Introduction: Metagenomics in Biology and Medicine

Where microbiomes can be found?

- Charleston harbor
- Islands and beaches
- Residential and agricultural areas
- Built environment
Charleston Waterkeeper: monitor bacteria levels at fifteen of the most frequently used recreational locations around Charleston so you can splash safely.

Medical University of South Carolina
Modern precision medicine decision support tools

So are these!
Why not these?

+ Need massive data on microbiomes at clinically relevant time points linked to detailed health information.
Major challenge: Silo-ing of clinical practice and research
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Microbiology specimen lifecycle

ORDER  COLLECT  TEST  STORE  DISCARD
Living μBiome Bank

MUSC Infection Surveillance Culture Program

- Every inpatient admitted to the hospital is subject to the program
- Swabs for MRSA and VRE are obtained as soon as possible after admission
- Samples are processed within 24-48 hours to determine colonization
- BD auto-streaking instrument is used to minimize bias
- 75% of the specimen volume remains in excess and is discarded within a week of collection
- Thousands of specimens pass through this program every month
Specimen sources for $\text{L}_{\text{µBB}}$

Why infection use surveillance specimens?

- **Sampling uniformity**
  - Limited number of nurses collect the swabs, minimizing collection biases.
  - Sample handling is semi-automated, minimizing handling bias.
- **Participation reach**
  - All patients are subject to the program.
  - ~15,000/year patients swabbed for MRSA.
  - ~7,500/year patients swabbed for VRE (select units, e.g. surgery, etc.)
- **Impact potential**
  - Specimens are collected early in the course of providing care.
  - Specimens are collected throughout care provision timeline.
  - Outcomes available in EHR for correlative research.
What is a microbiome after all?

Informally define it

Ontology for Host-Microbiome Interactions (OHMI) terms

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https://github.com/OHMI-ontology/OHMI
Unique aspects of microbiome analysis

• Expression Analysis
  • Unit of analysis: Transcript (an isoform of mRNA produced from a gene)
  • Measurement: Quantitative

• Variant Analysis
  • Unit of analysis: Variant (a version of a genomic segment)
  • Measurement: number of copies, usually 0, 1, or 2; or 0 vs 1+

• Microbiome Analysis
  • Unit of analysis: Abundance of variants of a 16S rRNA gene amplicon region
  • Measurement: Count of observed sequence variants in the specimen
  • Analogy: variant analysis in an omniploid organism

Additional caveats

• Sequencing errors
  • A big problem when you need to get 100x coverage of a gene in a diploid organism;
  • A huge problem, when the coverage is 10,000x in an omniploid microbiome.

• Functional multiplicity
  • Many microbes fill the same role

• Compositional effects
  • Inference about absolute quantity of microbes is hard with amplicon data

• Causal considerations
What is the role of microbiome in human health?

We are more microbes than we are humans?

- Human shelter 10 trillion microbes ($10^{13}$) in their gut alone, (we are made of 10 trillion cells).
- Only 1 in 10 cells in your body carries ‘your’ DNA. Recent evidence suggests as many bacterial cells as human.
- It is estimated that there are 1000 species of bacteria living in the human gut.
- Compare also the number of human genes (~25,000) to the number of genes and variants that bacterial communities may carry (~4,000,000, see e.g. doi:10.1038/ncomms3151).
Mechanisms for host-microbe interactions

- With each other
  - Via regular ecological mechanisms (competition)
- With the host/environment
  - Produce and metabolize hormones and common nutrients
  - Host immune system

Differential metabolic gene expression in the diseased periodontal microbiome.
A conceptual role of the microbiome in human disease, an infectious disease approach

Robert Koch’s (1843 - 1910) postulates:

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Do these apply to specific microbiota?

Causality via germ-free experiments (postulate 3)

Altering the Intestinal Microbiota during a Critical Developmental Window Has Lasting Metabolic Consequences

Lauren M. Cox, Sung Hee Park, Seung Yeon Yoon, Alexander Y. Aleksseyenko, Jacqueline M. Leung, Ilseung Cho, Arlin B. Rogers, Nicola A. Clinton, Martin J. Blaser

Cell
Microbiome as a mediator in human health

Experimental conditions
- Healthy vs. disease
- Exposure/Treatment

Changes in the host
- Gene expression
- Inflammatory
- Metabolic
- Immune, etc.

Microbiome

Antibiotic Perturbation of Gut Microbiota Dysregulates Osteoimmune Cross Talk in Postpubertal Skeletal Development

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See related Commentary on page 229
Antibiotic Perturbation of Gut Microbiota Does Not Alter Osteoblastogenesis

Static histomorphometric analysis was performed on toluidine blue-stained sections. Antibiotic treatment did not induce harmful effects on normal versus vehicle-treated mice further support the notion that antibiotic effects on osteoblast cellular end points lack differences in proliferative zone height had similar growth plate chondrocyte zone morphology, which suggests that antibiotic- versus vehicle-treated mice had similar growth plate morphology.

The American Journal of Pathology


High-dimensional host-microbiome characteristics

Psycho-physiological assessment Tissues specific gene expression Clinical and health record data Immune cell populations Epigenetic variation Somatic variants Genetic variants Nutrition

Bacterial, viral, and fungal composition and abundance Metagenomic composition Phylogeographic data Phylogenetic data Taxonomic data Metaproteome Metabolome Glycome

HEALTHY?
‘New’ ways to ‘look’ at microbiomes