Project

2015-DN-BX-K430: Characterizing Microbial Assemblages as Trace Evidence as Following Residential Burglaries

Project subjects

We enrolled individuals from 10 households; 5 in Chicago and 5 in Florida. Since surfaces within a house will be swabbed, all members of a family residing in the house will be subjects of the study. In total, 30 individuals were enrolled across the sampled households from birth and up.

Project design and methods

The human skin and mucosal surface microbiome has a unique signature that represents a composite of all the physical and environmental interactions a human has throughout their life. This microbial signature is preserved when it is transferred to a new environment for a defined period of time. The unique character of the human skin microbiome can provide clues as to that person's lifestyle, diet, ethnicity, type of work, and even whom they regularly interact with. That this signature is left behind in a space provides unique forensic potential to provide trace evidence that can be used in investigations. Once the microbiome stabilizes around age 2-3, a unique microbial composition and structure has formed, that can fluctuate, but retains a core identity. Based on our extensive experience examining human microbial signatures associated with the indoor and outdoor built environment we have been able to demonstrate the integrity of the signature we leave behind, and even show the hour by hour way in which

that signature changes on different surfaces. Using this information, we can track time since last occupancy for a space, and the potential for the signature left behind to capture information about our lifestyles, geographic origin, and personal relationships. We will also explore how architectural and building material choices can shape the way humans interact microbially with their environment, and what this means for how we can optimize the forensic potential of indoor environments.

We aim to characterize the microbiome left behind at mock crime scenes. We recruited 'home owners' and 'invaders' and performed repeated reciprocal invasions on properties in Chicago and Fort Lauderdale. We aimed to determine (a) the length of time an invasion has to occur for to allow for detection; (b) whether wearing gloves interferes with signature detection; (c) whether lifestyle traits of 'invader' can be detected.

We sampled the hand and nasal microbiome of 'home occupants' (including cats or dogs) and of the 'invaders', and up to 10 home surfaces, 4 samples of floors, 2 samples of door knobs, 2 samples of counter tops, and 2 miscellaneous surfaces. Residents of the home were instructed to take swabbed samples of commonly touched surfaces and personal items within the residence using puritan sterile cotton swabs (model no. 25–806 1WC) prior to the mock home invasion. A dry cotton swab was rubbed over the 10 surface for 10 seconds turning the swabbed head to collect as much biomass as possible. Surface texture (metal, wood, etc), time and location in the household was recorded for each swab. 'Home Occupants' were asked to swab their body (nose and dominant hand) as well as those of their children and any relevant pets (Paw and external nose surface) in the home. After the residents exit the home, two

members of the research team entered the home and conducted a mock invasion. Both research members accessed the home at single entry point indicative of forced entry. Prior to entering the home, the research team members swabbed their nose and hands to obtain microbiome prior to interaction with home. They were given 30 minutes to touch as many random surfaces within the home; research members acting as invaders were not given instructions on which surfaces to touch, but they had to interact with surfaces. Invasions were performed, with 1 invader wearing nitrile gloves and the other invader not wearing nitrile gloves. For communal areas within the household, both team members interacted with surfaces and objects. For private rooms (bedrooms, office, etc), team members were instructed to avoid entering a room if one of the invading team member has already entered that space, which allowed for a sole invader microbiome contribution. To help track surfaces touched, the invading team members placed a posted-note on each surface touched with an indication of time they interacted with that surface. Following the 30-minute invasion time a second group of research team members entered the residence for sample collection. To help minimize the spread of contamination, researchers wore shoe covers, facemasks and hair-coverings.

Microbiota samples were collected and stored on Dry Ice during shipment to the laboratory, where they were stored at -80°C until DNA extraction. All samples were processed using a modified version of the manufacturer's protocol of the Extract-N-Amp kit (Sigma-Aldrich). Swabbed tips were placed into 2ml 96-well Deep Well plates (Axygen). 200µl of Extract-N-Amp Extraction solution was added, vortexed for 5 seconds, and incubated at 90°C for 10 minutes. Samples were centrifuged a 2,500 x g for 1 minute. 200µl of Extract-N-Amp Dilution solution was added to each sample to

obtain a 1:1 ratio of extraction to dilution solution. Genomic DNA was amplified using the Earth Microbiome Project barcoded primer set, adapted for Illumina HiSeq2000 and MiSeq by adding nine extra bases in the adapter region of the forward amplification primer that support paired-end sequencing. The V4 region of the 16S rRNA gene (515F-806R) was amplified with region-specific primers that included the Illumina flowcell adapter sequences. Each 20µl PCR reaction contains 5µl of MoBio PCR Water (Certified DNA-Free), 10µl of Extract-N-Amp Ready Mix, 1µl of Forward Primer (5uM concentration, 200pM final), 1µl Golay Barcode Tagged Reverse Primer (5µM concentration, 200pM final), and 4µl of template DNA. The conditions for PCR were as follows: 94°C for 3 minutes to denature the DNA, with 35 cycles at 94°C for 45s, 50°C for 60s, and 72°C for 90s; with a final extension of 10 minutes at 72°C to ensure complete amplification. Following pooling, amplicons were quantified using PicoGreen (Invitrogen) and a plate reader. Once quantified, different volumes of each of the products were pooled into a single tube so that each amplicon is represented equally. This pool was then cleaned using the UltraClean® PCR Clean-Up Kit (MoBIO) and quantified using Qubit (Invitrogen). After quantification, the molarity of the pool was determined and diluted to 2nM, denatured, and then diluted to a final concentration of 4pM with a 30% PhiX spike for loading on the Illumina HiSeq2000 sequencer. Amplicons were then sequenced in a 151bp×12bp HiSeq2000 run using custom sequencing primers and procedures.

Data analysis

Sequence data and patient questionnaires were analyzed using standard methods, and correlated with ancillary data such as building, time of sampling, occupant density, etc. We employed standard non-parametric multivariate statistical tests to determine whether specific bacterial taxa (16S rRNA oligotypes) and combinations of taxa were predictive of occupants and invaders on different surfaces. We employed machine-learning algorithms to determine whether the microbial signature of an individual or a surface can be used to predict specific individual life style traits.

Project findings

Homes (n = 5) were burglarized at both sites (Chicago and Ft Lauderdale) in the morning; these burglaries were conducted in August as well as March to account for seasonal variation. Samples were collected from burglars and various surfaces within the homes before and after the burglary while homeowners provided samples prior but did not provide samples thereafter. DNA was successfully extracted from all samples, and 16S rRNA amplification and library construction and sequence was performed. Microbial 16S rRNA amplicon sequence data was analyzed using QIIME and R software toolkits. Using Bray-Curtis dissimilarity distances, we looked at the stability (0) and variability (1) of the microbial community structure where we detected noticeable shifts in the similarity of the home prior to and following burglaries among homes.

The 16S amplicon data was split into it the built-environment and human components in order to ascertain which microbial assemblages changed following the burglary. In order to eliminate signature variants, we applied DeBlur to all sequences to cluster sequences at 100% nucleotide identity. Bacteria commonly associated with the

skin microbiome (*Corynebacterium, Streptococcus, Staphylococcus*, etc) were shown to be significantly differentially abundant in the homes, homeowners and burglars when looking at the before- and after-burglary associated samples. Approximately 80% and 65% of the resident microbial populations were altered among individuals when analyzing nare- and hand-associated samples, respectively, suggesting that microbial assemblages could serve as potential trace evidence (Figure 1).

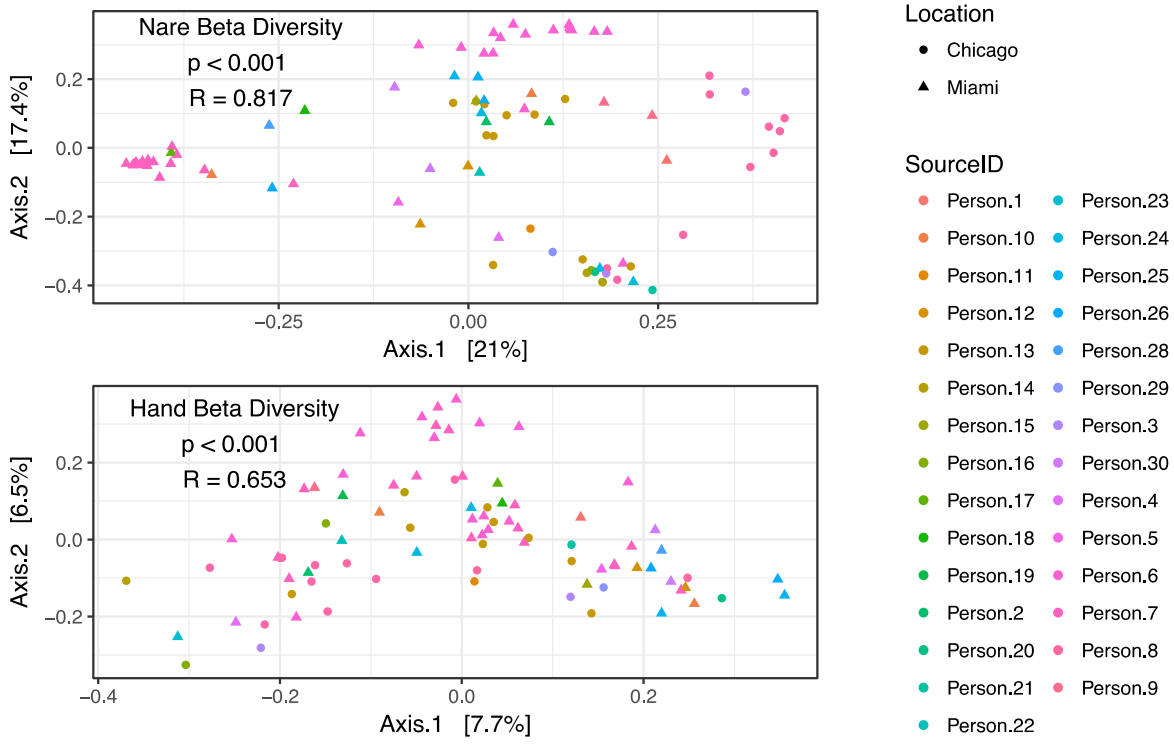

Figure 1 depicts the similarity via Bray-Curtis distances in microbial community structure for each participant in Chicago and Fort Lauderdale. Samples were split in into hand and nares, and permutational models showed both human sites were significantly ($p < 0.01$) different among individuals and highly distinguishable based on microbial community structure.

Hand samples were grouped either by the specific burglar or household resident. OTUs unique only to each individual were analyzed to discern whether identifiers could be used to profile potential suspects. OTUs that did not appear at least twice were discarded to reduce the risk that the OTU was an artifact of contamination. Preliminary

data demonstrates that we can extract biomarkers unique to burglars and resident (Figure 2). The majority of the unique bacteria recovered in residential homes come from either the Gammaproteobacteria or Alphaproteobacteria group. Many of the bacteria within these two groups are unidentified, which is not uncommon given their rarity. Approximately 70% of the unique OTUs identified among participants were derived from hand-associated samples with the remaining 30% sourced from the nares. We are currently analyzing these unique microbial signatures and their dynamic community contribution before and after the burglary for various surfaces to determine which surface is the best predictor of unique microbiomes and their temporal stability for detection.

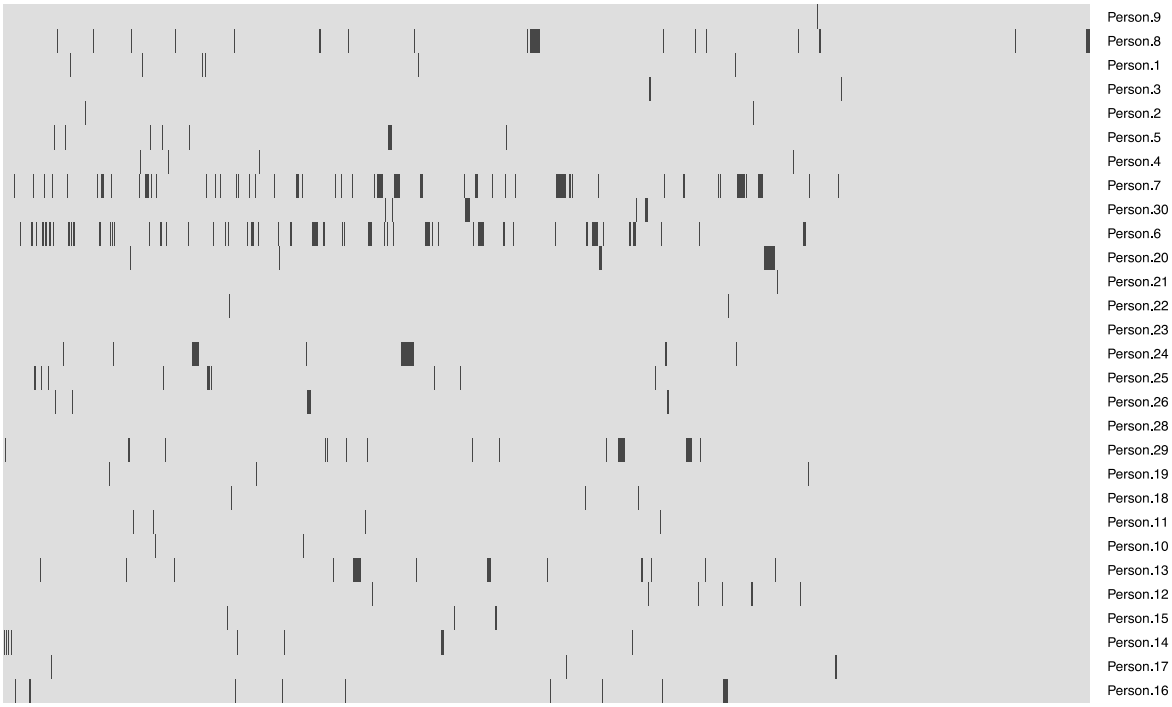

Figure 2 depicts a heatmap containing unique microbial taxa for each participant in Chicago and Fort Lauderdale.

It is well characterized that human microbiome is continually shaped by internal and external factors due to the host and/or environment. Because many of the taxa associated with an individual is considered to be part of the rare biosphere, we observed the change in these rare taxa over time in a controlled setting. The relative change of unique taxa for a single individual showed discrete rates of decay on a touched surface, which were unaffected even when disturbed (marked by the presence of other individuals). However, the decay of rare signatures was relatively rapid usually showing completely turnover within 30 minutes of initial contact suggesting detection of an individual's unique microbial combination is time sensitive.

Implications for criminal justice policy and practice in the United States

This research has built upon former work entitled; "Evaluating the Skin Microbiome as Trace Evidence" and built and extended its premise which is; the microbiome can be an important tool in the forensic scientist's toolbox. This research has shown several points including that unique and identifiable taxa may be identified back to a particular individual, that individual being the possible burglar or entrant to the crime scene. This then implies that crime scene individuals in the future MUST protect a crime scene from contamination by police, crime scene personnel, and others who might have entered the scene later after the crime has occurred. It has also been shown that further work needs to be done including identification of the bacteria which could NOT be identified as there represent a rare event or part of an individual's microbiome. This research has significantly lowered the bar ahead since it has involved data gathered by mock crime scenes which mimic real-time crime scenes. The first question which crime scene personnel ask is how much impact is made by the individuals entering a crime scene

and using their hands to touch objects within the crime scene? A second question is how unique is the individual microbiome can be traced back to an individual? The third question they ask is how long can this microbiome from an individual last at the scene? These questions have been addressed in the research.

Bacterial cells and their inherent genetic and taxonomic signatures are more likely withstand decomposition and degradation by the environment (temperature, sunlight, moisture etc) compared to residual human biological material. This may result from the bacterial cell wall or vegetative bacterial phases which can protect their hereditary material. Therefore, when microbial oligotypes which assist in individual identification can be reliably determined at each crime site, this valuable information can be applied to forensics applications in conjunction with more traditional methods. As demonstrated in this study, sampling for microbes with the routine swab method is not too onerous and relatively inexpensive (sans DNA sequencing), and probably should be included at most crime scenes. Sequence and statistical analyses of microbial 16S rRNA sequences would require an advanced level of trained personnel, or could be funneled to a central data processing center or expert collaborators.