Perspective

Hospital microbiomics: benchmarking from the first longitudinal study of microbiome dynamics among patient, staff and hospital habitats

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Introduction

A recently published analysis of microbial dynamics within the hospital setting is the first longitudinal study of its kind and provides baseline evidence to better understand how human-borne microbes colonize and distribute through a monitored indoor environment.[1] Equipped with subject matter expertise and modern methodologies in medicine, patient healthcare, and microbiome metagenomics research, the authors developed community-level knowledge about microorganisms unintentionally transferred between patient, staff, and building surfaces over the course of one year at a newly commissioned in-patient university hospital.[1] Their published results clarify some long-held notions about how people serve as vectors for extensive transmission of commensal microbes amongst themselves across commonly contacted surfaces. This perspective discusses some notable aspects of their findings on the dynamics of microbial communities shared among people and many surface habitats in the patient rooms and nurses' stations, and proposes that the study may be considered in beginning to design standardized microbiomics methodologies. Such benchmarking and baseline understandings are relevant and applicable to similar analysis in other health-related as well as to other high risk settings requiring awareness and control of infection or microbial contamination.

Microbes and microbiomics

Microorganisms are the most abundant and widely distributed form of life on Earth.[2] Diverse biochemical capabilities and highly adaptive genetics enables microbes to colonize almost any living or non-living surface, many with the facility to convert between active metabolism and various suspended states (spore, resting phase, VBNC, etc.). It is no surprise to a microbiologist, and with growing general awareness, that microbes may easily transfer by touch to other people, food, and inanimate objects and possibly persist. A recently cleaned, sanitized, or sterilized surface may have a very brief aseptic lifetime, as confirmed by this published study and others. Repeated handling without frequent washing may even build deposits of nutrients from shed skin cells, oils, and prior biofilm sufficient to sustain extended periods between human hosts or successive transfer to other surfaces. The reported study confirms that several commensal bacteria, monitored at the genera level, are indeed transferred amongst human and inanimate habitats and persist on environmental surfaces for several days.[1] Bedrail and hot water faucet habitats were among the most dynamic sources for microbial transfer, and floor habitats were the least dynamic. This is in a hospital environment having rigorous cleaning, disinfection, and hand washing policies and procedures. This report further confirms that practices for hand washing and facilities cleaning be monitored for compliance wherever patient safety and public health is of concern.

Microbiomics is the analysis of microbes, or their genomes, that populate a certain niche as delimited by host organism, environmental location, or other grouping parameters and characteristics. The practice of this science involves selection and application of several consecutive laboratory procedures and computational analysis tools which have evolved considerably since its origin in microbial genome sequencing.[3,4] The study authors, Drs. Lax, Gilbert and colleagues, used established microbiomics methods and formal clinical guidelines for the collection and analysis of non-cultured microbial communities, as sampled by swab from people and surfaces under an approved plan.[1] Their study design appears to be complete within finite scope, schedule, and budget, and is largely exemplary for practices in human subjects research and laboratory quality controls. As such, the report and underlying references may represent a baseline methodology for standardization, with one noted exception (See point "f", and reference.[5]

Considering standardization of microbiomics methodologies

Biological methods are inherently variable. This is particularly evident in the measurement of microbial samples, and variability is a known concern for microbial genomic analyses.[6] Variable test results challenge scientific and regulatory objectives for quality and reproducibility, and standardization enables normalization of input and processes for comparison of results and conclusions between different studies.[7] For example, microbiological test result variability is accounted for by standardized averaging and two-fold tolerance in regulatory guidance and industry standards for quality control testing for pharmaceuticals.[8,9] Beyond the naturally high variability of microbial test samples, several options for computational analysis tools are available to extract the non-linear relationships and trending within large scale omics data. The power of microbiomics, as demonstrated by this publication,[1] presages increased application even while the practice of microbiomics is resource intensive and rapidly evolving. As data and decisions about patient and product safety becomes increasingly affected by microbiomics, there will follow future updates to regulatory policy. Thus, industry standardization becomes increasingly important to generate unbiased assessments of

microbial content and temporal dynamics as well as to meet evolving compliance guidelines. In the case of the Lax et al.,[1] study referenced here, the same methodology serves well for their own follow-on studies, both to verify any identically performed sub-procedures for qualification and to support robust comparisons in the analysis of new data. However, their approach can also be considered more generally for benchmarking procedures and processes in discussions to design harmonized or standardized methodology, in entirety or in sub-steps as applicable to a field of testing where the utility of microbiomics becomes established.

Microbiomics can benefit other industrial or public health settings where awareness about the content and flow of microbes from people, product, packaging, and local environments is critical for contamination control. The depth of awareness and knowledge that can be provided my microbiomics will benefit other high risk industries employing cleanrooms and controlled environments, supporting facilities design, operational control, and risk management. For example, the manufacture and compounding of pharmaceuticals, biologics, and medical devices under sterile or aseptic processing controls requires trending of all site-isolated microorganisms with mitigation beyond specific thresholds.[8–10] Although testing requirements for sterile bio/pharmaceutical product manufacture requires awareness at the species level for contamination observed in controlled spaces,[10] a broader awareness of microbial flux through a manufacturing plant can provide important auxiliary or supporting evidence in root cause investigations of discrepancies or out of specification results. Industry guidelines also require contamination controls for aerospace manufacturing and assembly.[11]

Components for draft standardization are outlined next, as derived from the referenced Lux et al.,[1] publication[1] and suggested here as an initial talking point for further consideration.

Draft components toward a hospital microbiomics standard

Specifications drawn from this report are listed here for consideration in creating components for baseline standardization. This list is neither comprehensive nor universal, but instead is meant to be a starting point for standards development as would be most directly applicable to the hospital setting. Importantly, the draft list reflects study design and assumptions: a) data are observational rather than interventional; b) analytical power for community-level analyses and not species-level (i.e., does not track potential virulence, antimicrobial resistance, or metabolic profiles). Draft microbiomics method components as follows:

a. Compliance with an Institutional Review Board (IRB) protocol. Sampling sites are to be pre-defined, with consideration for randomized selection if appropriate (not used in this case).

b. Use of commercial reagents for DNA extraction, PCR amplification, and clean-up of amplicons prepared in triplicate and tailored for industry standard next-generation sequencing (HiSeq™ 2000 Sequencing System, Illumina) using standardized barcode oligonucleotide primers and protocols (Earth Microbiome Project).

c. Amplification across the 16S ribosomal RNA gene V4 region with a threshold minimum of 5000 high-quality amplicons per sample.

d. Quality controls in trimming and processing amplified sequences for clustering in the construction of operational taxonomic unit (OTU) using an open reference method (QIIME),[12] sequence identity cutoff set at 97%, and taxonomy assigned to the high-quality candidate OTUs (<1% incorrect bases and discarding OTUs of <5 reads).

e. High-quality oligotype specified by minimum criteria of 500 reads substantive abundance threshold, 1800 samples, and 5% abundance cutoff per oligotype.

f. Normalizing[5] or rarefying unequal OTU tables, with documented justification. While this study rarefied OTUs to an even depth of 5000 reads, the research community[5] has cautioned against rarefying due to loss of statistical strength in subsequent component analysis.

g. Use of a verified oligotyping pipeline (e.g.,[13]) to identify variation within OTUs and for reads back onto the OTU (e.g.,[9]) from targets selected by a justified rationale (e.g., the four most highly abundant genera which were *Acinetobacter*, *Corynebacterium*, *Streptococcus*, and *Staphylococcus* in this study).

h. Use a verified method[14] for weighting of UniFrac distances between each pair of samples, construction of principal coordinates (PC, eigenvectors), and calculation of PC correlations (r) as an average of the Pearson correlation among eigenvectors weighted by their corresponding eigenvalues. In this case, data complexity and noise were first reduced to the minimum set of eigenvectors whose eigenvalues summed to 50% of the variance which was represented by the first 10.

i. Quality check correlations along each eigenvector for significance (e.g., two-sided; confidence level, 95%), and reduce to zero all non significant correlations ($P>0.05$) before averaging.

j. Determine the diagnostic power of microbial community profiles and confirm that paired samples were collected as intended by applying a supervised learning model (e.g., Random forest method).[15]

k. Perform multivariate analysis to infer relationships between the microbiome taxonomic compositions of the tested habitats (patient/staff skin and medical and environments), as justified and supported by published procedures. In this case canonical component analysis (CCA) is applied by established methods with supporting statistical analyses to verify strength of correlations.[1]

l. Metagenomic sequencing was performed as described and referenced[1] and is not repeated here. Procedures described for library construction, quality-trimming, assembly of contigs, estimation of percentage completion using single-copy markers, genotype assembly and assignments for strain level inter-habitat analysis, alignments and other quality checks finish the basic subset of methods.[1] Similarly, tools applied for calculating a and b-diversity among samples are not summarized here as these will depend on method (e.g., see[12]).

Although the above listed specifications are directly applicable to a hospital microbiomics analysis at the same facility, they form a draft template from which to customize an approach for other purpose and setting. Sampling locations are not included here as these would be unique to the scope of each study or procedure. Some notable findings regarding the locations analyzed and their dynamics discovered in this study are summarized next.

Summary findings

The reported study[1] shows microbial dynamics that supports and clarifies prior understandings about the transmission of microorganisms and their survival on indoor environmental surfaces. The survey of microbial diversity began 2months before the hospital opened and continued for a year afterward, collecting 6523 microbial samples from multiple sites in 10 patient care rooms and two nurse stations divided evenly across two hospital floors. Sampling sites represented high contact locations for human and environmental surfaces, including: hand, nose, axilla (armpit), and inguinal fold (groin), staff clothing and personal effects (e.g., shoes, pagers, and cell phones), handrail, nurse's station phone, computer mouse and countertop, hot water faucet, and patient and station floor. Some notable findings are as follow:

I. Immediately upon opening, the nurse station floor and surface microbiota trended toward human skin–associated genera (*Corynebacterium*, *Staphylococcus*, and *Streptococcus*) and decreased in environmental genera which had dominated before opening (*Acinetobacter* and *Pseudomonas*); the increase in human skin microbes was significant for commonly contacted nurse station surfaces but not for floor samples.

II. Bacterial communities among different habitats in a patient's rooms consistently resembled the skin microbiota of the patient occupying the room, particularly the bedrail.

III. The patient's skin microbial signature increasingly influenced all room habitat communities which increased over time of the patient's stay.

IV. Patient's ambulatory status was the dominant factor associated with microbial similarities between patient and room surfaces.

V. Clinical factors (antibiotic use, chemotherapy, surgical recovery) were only weakly or non-significantly associated with microbiome dynamics.

VI. Antimicrobial resistance genes were more abundant on room surfaces than on patient's skin, and some persistent unique genotypes were identified including *Staphylococcus* and *Propionibacterium*.

VII. Significant correlation (r=0.47) suggested consistent reduction in certain bacterial taxa by pre-surgical preparation with antimicrobials such as chlorhexidine.

VIII. By end of study, skin microbiota (patients and nurses) were the least diverse of all sample types, while samples that interact with the outdoors (shoes, floors, and recirculated indoor air) remained the most diverse.

IX. Hand microbial communities of staff were more similar to those of hospital surfaces than were hand microbial communities of patients, reflecting the greater mobility of staff within the hospital.

X. These results confirm the rapid transfer of skin microbes to indoor surfaces and their persistent survival due to subsequent re-contamination and transfer. Bacteria and viruses deposited on indoor surfaces are generally understood to be nonviable within a 24-48 hr, although some infectious *Staphylococcus* strains have been shown to survive and re-infect after more than a week.[16] The results also concur with an idea of sequential transmission across indoor surfaces, e.g., from patient to bedrail to staff and vice versa. Although not specifically reported, these findings might concur with a likelihood that gloved hands can also transfer microbiota from a pre-contaminated surface. In summary, findings form the report further strengthens the need for care and vigilance in training and maintaining procedures and practices for cleaning hands and surfaces. This also reaffirms our understanding that human skin, and particularly direct contact, represents the greatest risk for introduction of microbial contamination.

Conclusion

The research approach and findings reported by Lux, Gilbert and colleagues[1] represents foundational knowledge in hospital microbiomics and a solid demonstration of methodology that can measure "the extent to which the microbial ecology of patient skin and of hospital surfaces are intertwined". Their confirmation of human microbial communities on and between frequently-contacted surfaces is notable because such habitats (hands, bedrail, faucet) continue to function actively in microbial transfer between patient, staff and common surfaces even though the hospital-controlled habitats are cleaned and disinfected regularly, the benefit of hand hygiene has been known for many years,[17,18] and many efforts have tried to improve compliance remarkably found to be low as 40%.[19,20] These findings underscore a necessity for any facility concerned with contamination control to periodically reassess the role and dynamics of microbial transmission so as to reaffirm policies, procedures, and practices with frequent monitoring for compliance. Regarding methodology, the study certainly provides baseline information for their follow-on studies, and more generally is suitable for drafting benchmark procedures useful in similar or related studies for other health-related settings that require contamination control, such as the pharmaceuticals and aerospace industries. Most directly and urgently, the reported method represents a potential standard to aid wider and improved monitoring for awareness and control of microbial transmission in the global effort against healthcare associated infections and emerging infectious diseases.[21–24]

With increased application of any method there comes a growing role for some form of standardization. Microbiomics methods are now supporting research, development, and clinical studies of traditional and new treatments including personalized medicine,[25–27] as well as an expanding list of non-medical areas of research and commerce, such as oceanography, agriculture, soil sciences and plant pathology to list but a few examples.[28–30] The application and impact of microbiomics is increasing rapidly. While specific methods and applications vary greatly per industry or area, and standardization will be a complex endeavor, the time has arrived to begin outlining best practices to benefit all stakeholders. Methodologies from the referenced Lax et al.[1] are suggested here as part of many exhibits to be considered toward the drafting of harmonized standards that will aid future application of microbiomics testing.

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Conflict of interest

The author declares no conflict of interest.

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