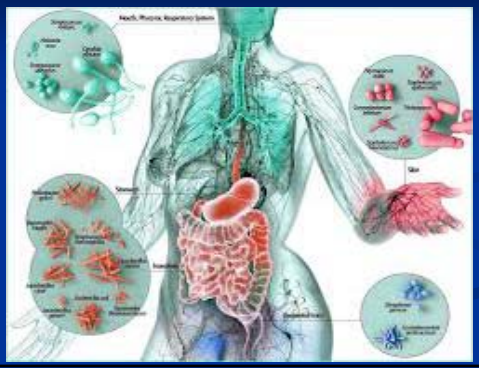
Biofilm Understsanding

Dr. Matthew Regulski DPM
Director, Wound Institute of Ocean County NJ

Don't Stop Your Curiosity

Human Microbiome



"Good Bacteria" in a wound ?

Commensals are considered microbes that provide benefits to the host organism

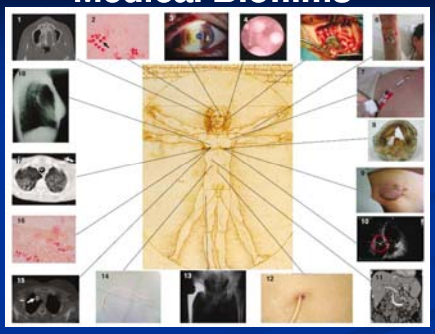
Notably, these interactions require redundant, complex host/microbe interactions that involve various host systems, including

- dendritic cells, keratinocytes, and antimicrobial peptides
- (defensins, alarmins, phenol soluble modulins, lipopeptides),

which do not exist in the wound bed.

Answer – NO Good bacteria in a wound

Medical Biofilms



Del Pozo and Patel *Clin Pharm Ther* Vol 82, 2007
Medical biofilm paradigm Journal of Wound Care Vol 19(2), 2010
Biofilm and Chronic Infections JAMA Vol 299(22), 2008

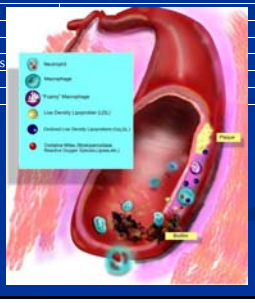
Medical Biofilms

	Medical Biofilm	US Incidence	Annual Cost
General Infections	Diabetic foot ulcers	(P) 3 M	50,000 deaths, 30% of hospital cost for diabetics
	Venous leg ulcers	(P) 2.5 M	
	Decubitus ulcers	(P) 3-5 M (P) 27%NH	> 50,000
	Surgical site infections	500,000	$5-10 B, 5,000 deaths
	Burn wounds	1.1 M	15,000 deaths
	Chronic meningitis	1,400-2,800	140-390 deaths
	Bacterial prostatitis	(P) 162,800	
	All odontogenic infections		
	Chronic tonsillitis	11,000	$121.5 M
	Gallstones	430,000	$5 B
	Crohn's disease	36,000-60,000	
	Ulcerative colitis	24,000-40,000	
	COPD	(P) 30 M	$37.2 B, 120,000 deaths
	Bronchiolectasia	110,000	
	Pneumonia (non-VAP)	1.2 M	$14-$25 B, 54,000 deaths
	Medical Biofilm	**US Incidence**	**Annual Cost**
Nosocomial	Vascular graft infection	16,000	$640 M
	Cardiac pacemakers	4,000-20,000	
	Peritoneal dialysis peritonitis	~20-25,000 on CPD	
	Ventilator acquired pneumonia	135,000	$1.5 B, 61,000 deaths
	Endotracheal tubes	100s of thousands	$5 B
	Urinary catheter cystitis	Millions	4,500 deaths
	Central venous catheters	250,000	$296 M-$2.3 B, 30-62.5 K deaths
Total		20 Million	$100 B, >500k deaths

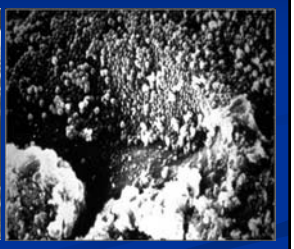
Medical Biofilms Context

For Comparison

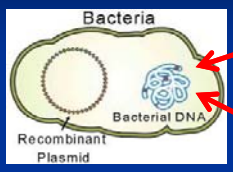
Disease	Incidence
Cardiovascular Disease	2.28 M per year
Cancer	1.5 M per year
Diabetes	1.5 M per year, ages
Medical Biofilm	> 10M per year



First Medical Device Biofilm



Microbial DNA



PCR - Panel of Microbes 8-20

NGS - Next Generation Sequencing

25,000 Species

Barrier to Technology
Cost and Time to Deliver

The New York Times

HEALTH

In a First, Test of DNA Finds Root of Illness

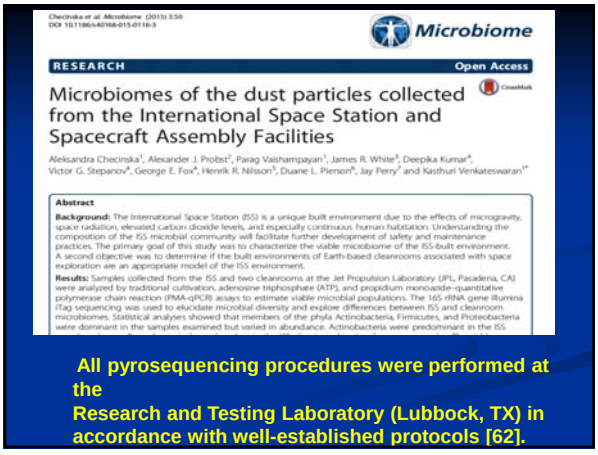
By CARL ZIMMER JUNE 4, 2014

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

Joshua Osborn outside his home in Cottage Grove, Wis., with his father, Clark Osborn. Joshua suffered from swelling in the brain, but tests, a spinal tap and a biopsy were inconclusive.



Microbiome

RESEARCH Open Access

Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities

Aleksandra Checinska[1], Alexander J. Probst[2], Parag Vaishampayan[1], James R. White[3], Deepika Kumar[4], Victor G. Stepanov[4], George E. Fox[4], Henrik R. Nilsson[5], Duane L. Pierson[6], Jay Perry[7] and Kasthuri Venkateswaran[1*]

Abstract

Background: The International Space Station (ISS) is a unique built environment due to the effects of microgravity, space radiation, elevated carbon dioxide levels, and especially continuous human habitation. Understanding the composition of the ISS microbial community will facilitate further development of safety and maintenance practices. The primary goal of this study was to characterize the viable microbiome of the ISS-built environment. A second objective was to determine if the built environments of Earth-based cleanrooms associated with space exploration are an appropriate model of the ISS environment.

Results: Samples collected from the ISS and two cleanrooms at the Jet Propulsion Laboratory (JPL, Pasadena, CA) were analyzed by traditional cultivation, adenosine triphosphate (ATP), and propidium monoazide–quantitative polymerase chain reaction (PMA-qPCR) assays to estimate viable microbial populations. The 16S rRNA gene Illumina iTag sequencing was used to elucidate microbial diversity and explore differences between ISS and cleanroom microbiomes. Statistical analyses showed that members of the phyla Actinobacteria, Firmicutes, and Proteobacteria were dominant in the samples examined but varied in abundance. Actinobacteria were predominant in the ISS

All pyrosequencing procedures were performed at the Research and Testing Laboratory (Lubbock, TX) in accordance with well-established protocols [62].

Original Article
European Journal of Clinical Microbiology & Infectious Diseases
February 2016, Volume 35, Issue 2, pp 293-298

First online: 15 December 2015

The microbiome of diabetic foot osteomyelitis

S. A. V. van Asten ✉ , J. La Fontaine, E. J. G. Peters, K. Bhavan, P. J. Kim, L. A. Lavery

10.1007/s10096-015-2544-1

No pathogens were identified in 8 out of the 34 bone samples (23.5 %) with conventional culture techniques. Two out of those eight negative samples did not sequence either.

Our results show that, by using a 16S rRNA sequencing technique, anaerobes were detected in 86.9 % of the positive bone samples (vs. 23.1 % with conventional techniques).

Biofilm Infection

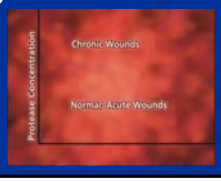
- (a) Bacteria adhered to surface — Surface selects (but is not necessary) for biofilm formation
- (a) Direct visualization of biofilm morphology — The current "gold standard" for diagnosing biofilm
- (a) Confined to a particular location — Biofilm seems to limit its size (quorum sensing)
- (a) Resistant to appropriate antibiotics — A hallmark of biofilm is high resistance to antibiotics
- (b)Resistant to biocides — A hallmark of biofilm is high resistance to biocides
- (b)Large number with high diversity in a host lesion
- (b)Infections that wax and wane with exacerbations
- (b)Secondary signs of infection

(a)Parsek Annu. Rev. Microbiol. Vol57, 2003
(b) Wolcott JWC Vol19(2), 2010

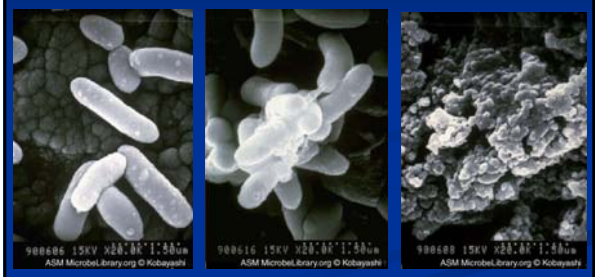
Costerton and Stewart Sci Am Vol 285, 2001

Biochemical Impairment of Chronic Wounds

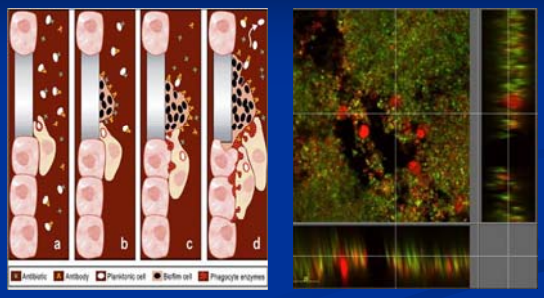
- Elevated proinflammatory cytokines
- Elevated proteinase activity – MMPs
- Diminished activity of growth factors
- Degraded receptor sites (degradation blocked by the addition of MMP inhibitors)



Biofilm Development



Host Defenses

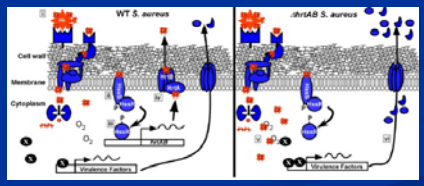


Leid, JG Infect Immun Vol 70, 2002

Molecular Mechanisms

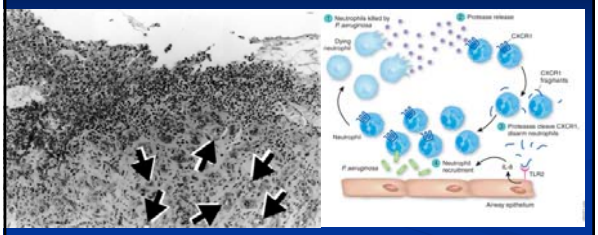
- Staphyloccocus Heme Sensing System

"… resulting in the tempering of virulence to avoid excessive host tissue damage."



Torres A Staphylococcus aureus Regulatory System that Responds to Host Heme and Modulates Virulence Cell Host and Microbe Vol 1, 2007

Neutrophils

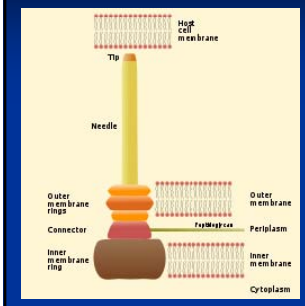


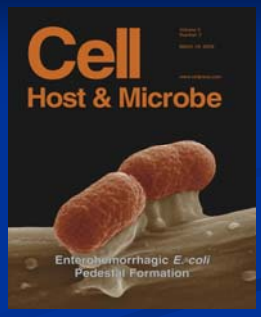
Diegelmann RF *Wound Repair Regen* Vol 11 2003

Hartl, D Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease Nature Medicine Vol 13, 2007

Biofilms and Chronic Wound Inflammation JWC Vol 17, 2008

Senescence

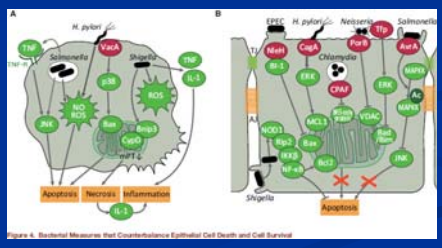




Putting Enterohemorrhagic E. coli on a Pedestal (Mar2009)

Molecular Mechanisms of Senescence

"…new paradigm of bacterial pathogenesis"



Kim Cell Host and Microbe Vol 8(1), July 2010

Planktonic Bacteria Single Cell

- Cause of Acute Infection
- Will grow in traditional Culture

Bio Film "Community of Bacteria"

- Cause of 80% of Infections
- Cause of Chronic Wounds
- **Will not grow in traditional culture**
- Biofilm cells express a radically differ phenotype than planktonic bacteria

Only Diagnostic tool is Molecular

Samples With Clinical Signs of Infection

- 50 % of Nail Samples had fungal Species Identified
- 50 % of Nail samples had no fungal species but did have Bacteria species identified

Nails with Fungal species identified also had Bacteria

10,000 Nail Samples

Economic Impact in Diabetic Foot Wounds

Treatment in 2005	Treatment in 2013
189 patients	215 Patients
Culture and Systemic Antibiotics	Molecular and Topical Antibiotics
Total Payments for related codes	Total Payments for related codes
$11,444	$3,060

Reduction in total charges of 68%

DNA Analysis vs. Traditional Cultures

51 Chronic wounds- Parallel Samples

16 S DNA Sequencing

- 46 /51 Staph Identified
- 32 / 51 Pseud Identified

Traditional Culture

- 28/51 Staph Identified
- 8 / 51 Pseud Identified

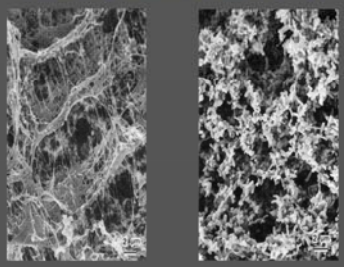
DNA Sequencing Outperformed Traditional Cultures

DNA Sequencing identified 145 Genera – Cultures 14 !

Attachment

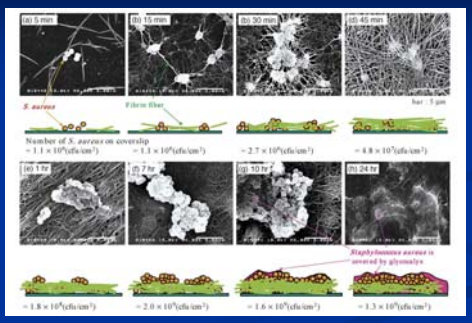
Extracellular Polymeric Substance

SEM of *P. aeruginosa* Biofilm



Control Clarithromycin

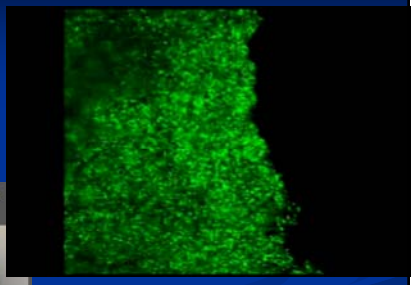
Biofilm Development



Masako,K Journal of Dermatologic Science June 2005

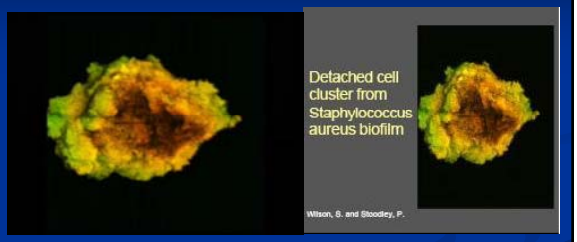
Biofilm 3-D Structure

Biofilm 3-D Structure

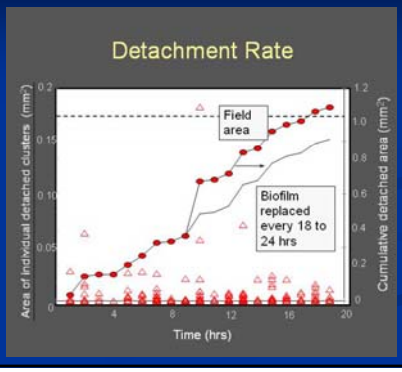


Biofilm Detachment

Biofilm Detachment

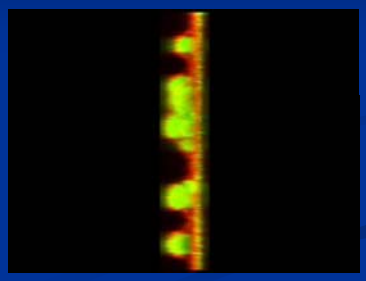


Biofilm Detachment

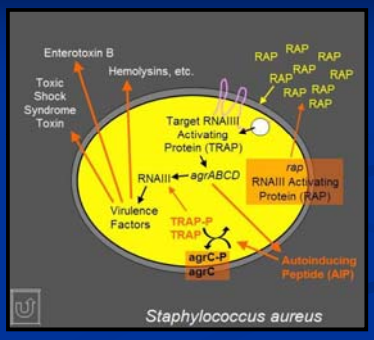


Eradicating Detachment Fragments

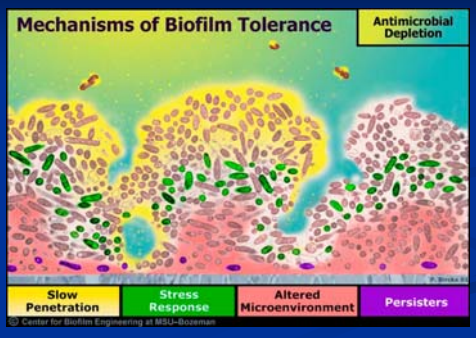
- Red is dead
- Reconstitute



Quorum Sensing

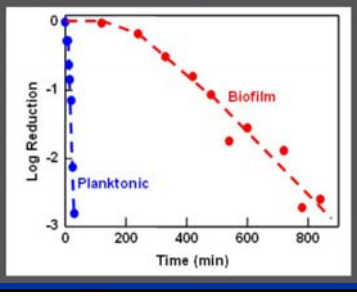


Biofilm Defenses

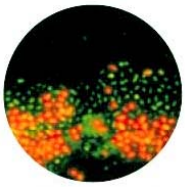


Slow Penetration

Glutaraldehyde (50 mg/L)
Against *Ps. aeruginosa* Biofilm



Biocides vs. Biofilm



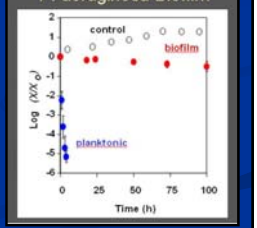
AFTER 60 MINUTES of exposure to bleach, many cells in this biofilm were dying (*green*), but many others, especially in the interior, still remained active (*red*).

Antibiotic Resistance

Biofilm

- **Vanc vs Staph biofilm >1000x**
- **Tobra vs Pseudomonas biofilm**

Tobramycin vs.
P. aeruginosa Biofilm



A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)[a]

Ellen Jo Baron,[1,2] J. Michael Miller,[3] Melvin P. Weinstein,[4] Sandra S. Richter,[5] Peter H. Gilligan,[6] Richard B. Thomson Jr.,[7] Paul Bourbeau,[8] Karen C. Carroll,[9] Sue C. Kehl,[10] W. Michael Dunne,[11] Barbara Robinson-Dunn,[12] Joseph D. Schwartzman,[13] Kimberle C. Chapin,[14] James W. Snyder,[15] Betty A. Forbes,[16] Robin Patel,[17] Jon F. Rosenblatt,[17] and Bobbi S. Pritt[18]

[1]Department of Pathology, Stanford University School of Medicine, Stanford, California; [2]Cepheid, R&D, Sunnyvale, California; [3]Microbiology Technical Services, LLC, Dunwoody, Georgia; [4]Department of Medicine and Pathology, Robert Wood Johnson Medical School, New Brunswick, New Jersey; [5]Department of Clinical Pathology, Cleveland Clinic, Cleveland, Ohio; [6]Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina; [7]Department of Pathology, NorthShore University HealthSystem, Evanston, Illinois; [8]Scientific Affairs, BD Diagnostics, Sparks, Maryland; [9]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland; [10]Department of Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin; [11]bioMérieux, Inc., Durham, North Carolina, and Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri; [12]Department of Pathology, William Beaumont Hospital to Beaumont Health System, Royal Oak, Michigan; [13]Department of Pathology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire; [14]Department of Pathology, Brown Alpert Medical School, Providence, Rhode Island; [15]Department of Laboratory Medicine, University of Louisville, Louisville, Kentucky; [16]Department of Pathology, Virginia Commonwealth University Medical Center, Richmond, Virginia; and [17]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

The critical role of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician and the microbiologists who provide enormous value to the health care

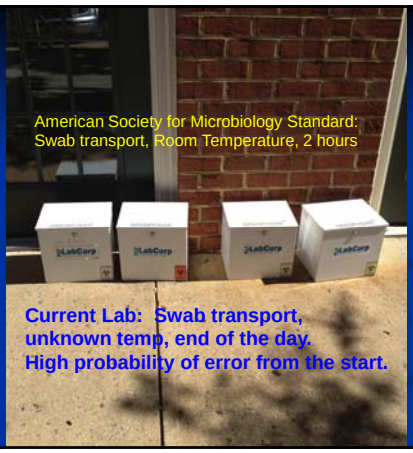
Table Introduction-1. Transport Issues (General Guide)*

Specimen Type	Specimen Required	Collection Device, Temperature, and Ideal Transport Time
Aerobic bacterial culture	Tissue, fluid, aspirate biopsy, etc	Sterile container, RT, immediately
	Swab (2nd choice) – flocked swabs are recommended	Swab transport device, RT, 2 h
Aerobic and anaerobic bacterial culture	Tissue, fluid, aspirate, biopsy, etc	Sterile anaerobic container, RT, immediately
	Swab (2nd choice) – flocked swabs are effective	Anaerobic swab transport device, RT, 2 h
Fungus culture; AFB culture	Tissue, fluid, aspirate, biopsy, etc	Sterile container, RT, 2 h
	Swab (2nd choice) (for yeast and superficial mycobacterial infections only)	Swab transport device, RT, 2 h
Virus culture	Tissue, fluid, aspirate, biopsy, etc	Viral transport media, on ice, immediately
	Swab – flocked swabs are recommended	Virus swab transport device, RT, 2 h
Suspected agent of bioterrorism	Refer to Centers for Disease Control and Prevention website: http://emergency.cdc.gov/documents/PPTResponse/table2specimenselection.pdf	
Serology	5 mL serum	Clot tube, RT, 2 h
Antigen test	As described in the laboratory specimen collection manual	Closed container, RT, 2 h
NAAT	5 mL plasma	EDTA tube, RT, 2 h
	Other specimen	Closed container, RT, 2 h

Abbreviations: NAAT, nucleic acid amplification test; RT, Room Temperature.

"The impact of proper specimen management on patient care is enormous"

"Specimen selection and collection are the responsibility of the medical staff and not the laboratory"



Nonviable Bacteria DNA

Bacterial Biofilms and Chronic Rhinosinusitis Kilty and Desrosiers

"Most convincing was the demonstration in a chinchilla model that live bacteria, although nonculturable, could persist in OME for weeks, whereas DNA strands and DNA from intact but nonviable bacteria could not exist for more than a day [21]."

"Given the extremely short half-life of mRNA, its presence was evidence that viable metabolically active bacterial organisms were likely present in OME."

"I need my Sensitivity for antibiotics"

- "Biofilm-growing microorganisms are significantly more tolerant to antibiotics [6] and corresponding breakpoints have not been established [34]. The S-I-R results can therefore not be used to predict therapeutic success in the case of biofilm infections and offer no guide to clinicians for treating such infections."

ESCMID Guidelines for the diagnosis and treatment of biofilm infections

PATHOGENIUS™ LABORATORIES
THE PATHOGENIUS DIAGNOSTIC SYSTEM

4321 Marsha Sharp Fwy Door 2 , Lubbock, TX 79407 | Ph: (806) 771-1134 | Fax: (806) 771-1168

Patient:	[redacted]	Specimen:	L HEEL	Physician:	Plishchuk, Michael
DOB:	02/21/1945	Received:	07/08/2016 12:43 PM	Phone:	(732)505-4500
Patient ID:	02214508731	Completed:	7/13/2016	Fax:	(732)505-6457
Gender:	Female	Accession:	92227	Collected:	07/05/2016 12:43 PM

Level 2: Next-Generation Final Comprehensive Diagnostics Results

Pathogenius Laboratories Level 2 testing (patent pending) is a relative quantitative universal test for bacteria/fungi. DNA sequencing 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)
Total Bacterial Load	High
Enterococcus faecalis	Medium
Methicillin resistance	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

Level 2 Results	
Detected Bacteria:	
Corynebacterium tuberculostearicum	46%
Bacteroides fragilis	27%
Enterococcus faecalis	11%
Porphyromonas bennonis	6%
Solobacterium moorei	2%
Staphylococcus epidermidis	2%
Peptoniphilus indolicus	2%
NO FUNGAL SPECIES DETECTED	

PATHOGENIUS™ LABORATORIES
THE PATHOGENIUS DIAGNOSTIC SYSTEM

4321 Marsha Sharp Fwy Door 2 , Lubbock, TX 79407 | Ph: (806) 771-1134 | Fax: (806) 771-1168

Patient:	[redacted]	Specimen:	TOENAIL	Physician:	Jamrok, Eric
DOB:	09/13/1953	Received:	03/24/2014 11:32 AM	Phone:	(410)208-8237
Patient ID:	219649184	Completed:	3/28/2014	Fax:	(410)208-0754
Gender:	Male	Accession:	27995	Collected:	03/20/2014 11:32 AM

Level 2: Next-Generation Final Comprehensive Diagnostics Results

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Level 1Q Results	Amount per g
Bacterial Load (Medium)	10^6-10^7

Level 2 Results	
Detected Bacteria:	
Brevibacterium luteolum	37%
Staphylococcus warneri	11%
Corynebacterium jeikeium	9%
Staphylococcus epidermidis	7%
Kocuria kristinae	7%
Kocuria rosea	4%
Staphylococcus lugdunensis	3%
Brevibacterium pseudoniens	3%
Corynebacterium tuberculostearicum	2%
Detected Fungi:	
Debaryomyces hansenii	
Alternaria malorum	
Stagonosporopsis cucurbitaceaurum	
Candida smithsonii	
Malassezia restricta	
Pyrenochaeta acicola	

Additional Information

Journal of Clinical Microbiology

Corynebacterium tuberculostearicum: a Potentially Misidentified and Multiresistant Corynebacterium Species Isolated from Clinical Specimens

Nevertheless, partial 16S rRNA gene sequencing still represents the gold standard for the identification of this species. Due to the challenging identification of C. tuberculostearicum, we presume that this organism is often misidentified and its clinical relevance is underestimated.

MOLECULAR DIAGNOSTICS AND PERSONALIZED MEDICINE IN WOUND CARE / *ASSESSMENT OF OUTCOMES*

Standard of Care Group	Group 1	Group 2
Traditional Culture with Systemic Antibiotics	Molecular Diagnostics with Systemic Antibiotics	Molecular Diagnostics with Customized Topical Antibiotics
% of Patient Healed	% of Patients Healed	%of Patients Healed
48.5% 244/503	62.4% 298/479	**90.4%** 358/396

Median Number of Days to Heal by Wound Type

Wound Type	Standard of Care Traditional Culture with Oral Antibiotics	Group 1 DNA Diagnostics with Oral Antibiotics	Group 2 DNA Diagnostics with Customized Topical Antibiotic
Pressure Ulcer	NA	107	**28**
Diabetic Foot Ulcer	168	84	**32**
Non-Healing Surgical Wound	176	75	**44**
Traumatic Abscess	39	33	**14**
Venous Leg Ulcer	177	98	**37**
Total	177	77 ($p<0.001$)	28 ($p<0.001$)

Benefits DNA Molecular Testing

- Fast Identification of Biofilms and their composition in patients referred to you—**Accuracy and Speed**.
- GOLD STANDARD of Microbial Diagnostics
- Medicare Covers the Test and pay $190
- Less Expensive than a Culture

God always takes the simplest way.
Albert Einstein