infants were associated with maternal vaginal microbiomes, whereas that of the C-section babies were composed of skin bacteria (Dominguez-Bello et al., 2010). The implications of these findings could be most important for infant health, as the percentage of C-section births is approximately 32% (Hamilton, Martin, Osterman, Curtin, & Matthews, 2015, p. 7) and babies born by C-section may have delayed establishment of their microbiomes (Dominguez-Bello, Blaser, Ley, & Knight, 2011). As the child matures, the microbiome gradually becomes more diverse (Borre et al., 2014), a trend that is reversed in older age when diversity decreases and a change in certain taxa occurs (Jackson et al., 2016). Fecal specimens from 13 young adults and 178 Caucasian, elderly individuals (64 to 102 years) showed that the gut microbiota changed with location of the elderly resident (assisted living, day-hospital, rehabilitation, long-term care or community) and diversity of the diet (Claesson et al., 2012); differences in the gut microbiome with diet, as well as with health outcomes such as increased fragility (significantly correlated), blood pressure and geriatric depression.

Brain–gut microbiome interaction—One of the most interesting and potentially productive areas for future gut microbiome research is the connection between the gut microbiome and the brain. Information about significant gut-brain research studies and their major outcome measures is tabled in Supplemental Digital Content 2 and discussed below.

There is direct communication between the gut microbiome and the brain, and many believe this communication occurs reciprocally (Bauer, Huus, & Finlay, 2016; Carabotti, Scirocco, Maselli, & Severi, 2015; Kelly, Clarke, Cryan, & Dinan, 2016; Q. Li & Zhou, 2016). Mouse studies have examined this interaction (Clarke et al., 2013; Davari, Talaei, Alaei, & Salami, 2013; Desbonnet et al., 2015; Diaz Heijtz et al., 2011; Tsurugizawa et al., 2009). The microbiome gut–brain axis has been directly linked to anxiety (Clarke et al., 2013), depression (Claesson et al., 2012), autism (Finegold et al., 2010), and Alzheimer’s disease (Bhattacharjee & Lukiw, 2013).

The immune (Erny et al., 2015) and endocrine systems (El Aidy, Dinan, & Cryan, 2015) may also be affected by the gut microbiome. These fascinating gut-brain connections are being examined; however, the complexity and state of early phase research limits a detailed review at this time. Evidence to support the interaction between gut microbes, the immune system, the neuroendocrine system, and the “psychoneuroimmunology network” is summarized in El Aidy et al. (2016). An example of this interaction is the role of the chronic stress predisposing humans to increased gut permeability and translocation of bacteria from the gut lumen into the circulation; some evidence suggests that probiotics can reverse this trend (Liu, Jiang, Zhou, Song, & Zhang, 2016).

Difficulties in gut microbiota sample processing and storage—Challenges involving specimen collection, processing, and storage are particularly pertinent to gut microbiome investigations, as most microorganisms reside in the gastrointestinal tract and fecal microbiome composition undergoes marked change over time, which complicates specimen storage and sampling protocols (Song et al., 2016). Effective sampling methods are needed to advance understanding of the gut microbiome (Gorzelak et al., 2015). However, no consensus exists regarding either standard sample collection and processing practices or sequencing protocols and storage methods are rarely sufficiently addressed in manuscripts (Thomas, Clark, & Doré, 2015), presenting significant obstacles for ongoing research. Gorzelak et al. (2015) proposed freezing feces within 15 minutes of defecation, storing specimens in a frost-free freezer for up to three days and homogenizing stool to reduce microbiome variability across sampled microenvironments. However, other storage recommendations include immediate freezing of samples at -80 °C, storage at 4 °C followed by freezing at -80 °C or addition of a stabilization agent (Thomas et al., 2015). Still, others maintain that variations in short-term storage conditions do not alter bacterial community composition significantly (Lauber, Zhou, Gordon, Knight, & Fierer, 2010). The choice of stabilization reagent is another challenge for researchers utilizing “omics” methods, as these procedures often necessitate the simultaneous preservation of DNA, RNA, and other metabolites, thereby preventing the use of an agent optimized for a single molecule (Song et al., 2016). Certainly, the literature presents a plethora of sample storage and processing options for the researcher, underscoring the need for standardized and optimized protocols for cross-study comparison (Gorzelak et al., 2015; Thomas et al., 2015).

Clinical Applications

Hospital-Acquired Infections and 16S rRNA Identification of Bacteria

Hospital-acquired infections are an increasingly important issue in clinical microbiology and epidemiology. Accurate tracking and identification of pathogens, particularly those resistant to antibiotics, is of utmost importance (Punina, Makridakis, Remnev, & Topunov, 2015). Currently, culture methods are usually employed to identify the responsible pathogen and broad-spectrum antibiotics are utilized during the period prior to identification of causative pathogens (Lax & Gilbert, 2015). Identification of the pathogen is fundamental for proper antibiotic stewardship. Use of 16S rRNA sequencing is a feasible, emerging tool for clinical microbiology laboratories to accelerate pathogen identification and identify bacteria that otherwise could not otherwise be successfully cultured. Using 16S rRNA to assess the entire bacterial community allows for the analysis of hospital surfaces and equipment that often harbor pathogens, informing hospital cleaning regimens and epidemiology practices to reduce the spread of infection.

Use of 16S rRNA sequencing is particularly useful in situations where a patient's specimen may contain hundreds of organisms, as traditional culture techniques may fail to isolate the organism of interest. Furthermore, 16S sequencing allows characterization of the complete bacterial community for an informed treatment decision. The 16S sequencing approach can be used to characterize bacterial communities that are typically hard to identify (Bharadwaj et al., 2012). For example, Bharadwaj et al. (2012) examined the effect of 16S sequencing on treatment outcomes in a clinical setting over a four-year period in 172 patients. These 172 patients had bacteria that were difficult to identify by standard clinical laboratory methods. In 140 patients, the organism identified did not change clinical management. However, in 24 patients with mycobacteria or other gram-positive bacilli with retrievable, clinical information, the additional information gained from the 16S sequencing led to treatment modification, initiation or discontinuation in 14 patients. Early hospital discharge was facilitated in many instances. Importantly, all 14 patients with treatment changes were alive 30 days postinfection, suggesting that using 16S sequencing to guide treatment potentially may save lives. Of the remaining 10 patients, six received appropriate therapy before the results of the molecular tests were available, two patients died and two left against medical advice (Bharadwaj et al., 2012).

Another clinical example involved a pipeline tested on samples from cystic fibrosis patients; it was especially useful for low-prevalence or hard to identify bacteria (Salipante et al., 2013). Additionally, 16S methodology was much faster and more cost effective for detailed analysis than traditional methods, producing results within one day.

Toma et al. (2014) used novel next-generation sequencing in mechanical ventilator patients, subjecting 44 tracheal aspirate samples to Single Molecule Real-Time (SMRT, Pacific Biosciences of California, Inc.) long-read 16S sequencing that uses approximately 95% of the 16S rRNA gene instead of merely one or two hypervariable regions. The authors in this study identified clinically important genera that were not identified in the clinical culture results (Toma et al., 2014).

In a review examining the microbiota as a target for infection control interventions, researchers outline emerging methods that harness the microbiome to combat antibiotic-resistant infections (Pettigrew, Johnson, & Harris, 2016). Proposed solutions include the introduction of commensals, or probiotics, to help patients maintain a healthy microbial community less susceptible to overgrowth and antibiotic resistant infection. At present, studies have produced mixed results and more research is necessary to understand the efficacy of probiotic administration for infection prevention.

16S rRNA and Bacterial Sensitivities

Although 16S rRNA sequencing has enlarged understanding of the human microbiome, taxonomic resolution is a limitation, as often the lowest classification possible is at the genus level. This is not as informative or powerful for identification of species- or strain-specific pathogens. Additionally, antibiotic sensitivities cannot be ascertained, making this an area where culture methods remain necessary. Whole genome sequencing is gaining traction over 16S sequencing, as improved resolution and strain-level mutations can inform antibiotic resistance. Researchers at the NIH Clinical Center used whole genome sequencing to track a carbapenem-resistant *Klebsiella pneumoniae* outbreak (Snitkin et al., 2012), demonstrating its utility in hospital epidemiology through the identification of the specific transmission route. In the clinical microbiological setting, whole genome sequencing for isolate characterization remains limited, primarily due to increased cost, time, education and training of staff (Kwong, McCallum, Sintchenko, & Howden, 2015). User-friendly bioinformatics pipelines are under development and represent a critical next step towards universal adoption of whole genome sequencing in clinical microbiology and epidemiology.

Clinical Implications and Future Directions for Nurse Scientists

Table 2 highlights nursing research questions related to the oral, lung and gut microbiome discussed below.

Oral Microbiome

The oral microbiome is a rich area for multidisciplinary studies between nurse scientists, dentists, and others interested in this complex environment. Because of nursing's role in direct patient care, from routine assessment to providing oral care and patient education, nurse scientists are especially well-positioned to design studies examining this important biome in special populations (Ames et al., 2012). Research is relatively more feasible because the oral cavity is accessible. Nurse scientists designing oral microbiome studies must think carefully, however, about which site or sites in the oral cavity they should sample. Sites selected should be based upon the populations of interest and the aims of the study, but training and ease of collection should also be considered. Development of an antimicrobial peptide that specifically targets *Streptococcus mutans*, a major cause of caries, illustrates the translational nature of oral microbiome research (Guo et al., 2015). Similar manipulations may have a role in the future treatment for disease and are important areas for nurse scientists to address.

Lung Microbiome

There are many opportunities for clinical studies examining the lung microbiome in healthy individuals and individuals with diseases such as pneumonia and asthma. This is especially true with regards to the challenges in obtaining samples. There is a procedure for bronchoscopy to prevent or decrease contamination from oral specimens (Charlson et al., 2012), but bronchoscopy is still not without risk—making justification for its use in research with healthy individuals somewhat problematic. Careful attention to study design and outcomes is essential. Research examining less invasive routes of effectively sampling the lung microbiome would be especially useful, as would larger studies examining associations between the lung and other microbiomes.

Gut Microbiome

Future studies investigating the role of the gut microbiome across the lifespan will have far-reaching implications. The health status of an elderly population might be followed by examining their gut microbiome at regular intervals and adjusting diet and nutritional supplements not only to improve physical function, but also to decrease depression and anxiety. Longitudinal studies in the elderly that collect reliable and valid measures of patient-reported outcomes in combination with frequent gut microbiome sampling are feasible in rehabilitation or nursing home settings. Maternal–child health studies are needed to further clarify the role of antibiotics in early childhood and the effects of C-section on the gut microbiomes of infant gut microbiome. Research is needed to identify specific bacteria that can serve as biomarkers in the gut microbiome at all ages, in healthy and diseased populations. An example of a direct clinical application of gut microbiome research and a future direction for nurses is the success of fecal transplants. In recent years, fecal transplants have been very successful in treating patients with recurrent *Clostridium difficile* infections. A recent systematic review that included two randomized control trials, 28 case-series, and two case reports showed fecal microbiome transplant provided resolution of symptoms in 85% of all cases (Drekonja et al., 2015)

An emerging research area that is particularly relevant for nurse scientists is the drug metabolism of xenobiotics or foreign substances by the bacteria in gastrointestinal tract (GI). In contrast to drugs metabolized by the liver, metabolism of foreign compounds by the gut microbiome converts hydrophilic compounds (water soluble) into a hydrophobic form (water insoluble) such that they can be absorbed into the body (Kim, 2015; Sousa et al., 2008). However, only a handful of drugs have been reported to be metabolized by GI tract bacteria (Haiser & Turnbaugh, 2013). In this review, the authors listed approximately 40 drugs metabolized by the human gut microbiota (Haiser & Turnbaugh, 2013). One important issue is that the majority of drugs are absorbed in the upper GI tract, whereas the majority of bacteria are located in the large intestine of humans (Klaassen & Cui, 2015; Sousa et al., 2008). There is also limited information available on xenobiotic metabolism in other microbiomes of the human body (Tralau, Sowada, & Luch, 2015). Specific drugs that are metabolized by the GI microbiome have been extensively reviewed in several recent publications (Haiser & Turnbaugh, 2013; H. Li, He, & Jia, 2016; Sousa et al., 2008; Tralau et al., 2015;). We refer the reader to these articles for an in-depth discussion on individual drugs.

Clinically important examples of drugs metabolized by the GI microbiome include sulfasalazine and lactulose. Sulfasalazine is metabolized by azoreductases from bacteria in the distal small bowel and colon to 5-aminosalicylic acid and sulfapyridine (Ardizzone & Bianchi Porro, 2002; Plosker & Croom, 2005). Lactulose is a synthetic metabolized by disaccharidases from bacteria in the colon to lactic acid and acetic acid (Clausen & Mortensen, 1997; Phongsamran, Kim, Cupo Abbott, & Rosenblatt, 2010). Furthermore, relevant issues that should be addressed to facilitate drug metabolism studies during drug development include identification of the specific bacteria involved in drug metabolism, further development of methods to identify metabolites, further development of animal models, and exploring the relationship between hepatic drug metabolizing enzymes and the microbiome (Kang et al., 2013).

Limitations of Current Microbiome Research

Microbiome research is largely descriptive science; few intervention studies exist. Researchers are attempting to describe the human microbiota to understand health and ultimately prevent diseases. Large studies such as the HMP have focused on healthy individuals. Overall, study samples are predominately white and lack significant racial or ethnic diversity. In fact, many studies fail to report the complete demographic information, yet give great detail about the molecular analysis. Evidence indicates microbiome may vary by geography (Nasidze, Li, Quinque, Tang, & Stoneking, 2009). In this study, saliva was sampled and compared with research participants from 12 different global locations. Further work comparing different ethnic and racial groups and their microbiomes will be important. Small sample size limits the generalizability of most microbiome studies. Larger studies will be more feasible as overall costs of molecular sequencing methods decline and analysis is streamlined. Already, the American Gut Project attempts to address this need through a crowd-sourced study design (<http://americangut.org/>). Nevertheless, cost remains a practical limitation and judging the most cost-effective option is difficult. Many companies offering molecular techniques exist and consistent prices are not advertised or easily-accessible. The decision to purchase a next-generation sequencer platform and perform DNA extraction and sequencing in-house or to outsource these processes is a major decision point in study planning and design. Limited funding or lack of experienced personnel, including laboratory scientists and bioinformaticists, may prohibit in-house investigation. Despite these limitations, microbiome research remains rich, capable of being harnessed to understand health and disease.

Conclusion

The human microbiome represents a fertile area of study for nurse scientists. Molecular sequencing is an essential tool in elucidating the microbiome. Each microbiome has large implications for advancing knowledge of disease occurrences and treatment. Understanding the strengths and the limitations of microbiome research and different methods of microbial identification is crucial to designing clinical studies. As metagenomics rapidly changes and new technologies constantly emerge, it is imperative for scientists to remain current and assemble the interdisciplinary teams required to operationalize, implement, analyze and interpret the human microbiome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Principal components analysis of samples from the Human Microbiome Project revealing variation by body site, as skin, urogenital, gastrointestinal, nasal and oral microbiome each cluster separately. From the Human Microbiome Project Consortium (2012b). Used with permission.

FIGURE 2. Dynamic changes in the microbiome throughout life as communication takes place between the central nervous system and the gastrointestinal tract. As development occurs, microbiome stability changes and during infancy, adolescence and old age may contribute to development of mental health disorders. ADHD = attention deficit hyperactivity disorder. From Borre et al. (2014). Used with permission.

TABLE 1

Useful Websites About Microbiome Research

Host	URL
University of Maryland Institute for Genome Science	http://www.igs.umaryland.edu
University of Chicago The Microbiome Center ^a	https://microbiome.uchicago.edu
NIH Human Microbiome Project Microbial Reference Genomes	http://www.hmpdacc.org/reference_genomes/reference_genomes.php
NIH Human Microbiome Project dbGaP Genotypes and Phenotypes	www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000228.v3.p1
American Gut Project	http://americangut.org/

Note. NIH = National Institutes of Health.

^aIn collaboration with Argonne National Laboratory, Lemont, IL and Marine Biological Laboratory, Woods Hole, MA.

TABLE 2

Microbiome Questions for Nursing Research

Microbiome	Priority questions for nursing research
Oral	• What sample location (saliva, tongue, buccal membrane or plaque) most fully represents the oral microbiome?
	• How does oral care influence the oral microbiome and, in turn, influence the lung biome? In high risk populations, e.g. elderly, critically ill), does systematic oral care decrease incidence of pneumonia?
	• What is oral care (regular tooth brushing, powered tooth brushing, chlorhexidine oral rinse, flossing) and how often should it be performed in high risk populations? How do different oral care measures change the oral microbiome?
	• Are there microbial “biomarkers” that can be sampled from the oral microbiome that predict disease? (e.g., inflammatory bowel disease)
Lung	• How does the lung microbiome change in disease states such as COPD or asthma?
	• In critically ill patients who are intubated, is a tracheal aspirate representative of the lung biome?
	• Over time, how does the lung biome change in patients who are intubated? How could this change influence clinical care and how does clinical care impact the microbiome?
Gut	• What is the relationship between changes in the gut microbiome and depression in the elderly?
	• Does administration of probiotics alleviate psychological conditions, (e.g., depression and anxiety) in certain populations?
	• What is the most efficient and valid method to obtain a stool specimen for gut microbiome analysis?
	• Does exposing babies born by Cesarean section to maternal vaginal microbiome decrease incidence of disease? What is the best practice to accomplish this exposure?
	• How rapidly does the microbiome change in neonates and young children? How do microbiome changes affect disease in this population?