Slide 1

Two Sides of the Same Coin: Human Bacteriome and Virome in Diagnostics and Disease

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Slide 2

Objectives

- Define and differentiate the human microbiome, bacteriome and virome.
- Describe the influence of metagenomic diagnostic methods on the diagnosis, prognosis and management of human health and disease.
- Examine and engage in discussions regarding the current knowledge related to the status regarding the human microbiome and roles in human health and disease.
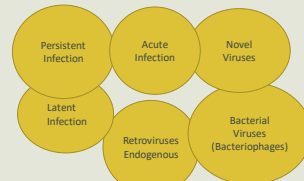
Slide 3

Microorganisms-Microbiome

- Human body contains bacteria, fungi, archaea and viruses
  - Gastrointestinal tract
  - Lungs
  - Skin
  - Other places
- Changes throughout development and life
  - Environmental factors
  - Genetic
- Manipulation of the **Bacteriome**
  - Prebiotics or probiotics
  - Constipation
  - Allergies
  - Inflammatory Bowel Syndrome (IBS)
  - Fecal Transplants (*C. difficile*)

Slide 4

Human Virome in Health and Disease

- Viruses have a MAJOR impact on health and disease
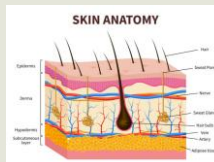- More diverse and abundant than the bacteriome

Persistent Infection
Acute Infection
Novel Viruses
Latent Infection
Retroviruses Endogenous
Bacterial Viruses (Bacteriophages)

Slide 5

Bacteriome + Virome + Fungi + Ectoparasites =

Human Microbiome

Slide 6

The Human Skin

- Complex environment
  - Cooler than body temperature
  - Externally exposed
  - Arid, in comparison to other body habitats
- Unique Habitat
  - Epidermis, outer layer-keratinocytes at various stages of development
    - Stratum corneum, superficial layer of epidermis
    - Semi-impermeable barrier composed of layers of dead, flattened, enucleated, polyhedral, keratin-filled cells termed corneocytes
    - Continuously shed, providing keratin as nutrients for growth of microorganisms
  - Dermis, connective tissue layer composed of blood and lymphatic vessels
    - Connective tissue and fat that separates the skin from the muscle and organs of the body

SKIN ANATOMY

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Slide 7: Skin as a Habitat

- Appendages (unique microenvironments)
  - Eccrine sweat glands-temperature regulation
    - Densely populated in the palms and soles of the feet
    - H_2O and NaCl, small amounts of antimicrobial peptides
  - Apocrine sweat glands
    - Densely populated in the axialla and anogenital area
    - Responsible for the characteristic body odor
    - Secretions consist of proteins and lipids that are odorless upon secretion
    - Microorganisms on the skin are responsible for distinct odor.
  - Pilosebaceous Unit (hair follicle and sebaceous gland)
    - Secretes sebum (primarily triglycerides)-emolliating and waterproofing
    - Prominent on face and scalp, found everywhere except palms and soles of the feet

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Slide 8: Acquisition and Colonization: Building your Skin Bacteriome

- 34 weeks gestational development, skin is ready to meet the outside environment
- Delivery mode contributes to early colonization
  - Vaginal (*Lactobacillus, Prevotella* and *Sneathia* spp.)
  - Cesarean (*Staphylococcus, Cutibacterium* and *Corynebacterium* spp.)
- Overtime, this will balance out, due to interactions in the infant's environment.
- Four dominant phyla
  - *Actinobacteria, Proteobacteria, Firmicutes, Bacteriodetes*
- Human microbiome project has demonstrated a low metabolic pathway diversity across skin organisms.

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Slide 9: Bacterial Identification- Skin Microbiota

- Culture-based isolation of skin microbiota
  - Coagulase-negative staphylococci
    - *Staphylococcus epidermidis* and *Staphylococcus hominis*
    - Mainly commensals but can cause nosocomial infections
      - Indwelling or implanted devices
  - *Cutibacterium acnes*
    - Gram-positive, facultative anaerobe
    - Inhabitant of hair follicles and sebaceous glands
    - Hydrolyzes triglycerides that release fatty acids (acidify and emolliate)
  - Intertriginous Regions
    - *Corynebacterium* spp. (lipophilic, gram-positive, slow growing)
      - *C. jeikeium, C. minutissimum, C. xerosis, Brevibacterium* spp.
    - Gram-negative (infrequent isolates)
      - *Acinetobacter* spp.

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Slide 10: Molecular Approaches –Skin bacteriome

NGS prokaryotic 16srRNA genes

Provides a much broader diversity of the skin microbiota

Hypervariable regions in 16srRNA (nine)

Skin shotgun metagenomics

These permit taxonomic classification

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Slide 11: Fungi Identification-Skin Microbiome

Culture-independent studies have lagged behind

Sequence analysis of the ribosomal RNA genes

Which gene or amplicon, is not as clear!
  Three commonly used are 18s rRNA, 5.8s rRNA, and internal transcribed spacer region
  The spacer is the most effective in species identification (species "barcode")
  18s rRNA, 5.8s rRNA are more conserved (phylogenetic placement)

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Slide 12: Fungi Inhabitants-Skin Microbiota

- Fungal inhabitants also vary topographically
- Most common:
  - *Malassezia* spp., dominates core body and arm sites
  - Less frequently identified on prepubescent individuals, lipid dependency
  - *M. restricta* (head and face); *M. globose* (trunk)
- Feet are characterized by more diversity
  - *Trichophyton, Penicillium, Aspergillus* spp.

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Ectoparasites- Skin Microbiota

- Arachnid skin mite-*Demodex* spp.
- *D. folliculorum* and *D. brevis* colonize the pilosebaceous unites
  - Increases in prevalence with age
  - Produce lipases and utilizes sebum as a nutritional source along with cellular debris and other bacteria such as *Cutibacterium* sp.
- These organisms may directly or indirectly influence skin microbiota based on their own gut microbiome
  - *Bacillus oleronius*, Gram-negative
  - Contribute to inflammatory disorders (rosacea)

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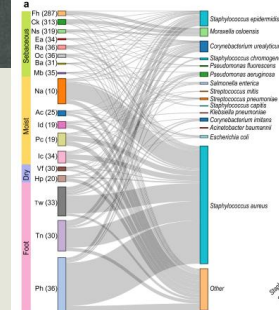
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Skin Microbiome-Virome

- Lack of a consensus gene that can be amplified and sequenced
  - Also complicated by genetic exchange and variability
  - Requires specific conserved genes for different viral agents
  - Whole genome metagenomic shot-gun sequencing allows more characterization
  - High amounts of host DNA from the skin and low biome DNA complicates the process.
- HPV (Human papilloma virus)- most extensively studied skin viruses
  - HPV L1 Gene conserved open reading frame
  - Identifies diverse community of types/strains on the skin
- HpyV (Human polyoma virus) – major commensal of the skin
- Multiple types HpyV6, HpyV7, Merkel cell polyoma virus
- Bacteriophages
  - Mediate the transfer of antibiotic resistance to bacteria
  - *Microviridae* and *Siphoviridae*

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Antibiotic Resistance Genes- Present on the Skin

Body site on the left

Bacteria that harbor the gene on the right

46 males and 248 female, health volunteers.

General population in Shanghai.

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Diseases or Conditions associated with Skin Microbiome

Bacteria
- Folliculitis
- Carbuncles
- Acne
- Atopic dermatitis
- Dandruff
- Wound infections
- Device associated infections

Fungi
- Tinea versicolor
- Dandruff
- Seborheic dermatitis
- Folliculitis
- Diaper rash
- Tinea pedis
- Tinea curis
- Oncychomycosis

Viral
- Common warts
- Plantar wars
- Merkel cell carcinoma
- Kaposi's sarcoma
- Roseola
- Chickenpox, shingles
- Molluscum contagiosum

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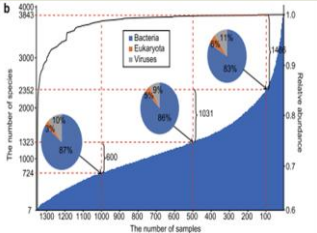
Potential Diagnostic Implications

Variants of disease may be identified based on colonizing microbiota

Personalized treatment regimens

May be used to predict disease risk
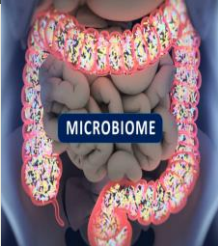
Downstream complications

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Gastrointestinal Microbiome-Habitat

- Complex and diverse community
  - Pathogens, commensals, uncultivable, unidentifiable
- Influenced by
  - Diet, age, host genetics, antibiotic treatment and the environment (psychological stress, hygiene, climate and allergies)
- Community has been demonstrated to influence the health of humans both positively and negatively
  - Gastrointestinal tract
  - Respiratory tract
  - Nervous system

MICROBIOME

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Identification- Gut Bacteriome

- Conventional Diagnostics (stool samples)
  - Gram-stain analysis
  - Stool antigen tests
  - Bacterial cultures
  - Targeted molecular detection of enteric pathogens
  - Pyrosequencing of variable regions of the 16s rRNA gene

- Little to no help in the detection and identification of uncultivable microbes
  - 80% of the gut microbiome
  - NGS has now identified more than 7,000 phylotopes in the human gut
  - Shotgun sequencing-based metagenomic studies

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Acquisition and Colonization of the Gut Bacteriome

- Native gut populations (estimated to be more than 100 trillion microorganisms) Inhibit the colonization by pathogens by:
  - Blocking attachment
  - Competing for nutrients
  - Producing bactericidal components
- Bacteria 60% of the total fecal mass
  - 1100 dominant species with approximately 90% from the phyla *Firmicutes* and *Bacteroidetes*
  - Genera associated with a healthy human gut microbiome
   - *Bacteroides*
    - *Bifidobacterium*
    - *Clostridium*
    - *Eubacterium*
    - *Faecalibacterium*
    - *Lactobacillus*
    - *Roseburia*

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Acquisition and Colonization of the Gut Bacteriome

- Human GI tract
  - Sterile at birth and undergoes a rapid and dynamic expansion following delivery of the fetus
  - Vaginal versus caesarean
- Profound changes occur in life stages based on
  - Food intake (Breast fed, formula fed, solid food) as a baby
  - Toddler (health status, malnutrition, antibiotic treatments)
    - Malnutrition; see an increase in *Campylobacter* and *Helicobacter* spp.
  - Adults (healthy or obesity)
- Geographical differences

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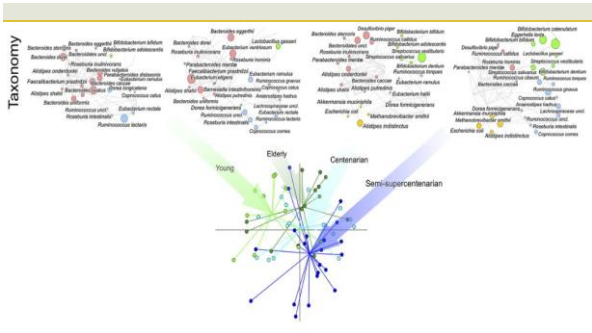
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Enteric Pathogens-Gut Bacteriome

- Progression of the gut bacteriome allows the identification of detrimental perturbations of the health gut
- Major pathogens
  - *Vibrio* (Cholera)
    - Watery stools and dehydration
  - *Campylobacter* spp.
    - Diarrhea, abdominal pain and fever
  - Order *Enterobacterales* (*Escherichia* [pathotypes], *Shigella* [inflammatory colitis], Yersinia [fever and gastroenteritis] and *Salmonella* [gastroenteritis])
  - *Clostridiodes difficile*
    - Diarrhea and severe colitis
  - *Helicobacter pylori* (affects 50% of the world's population)
    - Inflammatory

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Diet, Obesity and the Gut Bacteriome

- Geographic differences include dietary differences
  - Variety increases with a plant- and fiber-based diet
- Three enterotypes
  - *Prevotella* spp.
    - Rural society
  - *Bacterioides* spp.
    - Developed society
  - *Ruminococcus* spp.
- Other factors
  - Ethnicity, sanitation, hygiene and climate
  - Self-selected diets (high-fat/high-sugar)
  - Obesity – lower gut microbiome diversity

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Impact of Antibiotic Treatment-Gut Bacteriome

Antibiotic treatment
 Alters the microbial population of the body

Gut Bacteriome
 Longer lasting even after the last dose (days to several years)
 Decrease normal microbiota and/or increase the population shift to antibiotic resistant organisms

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Gastrointestinal Disease

- Irritable Bowel Disease (Crohn's and ulcerative colitis)
  - Linked to human gene- and microbiome associated factors
  - Decreased bacterial diversity
  - Overall reduction in *Firmicutes* spp.
  - Many studies on probiotic treatment and the affect on IBS
- Gut-Brain-Microbiome Axis
  - Gut microbiome profiles are associated with depression
  - Bacterial community interferes with neurotransmitter signals
  - Increased gut permeability contributes to increased IgA/IgM response against commensal bacteria
  - Brain development and behavior are also impacted by initial bacterial colonization of the gut
  - Autism spectrum disorders-alteration in gut microbiome has been identified

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Therapeutics and the Gut Bacteriome

- Probiotic treatments
  - Depending on microorganism content, may or may not alter the balance in the gut microbiome
- Fecal transplantation
  - Successfully utilized primarily to treat *C. difficile*
  - Also evaluated in metabolic syndromes (energy metabolism and sensitivity)
    - Weight loss

https://www.shutterstock.com/image-vector/fecal-microbiota-transplant-fmt-ibd-ibs-1688915851

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The Gut Bacteriome as a Diagnostic Marker

- Shift in diversity and populations definitively correlates with disease states
- *Bacteriodes fragilis* 60% of the biofilm in inflammatory bowel disease, 30% in self-limiting colitis
- Increased *Campylobacter* and *Helicobacter* spp.
- Too many markers, no standardization in processing
- Need much larger studies

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The Gut-Virome

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Identification of the Human Virome

- Viral culture, electron microscopy, antigen detection, nucleic acid detection, immunologic response
- Molecular and the Human Microbiome Project
  - 102 "normal" subjects
  - Sampled at 5 body sites (nose, skin, mouth, vagina and stool)
  - Main viruses detected
    - Papillomaviruses
    - Polyomaviruses
    - Herpesviruses (Roseolovirus)
    - Adenoviruses
    - Anelloviruses

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Characterization of Body Sites-Primary Efforts

- Gastrointestinal tract
- Respiratory tract
- Plasma/Blood
- Skin
- Cancer
- Attempting to define a baseline and effects of age, geography, environmental exposure and immune state of the human host.

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Bacteriome and Cancer

- Alteration in the bacteriome
  - Induce carcinogenesis
  - Proliferation of oncogenic bacteria
  - Effect of bacterial metabolites
  - Even in organs without bacteriomes
- Where?
  - Colorectal cancer
  - Hepatocellular carcinoma
  - Lung cancer
  - Breast cancer
  - Gastric cancer
  - Pancreatic cancer
  - Oral squamous cell carcinoma

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Virome and Cancer

Human Viruses and Bacteriophages

Tumors (HPV, HBV, HTLV-1, HCV, EBV, MCV)

Burkitt's Lymphoma

Nasopharyngeal carcinoma

Cervical cancer

Head and neck cancer

Bladder cancer

Gastric cancer

Colorectal cancer

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The Virome and Cancer

Molecular Mechanisms of carcinogenesis induced by the T-antigen of polyomaviruses

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Virome and Cancer

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Virome-Bacteriophages and Bacteriome

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Microbiome- Bacteriome and Virome Analysis (METAGENOMIC)

Metagenomic sequencing-method of choice	Data from mixed organisms and the host	Steps in Metagenomic Sequencing	Initial sample processing and nucleic acid extraction
Library construction (target amplification or capture)	Nucleotide sequencing	Primary sequence analysis (database comparisons)	Interpretation and additional analysis

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Metagenomics and Beyond!

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Disease

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Two Sides of the Same Coin! Diagnostics and Disease!

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