

**Background:
Hospital Acquired Infections (HAI)**

- The ten most common pathogens:
 - coagulase-negative staphylococci
 - *Staphylococcus aureus*
 - *Enterococcus species*
 - *Candida species*
 - *Escherichia coli*
 - *Pseudomonas aeruginosa*
 - *Klebsiella pneumoniae*
 - *Enterobacter species*
 - *Acinetobacter baumannii*
 - *Klebsiella oxytoca*

Accounted for 84% of the observed HAIs in 463 hospitals over a 21 month period. (Hidron, 2008)

Hidron, A. I. et al. Infection Control and Hospital Epidemiology 29, 996-1011 (2008).

**Background:
Hospital Acquired Infections**

	Contracted	Fatal (% Fatal)
Hospital	1.7 million	99,000 (6%)
Non-Hospital	1.5 million	15,743 (1%)

4.5 Infections per 100 Hospital admissions

Cause of Death – Total over U.S. in 2002	Number
1. Diseases of heart	696,947
2. Malignant neoplasms	557,271
3. Cerebrovascular diseases	162,672
4. Chronic lower respiratory diseases	124,816
5. Accidents (unintentional injuries)	106,742
Hospital Acquired Infection - associated	99,000
6. Diabetes mellitus	73,249
7. Influenza and pneumonia	65,681
8. Alzheimer's disease	58,866

Groseclose SL, et al. (2004) MMWR Morb Mortal Wkly Rep 51: 1-84. Anderson RM, Smith BL (2005) Natl Vital Stat Rep 53: 1-89. Hall-Baker PA, et al. (2010) MMWR Morb Mortal Wkly Rep 57: 1-100. Klevens RM, et al. (2007) Public Health Rep 122: 160-166.

Project Goal

- Determine which environmental parameters have the greatest influence on the development of microbial communities within a hospital.
 - Patient/Staff Microflora
 - Building Material
 - Temperature/Humidity
 - Light Level/Source
 - Demographic Exposure
 - High vs Low Traffic
 - Staff vs Patient Area
- Understanding how demographics interact with the succession of microorganisms in a hospital.

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Guiding Hypotheses

1. Microbial community structure on hospital surfaces can be predicted by human demographics, physical conditions (e.g. humidity, temperature), and building materials for each location and time.
2. A patient-room microbiota is influenced by the current patient and their duration of occupancy, and shows community succession with the introduction of a new occupant.
3. The colonization of the surfaces and patients by potential pathogens is influenced by composition and diversity of the existing microbial community derived from previous occupants of the space.
4. The rate of microbial succession is driven by demographic usage and building materials.

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Ideal Sampling Strategy: Daily Sampling of Bacterial Reservoirs for a Year.



<p>Patient Area Bed rails, tray table, call boxes, telephone, bedside tables, patient chair, IV pole, floor, light switches, air exhaust.</p> <p>Patient Restroom Sink, light switches, door knob, handrails, toilet seats, flush lever, bed pan cleaning equipment, floor.</p> <p>Additional Equipment IV Pump control panel, monitor control panel, monitor touch screen, monitor cables, ventilator control panel.</p>	<p>Water Cold tap water, hot tap water, water used to clean floors.</p> <p>Patient Stool sample, nasal swab, hand.</p> <p>Staff Nasal swab, bottom of shoe, dominant hand, cell phone, computer mouse, work phone, shirt cuff, stethoscope.</p>	<p>Travel Areas Corridor floor & wall, stairwell handrail & steps & door knobs & kick plates, elevator buttons & floor & handrail.</p> <p>Lobby Front desk surface, chairs, coffee tables, floor.</p> <p>Public Restroom Floor, door handles, sink controls, sink bowl, soap dispenser, towel dispenser, toilet seats, toilet lever, stall door lock, stall door handle, urinal flush lever.</p>
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240 Patient Rooms + 50 Staff
= 2,437,105 samples
= \$24 million in extraction/
sequencing consumables alone

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Reduced Sampling Strategy: Weekly Sampling for a Year of 187 Sites

<p>Human</p> <ul style="list-style-type: none"> ▪ Patients (≤ 20) <ul style="list-style-type: none"> - Nose - Stool ▪ Staff (x5) <ul style="list-style-type: none"> - Nose - Uniform cuff - Pen - Cell phone 	<p>Patient Room (x20)</p> <ul style="list-style-type: none"> ▪ Floor ▪ Bedrail ▪ IV control panel ▪ Faucet handle ▪ Outer door handle ▪ Air exhaust filter 	<p>Nurse Station (x1)</p> <ul style="list-style-type: none"> ▪ Countertop ▪ Computer mouse ▪ Phone handle ▪ Chair ▪ Corridor floor ▪ Hot tap water ▪ Cold tap water
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Sampling Airborne Microorganisms

- Each patient room has independent exhaust vents which can be fitted with removable filters for this study.
 - Replace filters weekly.
 - Use ventilation rate, filter efficiency, and microbial abundance to calculate the concentration of airborne microorganisms.

- Further interrogation via particle deposition onto blood-agar plates for a 1 hr period, followed by challenging of colonies with antibiotic susceptibility tests.



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Sampling Protocol: Compatible with Quantitative Analyses

- Sterile swabs moistened with saline solution will be used to sample a region of pre-defined dimensions.
 - qRT-PCR provides an estimate of genomes, yielding cells/cm²
 - Allows conclusions to be drawn regarding actual abundance of microbial taxa, rather than relative abundance.

- Hot and cold water supplies
 - Single location
 - Run for 15 sec
 - Extract from 100 uL



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Sample Selection

- Although 20 patient rooms and their occupants will be sampled, only 8 will be selected for sequencing.
 - Dramatically reduces expenses for sample prep & sequencing.
 - 4 rooms in which HAIs were contracted.
 - 4 rooms in which HAIs were NOT contracted.

- Addresses the hypothesis:

The colonization of the surfaces and patients by potential pathogens is influenced by composition and diversity of the existing microbial community derived from previous occupants of the space.

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Passive Monitoring

- Data loggers will be placed in patient rooms to take hourly measurements of:
 - Temperature
 - Relative Humidity
 - Dew Point
- National Oceanic and Atmospheric Administration records will be used to obtain data on outdoor environmental conditions.



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Patient Infections

- We will request IRB approval for accessing pathogen reports for patients participating in this study if they contract, or are admitted with, an infection.



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Project Timeline

- **November 2012**
 - Conduct survey of building materials.
 - Identify all sampling locations in the building, and begin collecting surface, air, and water samples.
 - Secure and activate data loggers in patient rooms.
- **December 2012**
 - Identify staff members who wish to participate and begin sampling them in their current working environment.
- **January 31st, 2013 – Hospital Opens**
 - Identify patients who wish to participate and begin sampling them a they are admitted to the rooms under observation.



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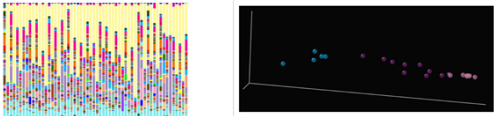
Sample Processing

- Swab tips are cut off and placed in a lysis/PCR solution.
- After incubation and thorough mixing, aliquots are distributed to 96-well PCR plates in triplicate.
- Amplification of 16S/18S/ITS takes place in a qualitative real time (qRT) PCR machine using barcoded primers.
- Samples are pooled into groups of 500 and sequenced to a depth of 3,000 read pairs (2 x 150 bp) per sample.
- Reads are filtered for quality, merged into 250 bp reads, and demultiplexed based on barcode.

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Data Analysis

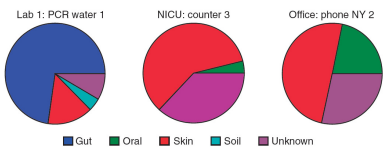
- The QIIME software suite will be used to:
 - Cluster reads into operational taxonomic units (OTUs).
 - Phylogenetically classify OTUs based on reference databases.
 - Calculate alpha and beta diversity among samples.
 - Visualize sample similarity via principle coordinate analysis plots.



Caporaso, J. G. et al. Nature Methods 7, 335-336 (2010).

Data Analysis

- SourceTracker will be used to:
 - Identify sources and proportions of contamination on surfaces.
 - Answer questions such as "What proportion of the air's microbes originate from a patient's nasal microbiome?"

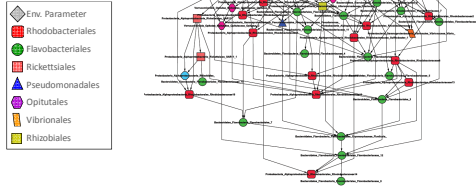


Knights, D. et al. Nature Methods 8, 761-763 (2011).



Data Analysis

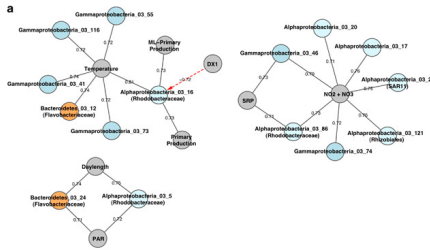
- Microbial Assemblage Prediction (MAP) will be used to:
 - Predict the relative abundance of microorganisms in an environment, given set of environmental conditions.
 - Simulate how community composition will shift if an environmental variable is altered.



Larsen, P. E., Field, D. & Gilbert, J. A. Nature Methods (2012).

Data Analysis

- Local Similarity Analysis will be used to:
 - Identify patterns in microbial succession. E.g. if organism A is blooming now, then organism B will bloom in a few weeks.



Gilbert, J. A. et al. The ISME Journal 6, 298-308 (2011).

Project Scope

- We are currently pursuing a grant that will enable processing of 4,732 samples collected over 52 weeks from 91 locations:

Human	Patient Room (x8)	Nurse Station (x1)
<ul style="list-style-type: none"> Patients (≤ 8) <ul style="list-style-type: none"> Nose Stool Staff (x5) <ul style="list-style-type: none"> Nose Uniform cuff Pen Cell phone 	<ul style="list-style-type: none"> Floor Bedrail IV control panel Faucet handle Outer door handle Air exhaust filter 	<ul style="list-style-type: none"> Countertop Computer mouse Phone handle Chair Corridor floor Hot tap water Cold tap water

- Additional funding could allow for:
 - More frequent collection
 - Additional sampling locations
 - Longer time-course
 - Increased replication
 - Introduction of variables
 - Cancer vs. surgery wards
 - North vs. south facing windows

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