The Culture of Your Wound Culture

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Private Practice
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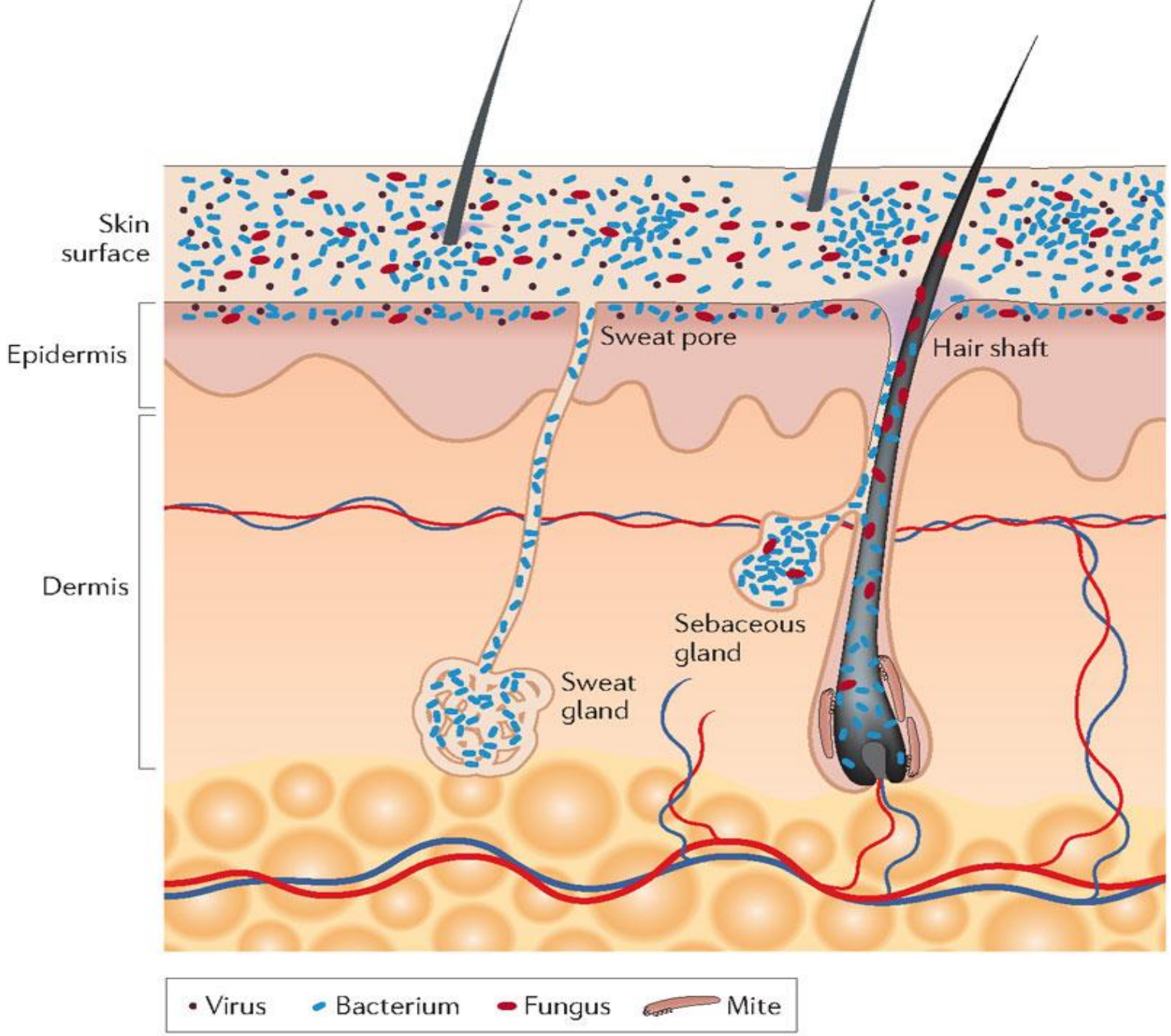
No Conflicts of Interest

Today's Topics

- Problem
- Hx of culture/techniques
- Colonization vs Infection
- Microbiome
- Sequence testing vs Wound Cultures
- Chronic wounds
- Culture technique


Skin surface
Epidermis
Dermis
Sweat pore
Hair shaft
Sebaceous gland
Sweat gland
Virus
Bacterium
Fungus
Mite

The Problem Scenarios

- My antibiotic dilemma
- “Your culture was negative. There is no infection.”
- “It just grew normal skin flora”
- “You should be really sick or dead based on your culture result”

Bacterial Cultures

- Standard since 1800's
- Detect roughly 1% of bacteria in chronic wounds
- Select for bacteria that thrive in nutritional and physical parameters set by a lab
- These organisms may not be relevant
- Reported organism - outcompetes others
- Anaerobes cultivation is problematic
- Ignores all other life forms

Grice, et al. *A diversity profile of the human skin microbiota*. Genome Res 2008;18:1043-50

"I just really hoped you'd have come up with something better by now."

Van Leeuwenhoek
Posthumous thoughts

The Great Plate Count Anomaly

- Observation that most environmental microorganisms seen in the microscope cannot be grown under laboratory conditions

Your Aerobic Wound Culture

- Gram stain
- Blood Agar
- Chocolate Agar
- CNA (gram +)
- MAC (gram -)
- Thiol

Your Anaerobic Wound Culture

- Gram stain
- Brucella blood agar
- CNA
- Laked Blood agar

*Both cultures – 24, 48, 72 hour reads

Wound Swabs

- Cotton, calcium alginate, Dacron-Rayon
- Collect < 0.1ml
- Tend to retain collected specimen
- Sterile loop is diluted (+1,+2,+3,+4)
- More testing = less material (aerobic, anaerobic, mycobacterial, and fungal)
- Transport dilemmas

JCM
Journals.ASM.org

Are Quantitative Bacterial Wound Cultures Useful?

George Kallstrom
Summa Health System, Department of Pathology, Akron, Ohio, USA

Determining if a nonhealing wound is infected can be difficult. The surface of a wound is not sterile and can be colonized with numerous commensal, environmental, and potentially pathogenic microorganisms. Different types of wounds have various clinical presentations, with some signs and symptoms more likely to be present than others depending on the type and location of the wound. Clinicians often order microbiology wound cultures to assist in determining if a nonhealing wound is infected. This minireview briefly summarizes the clinical microbiology of wound cultures, with an emphasis on the history and utility (or lack thereof) of the quantitative wound culture.

Quantitative bacteriology cultures are an important part of the modern clinical microbiology laboratory. Quantitative cultures assist clinicians in determining the threshold above which the bacterial burden of a culture will likely demonstrate clinical significance. Bacterial growth below established thresholds in quantitative cultures typically represents "background noise" of subclinical colonization or inconsequential growth of normal commensal microbiota. The most frequently used quantitative bacterial cultures are urine cultures, where a calibrated inoculation loop is used to inoculate media in order to yield accurate quantitative culture per milliliter of urine. Other less commonly utilized quantitative culturing techniques may be routinely performed depending on the size and scope of the clinical laboratory and can include the use of high-quality liquid specimens such as protected bronchial brushings. Quantitative wound culture techniques were described in large part by research microbiology laboratories in the 1960s and 1970s and were adopted into clinical use thereafter. Quantitative culturing of wounds, particularly biopsy specimens of wounds, involves extensive processing techniques that can be difficult for most clinical microbiology laboratories. Therefore, most nonurine bacterial cultures, including wound cultures, are plated using a semiquantitative technique where cultures are inoculated onto media using a sterile loop that sequen-

lection, processing, and inoculation can often confuse the interpretation of quantitative wound culture results.

Some clinicians are reluctant to perform tissue biopsy procedures in order to minimize patient discomfort, while others fear complications such as introducing bacteria deeper into noninfected tissue, so swab specimens are submitted for culture. It has been my observation that it is not uncommon for clinicians to aspirate wounds producing a purulent drainage with a syringe (ideal specimens) and then inoculate the aspirate onto a swab (a less than ideal specimen) for culture submission. Traditional swabs are made from cotton, calcium alginate, and Dacron-Rayon. Swabs tend to collect a small fraction of a milliliter of specimen (<0.1 ml), which greatly reduces the amount of bacteria that can be recovered from the swab for bacterial culture. In addition to limited volume collection, traditional swabs tend to retain the collected specimen. A newer generation of swabs made from a flocking process which allows more-efficient specimen release has emerged over the past decade. However, flocked swabs share most of the collection limitations of traditional swabs as they do not collect adequate specimens for comprehensive clinical microbiology wound cultures. Swab culture yields are reduced as multiple types of cultures (aerobic, anaerobic, mycobacterial, and fungal) are requested from a single swab, thus requiring inocula-

Colonization vs Infection

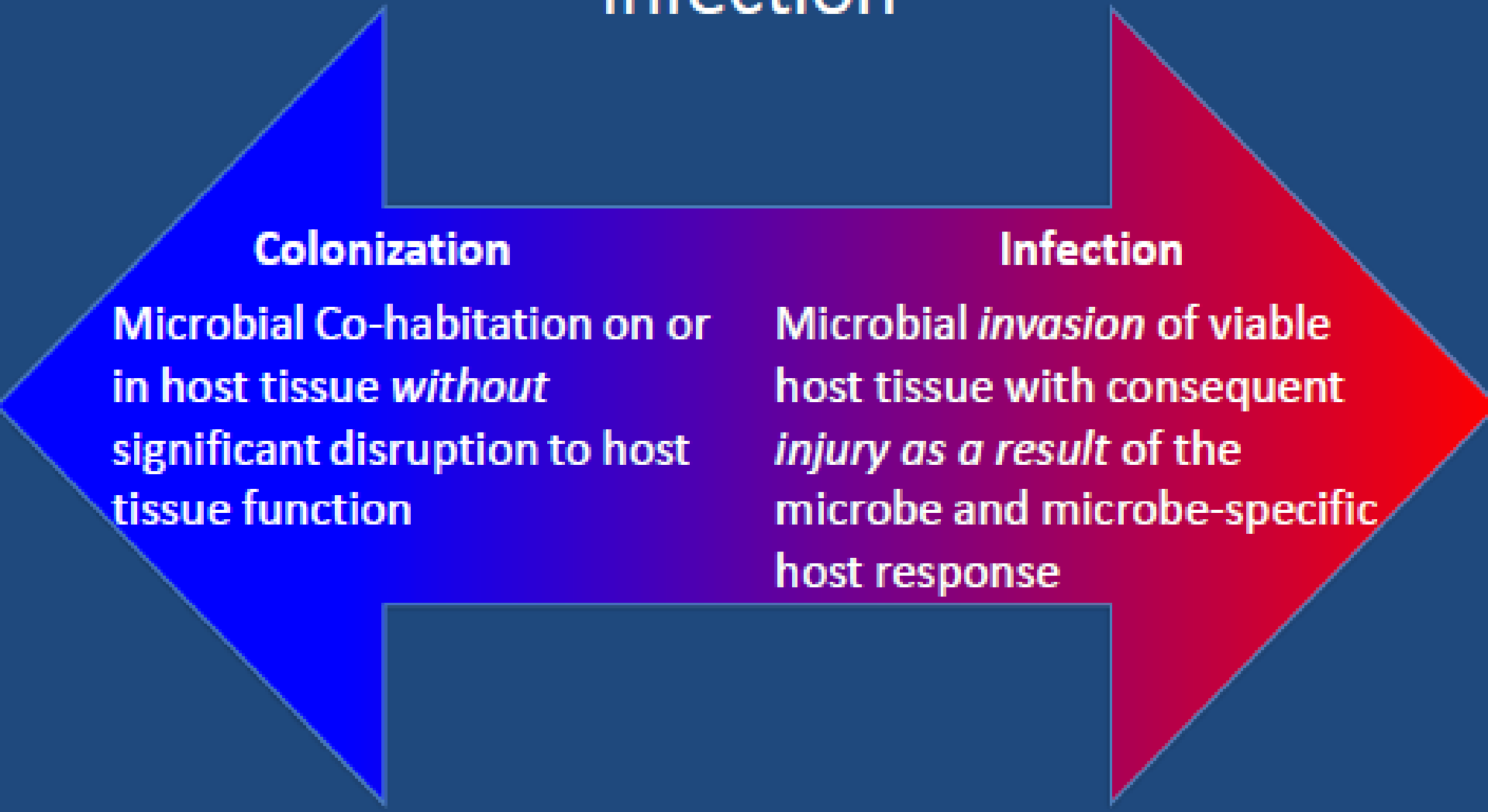
- Infection is **your diagnosis**. Not the lab's
- Organisms cultured from wounds do not define infection
- Antibiotics can have lasting effects

C. Diff Risk with Antibiotic

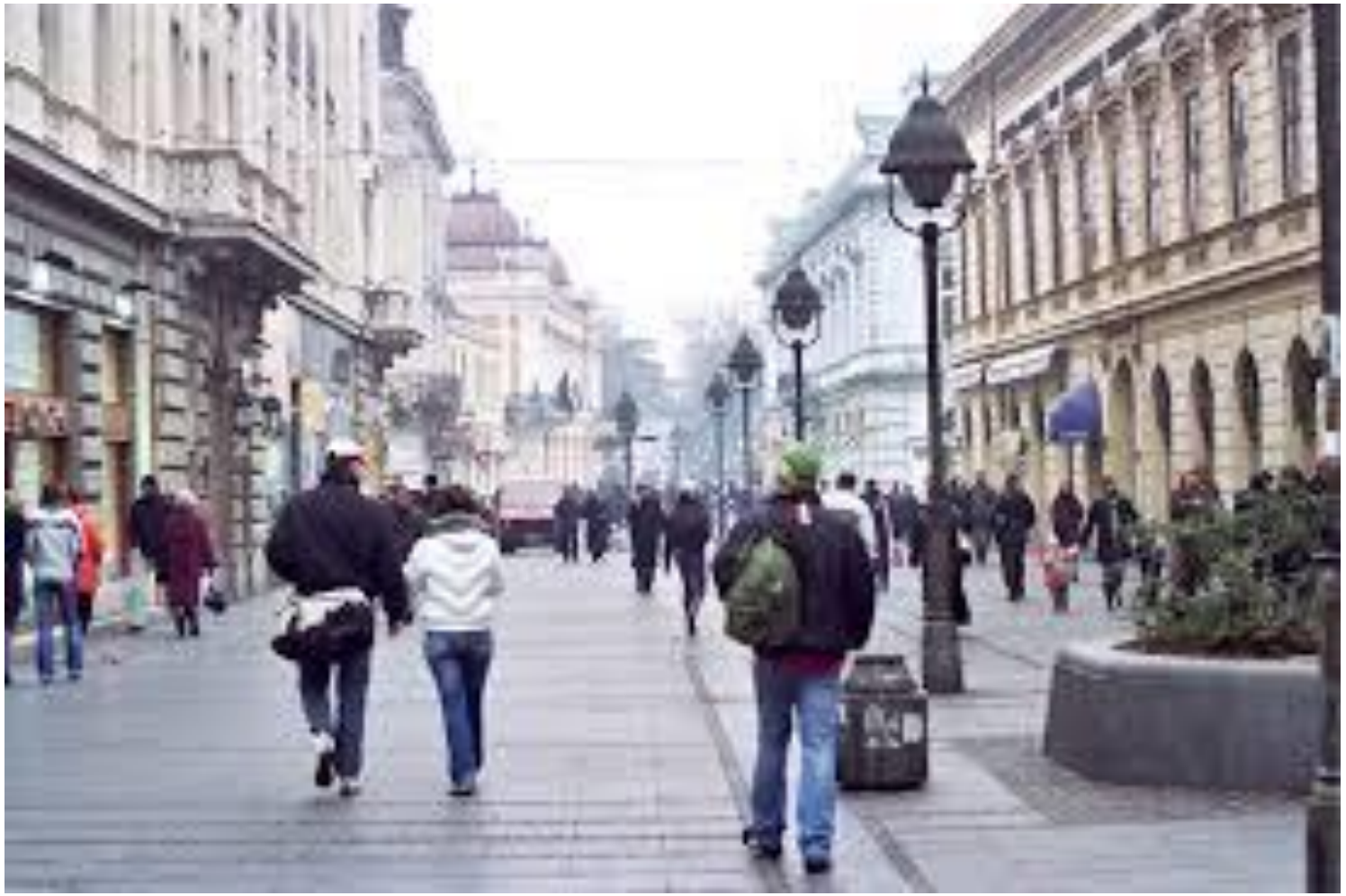
antibiotic	**Odds of CDI**
• **Flouroquinolones**	• **2.8-5.2**
• **Clindamycin**	• **2.8-20.3**
• 3rd Gen Cephal	• 3.2-4.6
• Penicillins	• 1.75
• Macrolids	• 1.4
• TMP-SMX	• 1.78
• Proton inhibitors	• 1.7-2.2
• **Doxycycline**	• **0.91**

J Antimicrob Chemother (2014)69(4)881-2

Distinguishing Colonization from Infection



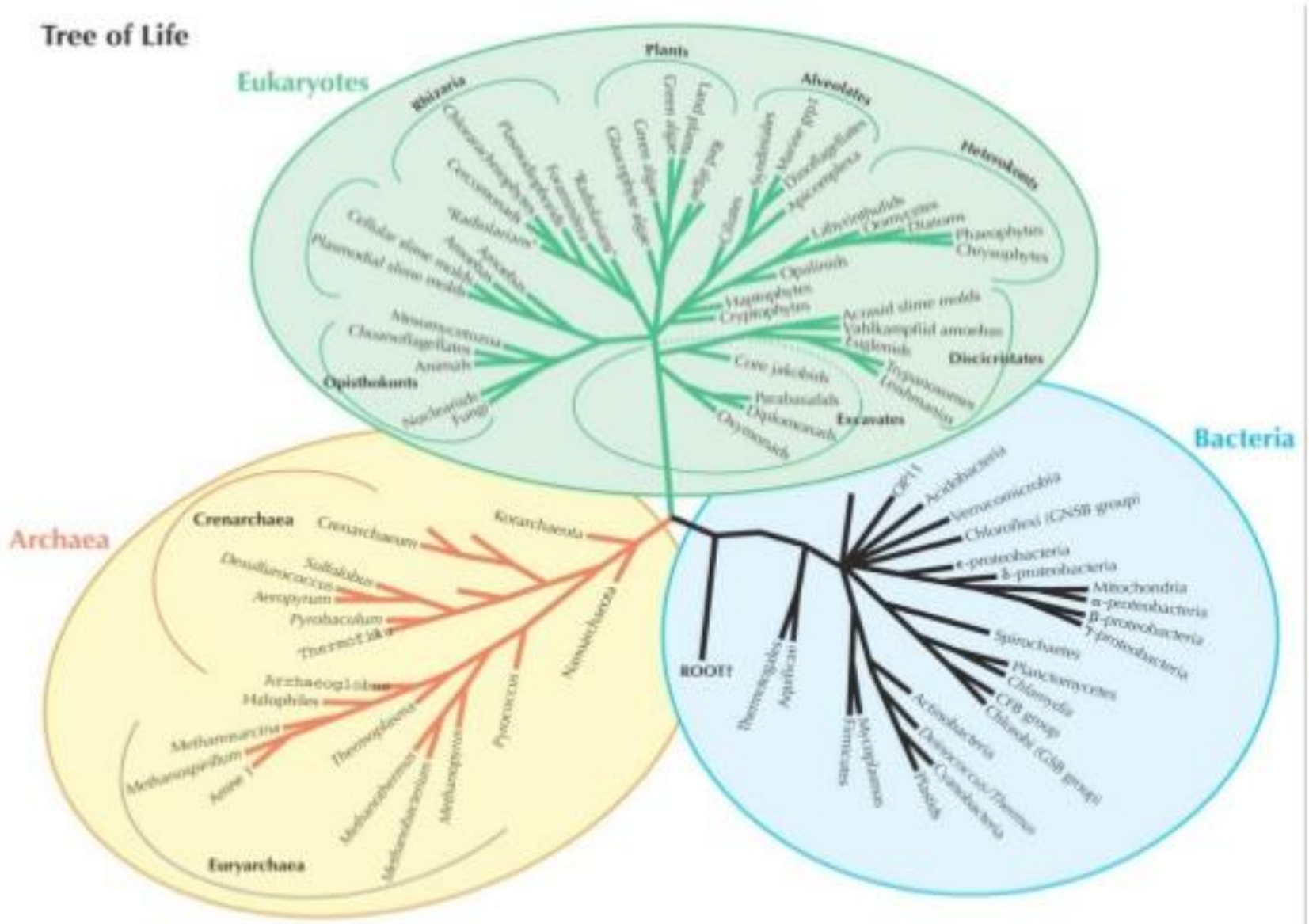
Healthy Skin



Chronic Wounds

Infection/Inflammation

Phylogenetic Diversity

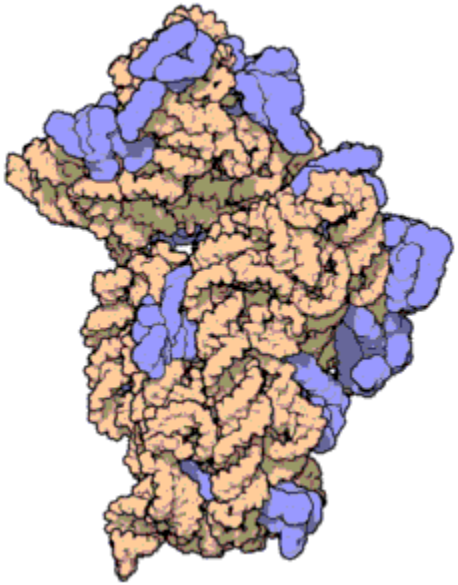


Sequenced Based Testing
16S rRNA

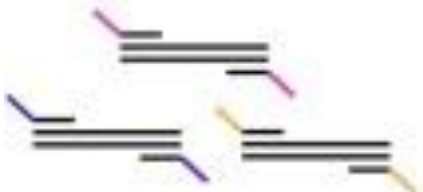
- “gold standard” among microbiologists
- >500,000 in public database (NCBI)
- Reportedly > 2 million in private database
- GreenGenes, EZ-Taxon e, Ribosomal Database Project, SILVA

16S rRNA

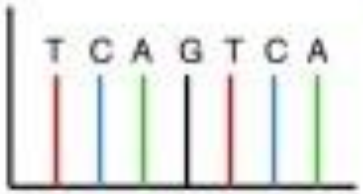
- 16S rRNA present in prokaryotes
- Encodes part of a ribosome
- Allows for identification and amplification (PCR)
- Slow rate of evolution



1) Extract DNA from wound sample



2) Amplify bacterial DNA using 16S rRNA gene primers



3) Sequence the PCR products

4) Analyze sequencing data:

a) Identify the microbial taxa by querying the sequenced amplicons against 16S rRNA gene databases

b) Measure similarity between wound microbiomes by analyzing shared phylogeny

c) Analyze microbial community membership, structure, and diversity

Mesenchyme | The Embryo Pro... | Mycobacteria Sequencing | Uni... | Home - Nucleotide - NCBI

www.ncbi.nlm.nih.gov/nuccore

Apps | Suggested Sites | Imported From IE | Canvas OCC | Places | Atlas Obscura | ucsf pathology

NCBI Resources How To Sign in to NCBI

Nucleotide

Nucleotide Search

Advanced Help

Nucleotide

The Nucleotide database is a collection of sequences from several sources, including GenBank, RefSeq, TPA and PDB. Genome, gene and transcript sequence data provide the foundation for biomedical research and discovery.

Using Nucleotide

- Quick Start Guide
- FAQ
- Help
- GenBank FTP
- RefSeq FTP

Nucleotide Tools

- Submit to GenBank
- LinkOut
- E-Utilities
- BLAST
- Batch Entrez

Other Resources

- GenBank Home
- RefSeq Home
- Gene Home
- SRA Home
- INSDC

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Pros and Cons of DNA/RNA sequencing

Pros

- Eliminates bias of culture techniques
- Not limited to bacteria
- Microbial load
- Microbial diversity
- Identifies “Pathogens”
- Cost is reasonable
- Primer tailored

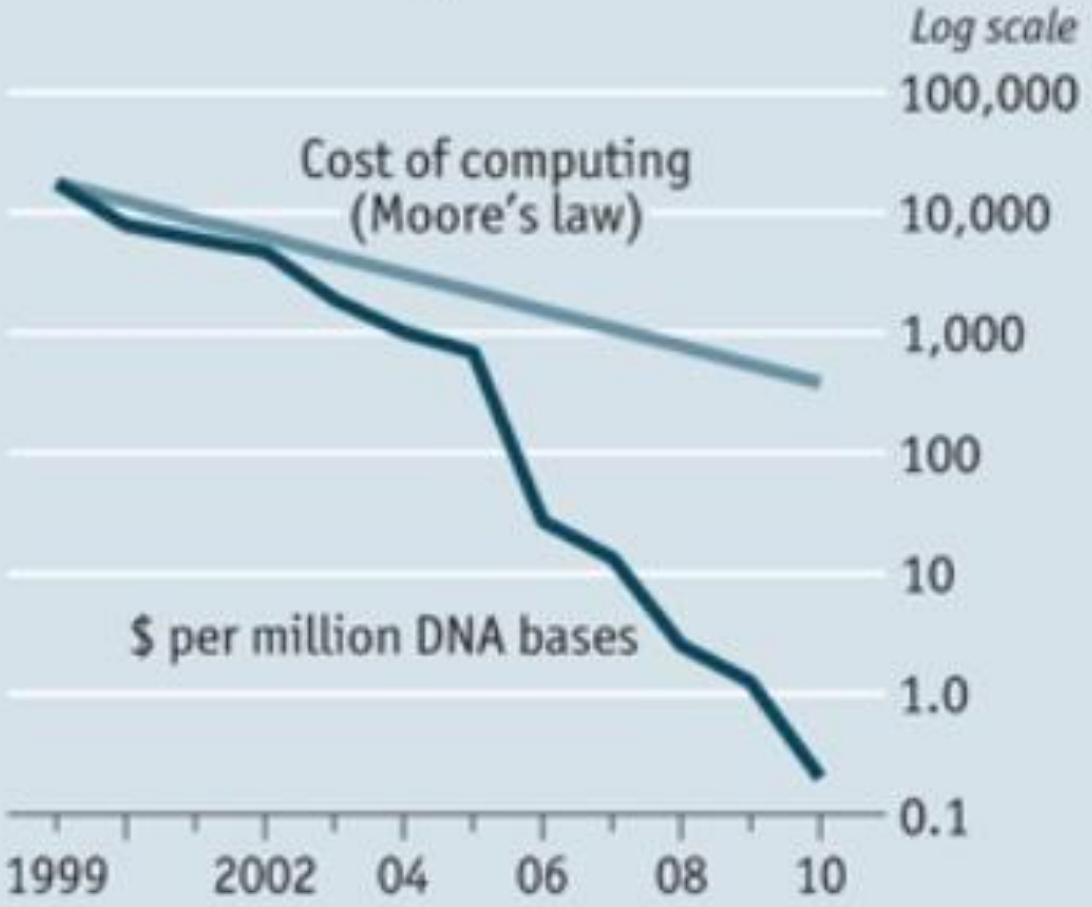
Cons

- Possible human contamination
- Viable vs non-viable
- ID’d organism may not be clinically relevant
- “Chain of Evidence”
- Primer bias*

Baseline information

1

Cost of genome sequencing compared with Moore's law for computers



Source: Broad Institute

Mycobateria (acid-fast)

- Good for slow growers
- Rapidly growing mycobacteria (RGM)- 65-KDa heat shock protein and RNA polymerase Beta subunit genes*

*Differentiate between *M. abscessus, M. chelonae, M. bolletii*, and *M. massiliense.*

Yeasts & Molds

- Phenotypic testing can be difficult
- Phenotypic variation within species*
- Can take weeks
- 26S ribosomal RNA (rRNA) and Internal Transcribed Spacer 1 and 2 regions (ITS1 & ITS2)

Culture-based and Sequence-based

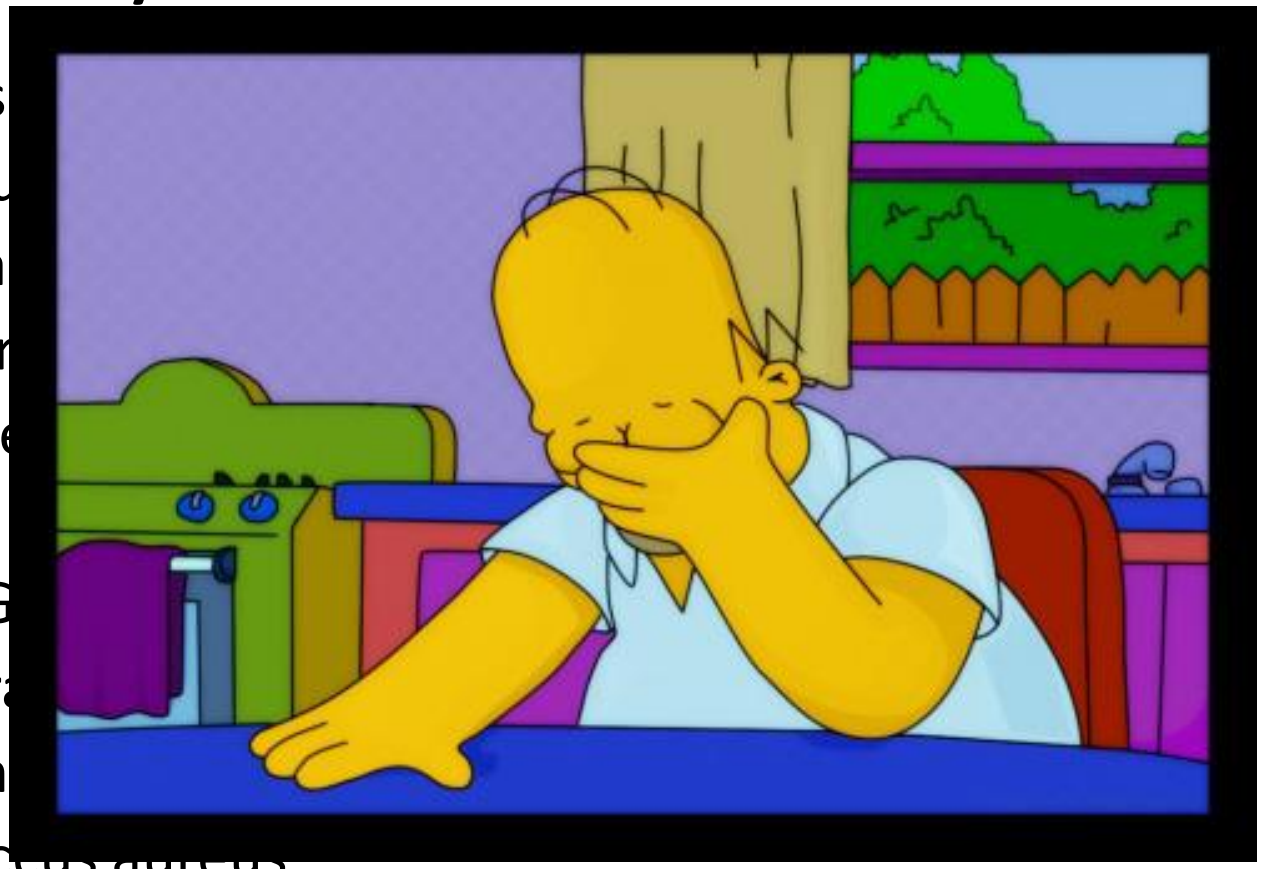
- Who is there?
- Not what's going on

Skin & Soft Tissue Infections (SSTI) by Real-Time PCR

- Bacteroides fragilis,
- Enterococcus faecalis,
- Escherichia coli,
- Group A Streptococcus,
- Group B Streptococcus,
- Klebsiella
- Prevotella Groups 1 & 2,
- Proteus mirabilis,
- Pseudomonas aeruginosa,
- Staphylococcus aureus,
- MRSA

Skin & Soft Tissue Infections (SSTI) by Real-Time PCR

- Bacteroides
- Enterococcu
- Escherichia
- Group A Str
- Group B Stre
- Klebsiella
- Prevotella G
- Proteus mira
- Pseudomon
- Staphylococcus aureus,
- MRSA



PRIMER BIAS!

We are…

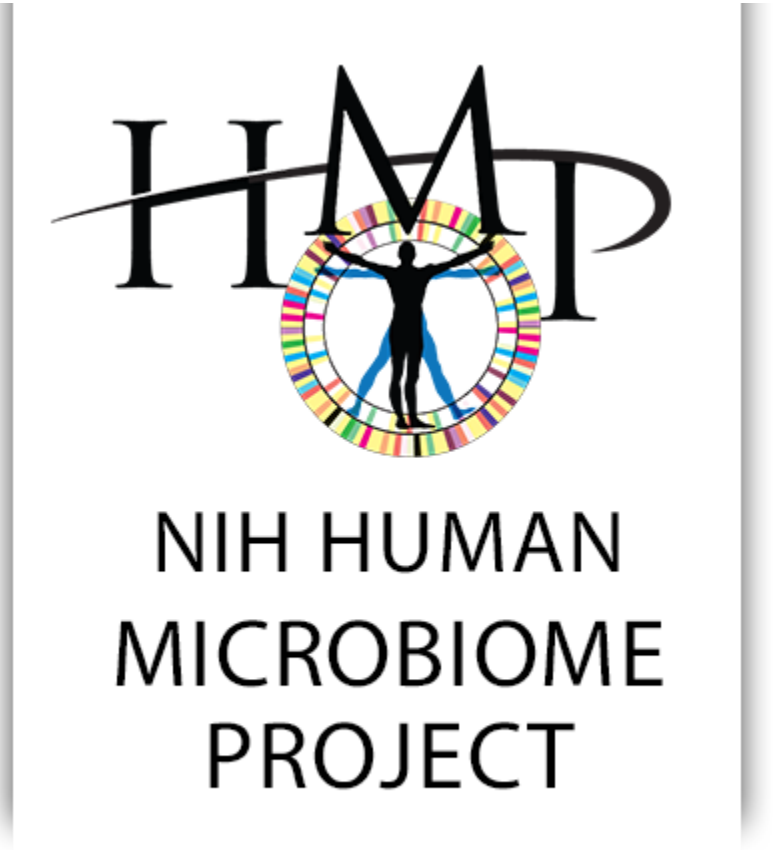
- 2 -5 lbs of bacteria
- 90% bacteria, 10% human by cell count
- 99% bacteria, 1% human by genes
- Largely ignorant of our microbiome
- 99.6% of human microbiome species cannot be cultured

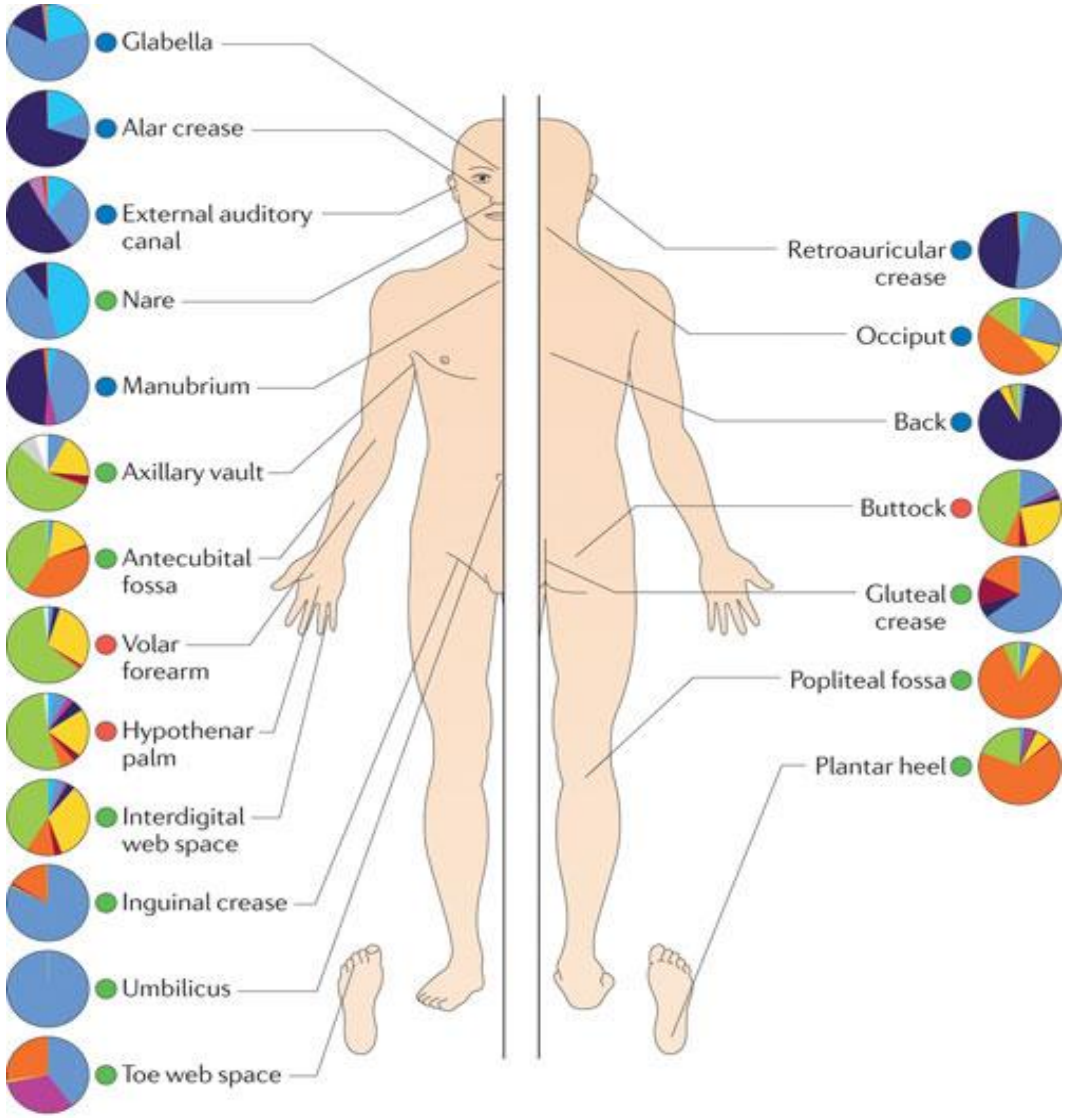
Square CM of Your Skin

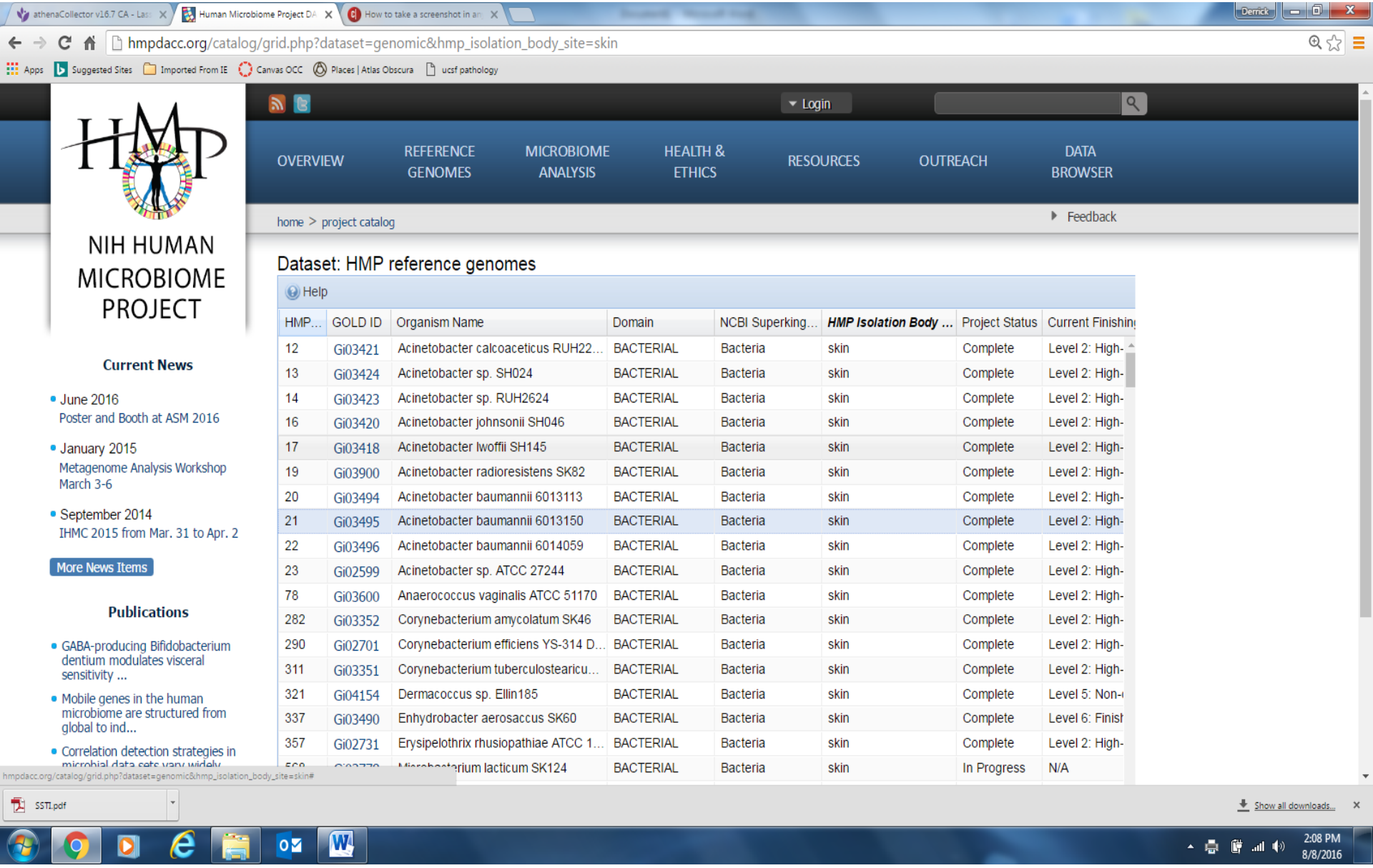
- Hundreds of distinct species
- Estimated 1 million bacteria
- Very site specific
- Quite resilient to change (*forehead licking)
- May affect immunity
- May affect physiology of keratinocytes

Human Microbiome

- 2007 NIH
- 242 **healthy** adults
- Gut
- Genitourinary
- Skin
- Spatial niches




Glabella
Alar crease
External auditory canal
Nare
Manubrium
Axillary vault
Antecubital fossa
Volar forearm
Hypothenar palm
Interdigital web space
Inguinal crease
Umbilicus
Toe web space
Retroauricular crease
Occiput
Back
Buttock
Gluteal crease
Popliteal fossa
Plantar heel
Actinobacteria
Corynebacteriaceae
Propionibacteriaceae
Micrococciaceae
Other Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Other Firmicutes
Staphylococcaceae
Proteobacteria
Divisions contributing <1%
Unclassified
Sebaceous
Moist
Dry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Human Microbiome Project DA
How to take a screenshot in an
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Apps
Suggested Sites
Imported From IE
Canvas OCC
Places | Atlas Obscura
ucsf pathology
Login
OVERVIEW
REFERENCE GENOMES
MICROBIOME ANALYSIS
HEALTH & ETHICS
RESOURCES
OUTREACH
DATA BROWSER
NIH HUMAN MICROBIOME PROJECT
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Current News
June 2016
Poster and Booth at ASM 2016
January 2015
Metagenome Analysis Workshop March 3-6
September 2014
IHMC 2015 from Mar. 31 to Apr. 2
More News Items
Publications
GABA-producing Bifidobacterium dentium modulates visceral sensitivity ...
Mobile genes in the human microbiome are structured from global to ind...
Correlation detection strategies in
Dataset: HMP reference genomes
Help
HMP... GOLD ID Organism Name Domain NCBI Superking... HMP Isolation Body ... Project Status Current Finishing
12 Gi03421 Acinetobacter calcoaceticus RUH22... BACTERIAL Bacteria skin Complete Level 2: High-
13 Gi03424 Acinetobacter sp. SH024 BACTERIAL Bacteria skin Complete Level 2: High-
14 Gi03423 Acinetobacter sp. RUH2624 BACTERIAL Bacteria skin Complete Level 2: High-
16 Gi03420 Acinetobacter johnsonii SH046 BACTERIAL Bacteria skin Complete Level 2: High-
17 Gi03418 Acinetobacter lwoffii SH145 BACTERIAL Bacteria skin Complete Level 2: High-
19 Gi03900 Acinetobacter radioresistens SK82 BACTERIAL Bacteria skin Complete Level 2: High-
20 Gi03494 Acinetobacter baumannii 6013113 BACTERIAL Bacteria skin Complete Level 2: High-
21 Gi03495 Acinetobacter baumannii 6013150 BACTERIAL Bacteria skin Complete Level 2: High-
22 Gi03496 Acinetobacter baumannii 6014059 BACTERIAL Bacteria skin Complete Level 2: High-
23 Gi02599 Acinetobacter sp. ATCC 27244 BACTERIAL Bacteria skin Complete Level 2: High-
78 Gi03600 Anaerococcus vaginalis ATCC 51170 BACTERIAL Bacteria skin Complete Level 2: High-
282 Gi03352 Corynebacterium amycolatum SK46 BACTERIAL Bacteria skin Complete Level 2: High-
290 Gi02701 Corynebacterium efficiens YS-314 D... BACTERIAL Bacteria skin Complete Level 2: High-
311 Gi03351 Corynebacterium tuberculostearicu... BACTERIAL Bacteria skin Complete Level 2: High-
321 Gi04154 Dermacoccus sp. Ellin185 BACTERIAL Bacteria skin Complete Level 5: Non-
337 Gi03490 Enhydrobacter aerosaccus SK60 BACTERIAL Bacteria skin Complete Level 6: Finish
357 Gi02731 Erysipelothrix rhusiopathiae ATCC 1... BACTERIAL Bacteria skin Complete Level 2: High-
Mierobacterium lacticum SK124 BACTERIAL Bacteria skin In Progress N/A
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Top 4 Skin Phyla

- Actinobacteria
- Firmicutes
- Bacteroidetes
- Proteobacteria

Top 4 Skin Phyla

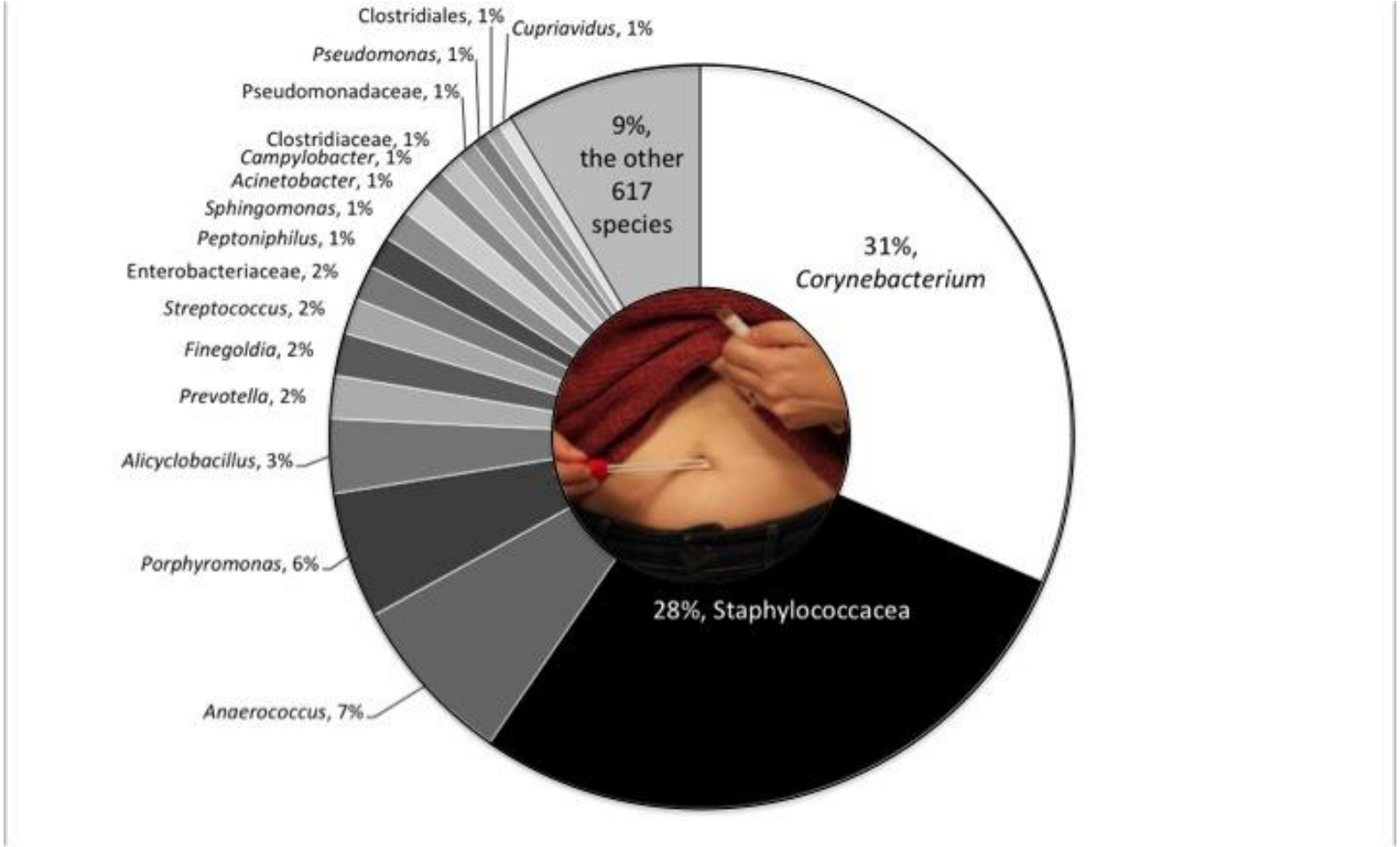
- Actinobacteria – *Propionibacterium Mycobacterium, Corynebacterium, Nocardia*
- Firmicutes – *clostridium, staph, strep*
- Bacteroidetes- *b.fragilis, prevotella*
- Proteobacteria – *e.coli, pseudomonas*

Palm Microbiome

- 51 healthy subjects
- 4742 distinct species
- Average 158 species coexisting on single palm

Fierer, et al. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci USA 2008;105:17994-9

The Belly Button Biodiversity Project



- Hulcr J, Latimer AM, Henley JB, Rountree NR, Fierer N, et al. (2012) A Jungle in There: Bacteria in Belly Buttons are Highly Diverse, but Predictable. PLoS ONE 7(11): e47712. doi:10.1371/journal.pone.0047712

Generalities

- *Propionibacterium* – sebaceous areas
- *Stapylococcus* – moist areas/intertriginous
- *Corynebacterium*- same as staph
- Antecubidal fossa – highest diversity among subjects
- Partially occluded sites (axilla/inguinal)- more stable

Human Microbiome Consortium: Srucutre, function and diveristy of the healthy human microbiome. Naure 2012;486:207-14

Grice, et al. Topographical and temporal diveristy of the human skin microbiome. Science 2009;324:1190-2

Surprise!

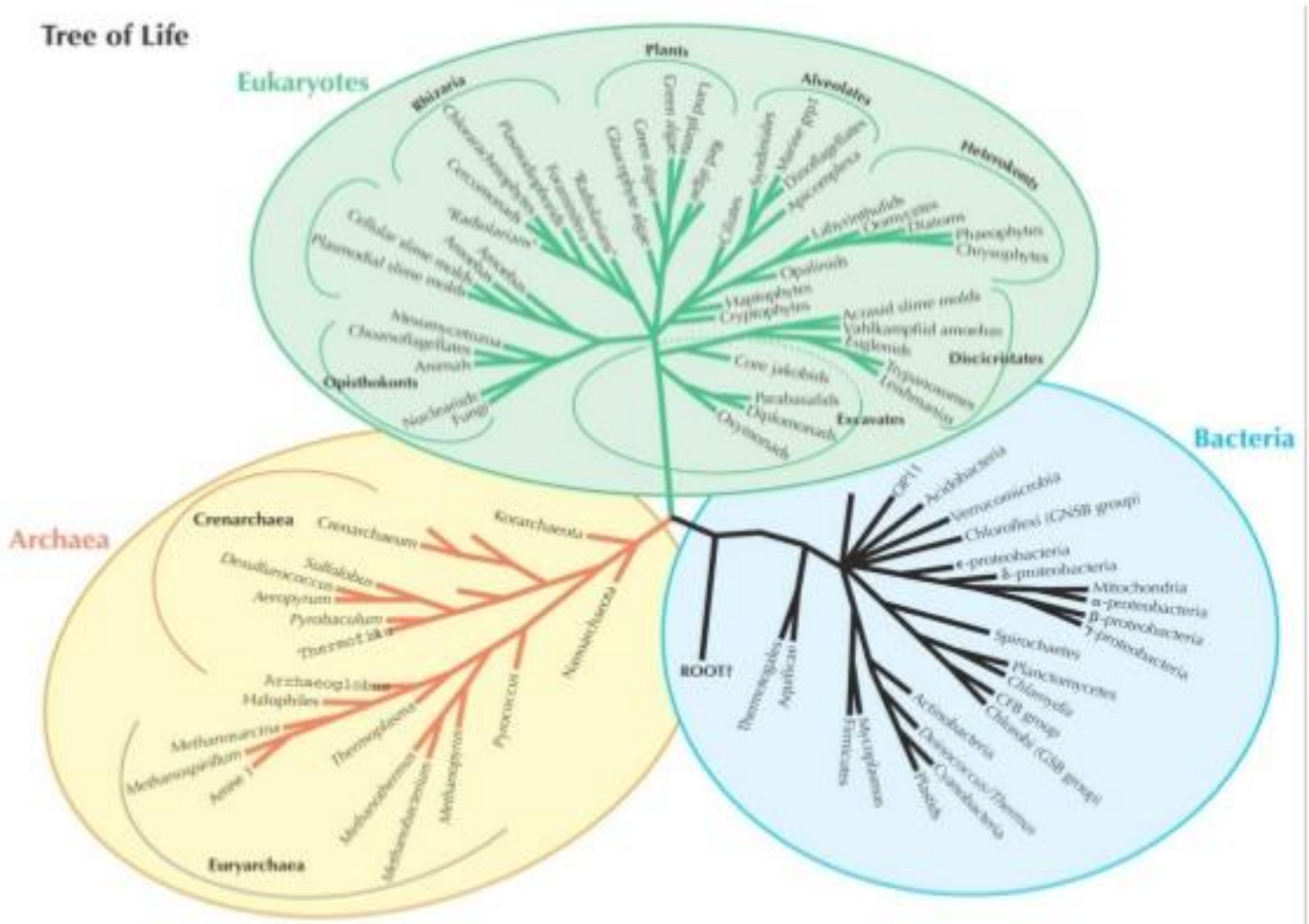
- Gram-negatives found in dry areas (forearm and legs)
- Not always fecal contaminant

Chen, Tsao. The skin Microbiome: Current perspectives and future challenges. Journ Amer Acad Derm. 2013; 143-52

- Low-abundance species may be “linchpins” of the skin ecosystem (soil fungal studies)

Baldrain, et al. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J 2012;6:248-58

Phylogenetic Diversity



- “Culture Everything!”
- Microbial Load
- Sensitivity Data
- Biofilm analysis
- Reasonable cost


CSI:
CRIME SCENE INVESTIGATION

The New York Times

In a First, Test of DNA Finds Root of Illness

Joshua Osborn, 14, lay in a coma at American Family Children's Hospital in Madison, Wis. For weeks his brain had been swelling with fluid, and a battery of tests had failed to reveal the cause.

The doctors told his parents, Clark and Julie, that they wanted to run one more test with an experimental new technology. Scientists would search Joshua's cerebrospinal fluid for pieces of DNA. Some of them might belong to the pathogen causing his encephalitis.

The Osborns agreed, although they were skeptical that the test would succeed where so many others had failed. But in the first procedure of its kind, researchers at the University of California, San Francisco, managed to pinpoint the cause of Joshua's problem — within 48 hours. He had been infected with an obscure species of bacteria. Once identified, it was eradicated within days.

The case, reported on Wednesday in The New England Journal of Medicine, signals an important advance in the science of diagnosis. For years, scientists have been sequencing DNA to identify pathogens. But until now, the process has been too cumbersome to yield useful information about an individual patient in a life-threatening emergency.

"This is an absolutely great story — it's a tremendous tour de force," said Tom

Wound culture: Pseudomonas

in

Level 1Q Results	Amount	Level 2 Results		Additional Information
Bacterial Load(High)	$> 10^7$	Detected Bacteria:		
Pseudomonas aeruginosa	3.6×10^6	Fusobacterium ulcerans	63%	
		Bacteroides ovatus	8%	
		Porphyromonas somerae	5%	
		Pseudomonas aeruginosa	5%	
		Clostridium bolteae	2%	
		Bacteroides stercoris	1%	
		Bacteroides xylanisolvens	1%	
		Clostridium ramosum	1%	
		Peptostreptococcus anaerobius	1%	
		Prevotella nanceiensis	1%	
		Anaerococcus lactolyticus	1%	

Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Level 1 Swab Results	Amount (N/A)
Total Bacterial Load	Med
Enterococcus faecalis	Low
Klebsiella pneumoniae	Not Detected
Streptococcus agalactiae	Not Detected
Streptococcus pyogenes	Not Detected
Vancomycin resistance	Not Detected
Candida albicans	Not Detected
Enterococcus faecium	Not Detected
Pseudomonas aeruginosa	Not Detected
Staphylococcus aureus	Not Detected
Serratia marcescens	Not Detected
Methicillin resistance	Not Detected

Level 2 Results	
Detected Bacteria:	
Staphylococcus epidermidis	42%
Enterococcus faecalis	24%
Staphylococcus hominis	24%
Staphylococcus lugdunensis	4%
Corynebacterium tuberculostearicum	4%
NO FUNGAL SPECIES DETECTED	

Only relative Level 1 Quantitation is obtainable from swab samples.

Antibiotics Report On Attached Sheet

Clinical erythrasma

Level 1 Swab Results	Amount (N/A)
Total Bacterial Load	High
Enterococcus faecalis	Not Detected
Klebsiella pneumoniae	Not Detected
Streptococcus agalactiae	Not Detected
Streptococcus pyogenes	Not Detected
Vancomycin resistance	Not Detected
Candida albicans	Not Detected
Enterococcus faecium	Not Detected
Pseudomonas aeruginosa	Not Detected
Staphylococcus aureus	Not Detected
Serratia marcescens	Not Detected
Methicillin resistance	Not Detected

Level 2 Results	
Detected Bacteria:	
Proteus mirabilis	78%
Staphylococcus aureus	13%
Proteus vulgaris	2%
NO FUNGAL SPECIES DETECTED	

Wound culture: “normal skin flora”

Only relative Level 1 Quantitation is obtainable from swab samples.

Antibiotics Report On Attached Sheet

Finegoldia magna

- Normal skin flora

methods are used to identify the pathogens' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Level 1 Swab Results	Amount (N/A)	Level 2 Results		Additional Information
Total Bacterial Load	Low	Detected Bacteria:		
		Finegoldia magna	33%	
		Corynebacterium glucuronolyticum	22%	
		Streptococcus parasanguinis	11%	
		Veillonella atypica	6%	
		Corynebacterium tuberculostearicum	5%	
		Actinomyces neuii	3%	
		Staphylococcus warneri	2%	
		Peptoniphilus gorbachii	2%	
		NO FUNGAL SPECIES DETECTED		

[redacted]

Level 1Q Results	Amount per g	Level 2 Results		Additional Information
Bacterial Load (Medium)	10^5-10^7	Detected Bacteria:		
		Brevibacterium luteolum	37%	
		Staphylococcus warneri	11%	
		Corynebacterium jeikeium	9%	
		Staphylococcus epidermidis	7%	
		Kocuria kristinae	7%	
		Kocuria rosea	4%	
		Staphylococcus lugdunensis	3%	
		Brevibacterium paucivorans	3%	
		Corynebacterium tuberculostearicum	2%	
		Detected Fungi:		
		Debaryomyces hansenii		
		Alternaria malorum		
		Stagonosporopsis cucurbitacearum		
		Candida smithsonii		
		Malassezia restricta		
		Pyrenochaeta acicola		

Top 5 Nail Fungus by Next Gen Sequencing

- Trichophyton Rubrum 38%
- Leptosphaerulina chartarum 17%
- Cladosporium uredinicola 14%
- Epicoccum nigrum 13%
- Malassezia restricta 9%

National Human Genome Research Institute (fungal studies)

- Heel – largest fungal diversity, 80 species
- Nail clippings – 60 species
- Toe web – 40 species

- (Head and trunk hosted between 2-10)

NIH/National Human Genome Research Institute. "First genomic survey of human skin fungal diversity." ScienceDaily. ScienceDaily, 22 May 2013.

“Normal Skin Flora?”

- Propionobacterium acnes – orthopedic and neurosurgery infections
- Elaborate biofilms in nonunion open fractures
- Very difficult to culture

Nisbet M, Briggs S. (2007) Propionobacterium acnes: an under-appreciated cause of post-neurosurgical infection. J Antimicrob Chemother 60:1097-1103

100 Adults Toe Web Spaces

- *Candida albicans*
- *Rhodotorula rubra*
- *Torulopsis and Trichosporon cutaneum*
- *Microsporum gypseum,*
- *Trichophyton rubrum*
- *Rhizopus stolonifer*
- *Trichosporon cutaneum*
- *Fusarium*
- *Scopulariopsis brevicaulis*
- *Curvularia*
- *Alternaria alternata*
- *Paecilomyces*
- *Aspergillus flavus*
- *Penicillium*

Oyeka CA, Ugwu LO (2002). "Fungal flora of human toe webs". Mycoses 45 (11-12): 488–91. PMID 12472726. doi:10.1046/j.1439-0507.2002.00796.x.

Microbial Diversity in Venous Ulcers

Number of taxa identified by each analytic method

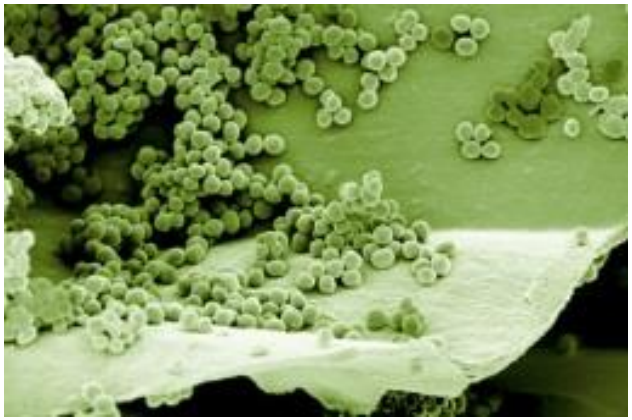
Taxon	No. identified by:								
	Pyrosequencing			Ibis T5000			Culture		
	Total	Range	Mean (SE)	Total	Range	Mean (SE)	Total	Range	Mean (SE)
Phylum	6	2–5	3.43 (0.25)	4	1–3	1.78 (0.19)	2	1–2	1.07 (0.18)
Class	11	3–7	4.64 (0.37)	8	1–5	2.29 (0.30)	3	1–2	1.07 (0.18)
Order	15	3–8	5.71 (0.51)	13	1–5	2.86 (0.36)	5	1–2	1.07 (0.18)
Family	27	3–12	7.86 (0.78)	15	1–5	2.93 (0.37)	7	1–2	1 (0.20)
Genus	43	3–17	9.64 (1.04)	20	1–8	3.50 (0.52)	7	1–2	1 (0.20)
Species	55	4–15	8.78 (0.87)	29	1–7	3.29 (0.50)	8	1–2	1 (0.20)

J. Clin. Microbiol. November 2011 vol. 49 no. 11 3812-3819

Two Faces of Same Microbe

Planktonic

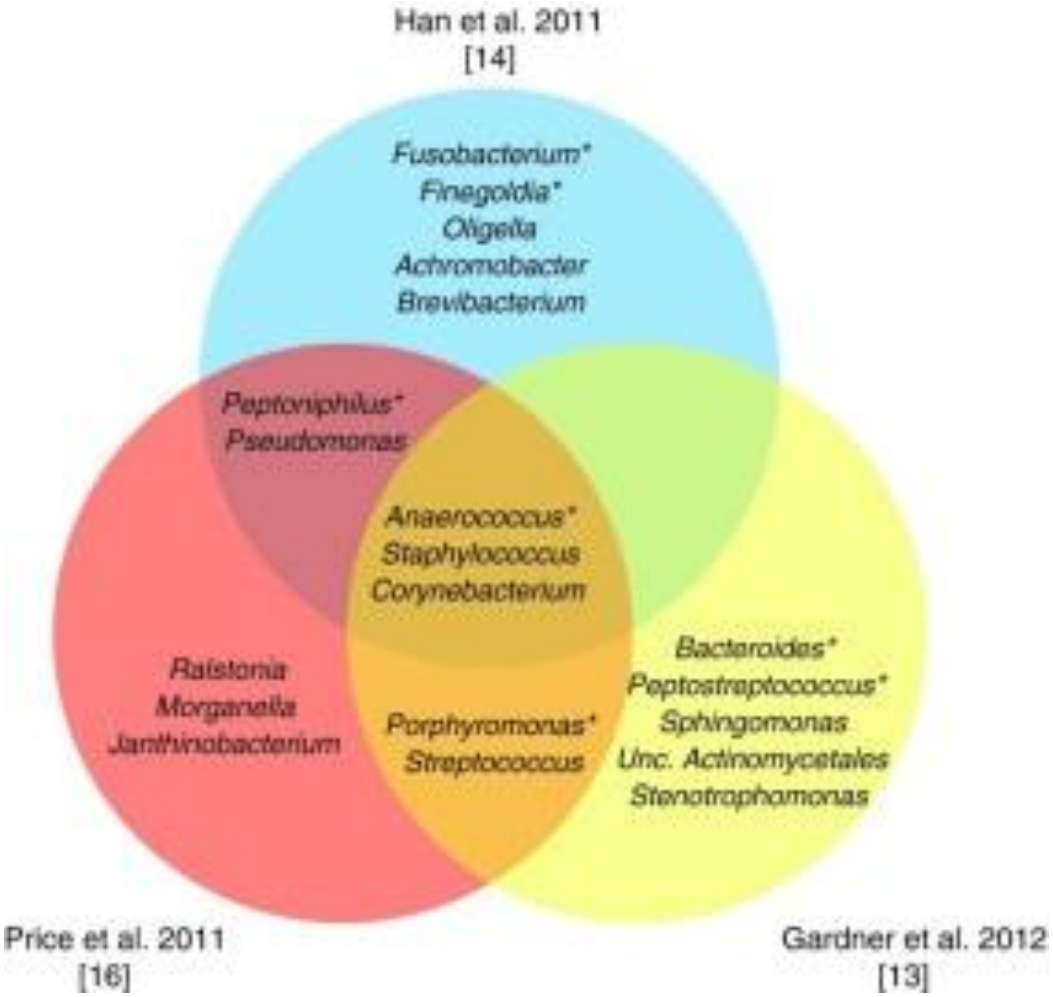
- Acute infections
- Grows easily



Biofilm

- Chronic infections
- Difficult to grow & treat
- Express a radically different phenotype than planktonic
- Only diagnostic tool is molecular

Top 10 Chronic Wound Genera



Take your Sample

- “Garbage in. Garbage out”
- Surface or deep?
- Are they on antibiotics?
- Immune status?
- How old is the wound?
- What are you looking for?
- Who is taking sample?

- Deep-tissue or punch biopsy
- Needle aspiration
- Swab culture (levine's vs "Z" technique)
- Sequence based swab/tissue

Wound Swab
(FYI – no standardized technique)

- Avoid a superficial sample
- Collect the culture before topical or systemic antibiotics.
- Viable wound bed – deep sample more useful
- No necrotic debris
- Swab 1cm2 (or Z-technique) for 5 seconds hard enough to get exudate
- Room temp – 2 hours

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Tissue Biopsy for Culture

- Debride and clean superficial area
- Resect viable tissue with aspectic technique
- Aerobic & Anaerobic orders

Needle Aspiration for Culture

- Disinfect overlying tissue
- Use 18-22 gauge needle to aspirate fluid
- Aerobic & Anaerobic orders

Superficial Swabs

- Carefully swab surface of wound
- Throw swab into garbage can

Sequencing Sample Collection

- Swab the deck!
- Throw everything in!
- Try to give get as much as possible
- Remember: a chronic wound is an ecosystem
- Topical lidocaine will degrade DNA

Summary

- Infection is a clinical diagnosis and not a culture diagnosis
- Most wounds will culture something
- Chose your culture/sequence technique wisely
- Comprehensive sequencing is available
- Today’s dogma is tomorrow’s heresy

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