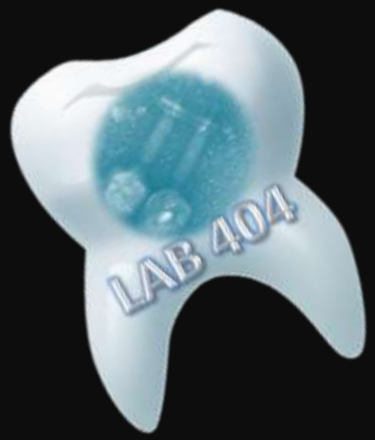
Phage Therapy as a promising tool against antibiotic resistant bacteria

Ronen Hazan

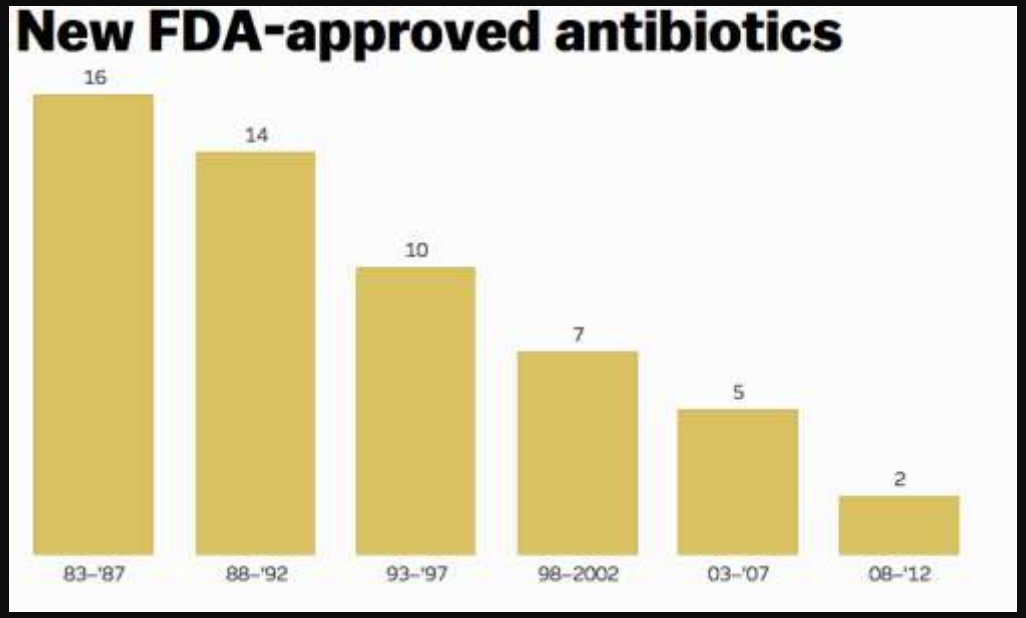
Institute of Dental Science

Hebrew University



March 2018

Do we need more antibiotics ?



Science

Science

First new antibiotic in 30 years discovered in major breakthrough

The discovery of Teixobactin could pave the way for a new generation of antibiotics because of the way it was discovered.

By Sarah Knapton, SCIENCE EDITOR

7 JANUARY 2016 • 5:44PM

The first new antibiotic to be discovered in nearly 30 years has been hailed as a 'paradigm shift' in the fight against the growing resistance to drugs.

Yes we need!

"Resistance to antibiotics poses **a major global threat** to public health that requires action across all government sectors and society." (WHO)

"Each year in the United States, at least **2 million people** become infected with bacteria that are resistant to antibiotics and at least **23,000 people die** each year as a direct result of these infections." (CDC)

Yes we need!

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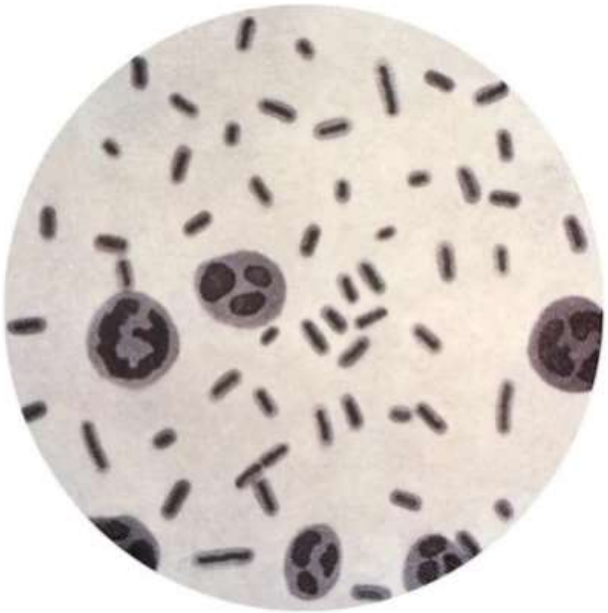
Woman Killed by a Superbug Resistant to Every Available Antibiotic

The "nightmare bacteria" could fend off 26 different drugs



A pan-resistant form of *Klebsiella pneumoniae* killed a 70-year-old woman in Nevada in September. *Credit: CDC*

News Alerts > Medscape Medical News

First US Case of *E coli* Resistant to Last-Resort Antibiotic

Robert Lowes

Disclosures | May 26, 2016

Solution: The last resort antibiotics

Emergence of antibiotic resistant strains

Sensitive *P. aeruginosa*

Hadassah, 2017

When and where do antibiotic fail?

1) Emergence of resistant bacteria

2) Biofilm

3) Microbiome dysbiosis

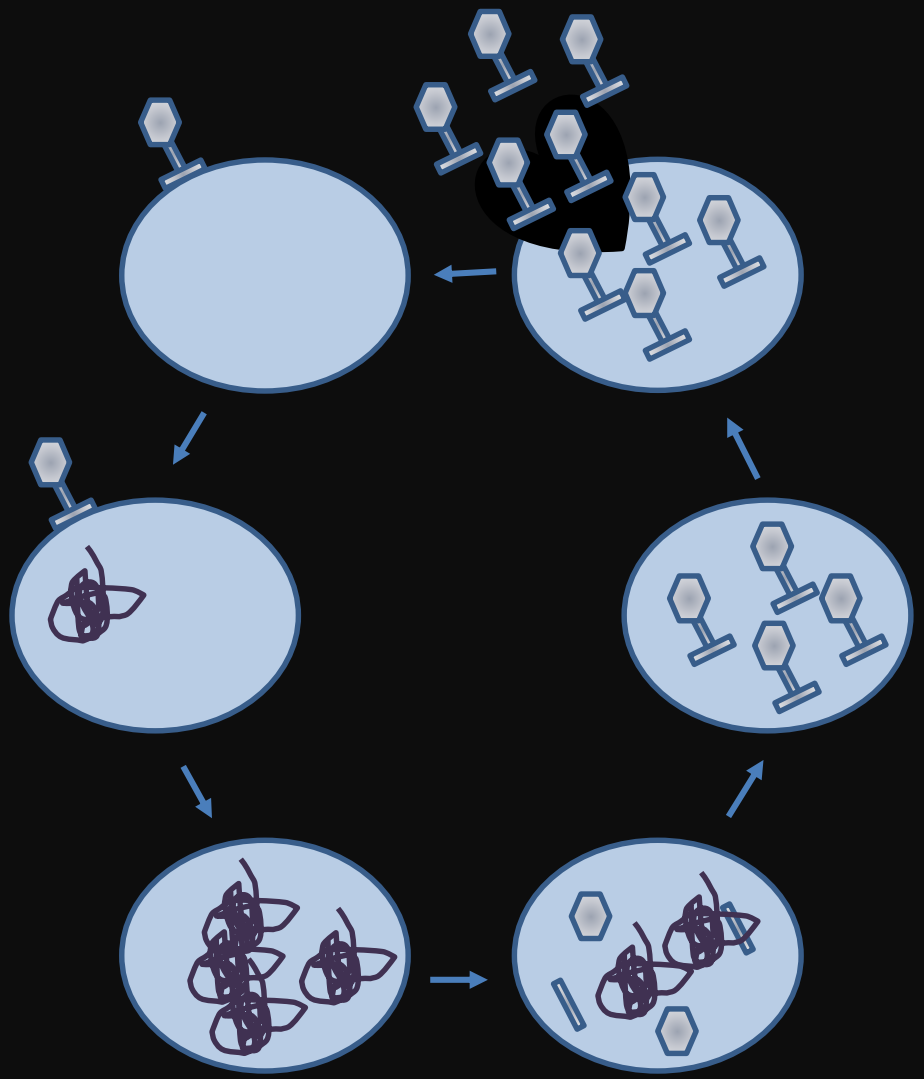
4) Persisters

So ? What can be done?

The promise of Phage Therapy

- Bacteriophages (viruses of bacteria) against bacteria
- “The enemy of my enemy is my friend”
- An old idea

Phages : Viruses of bacteria

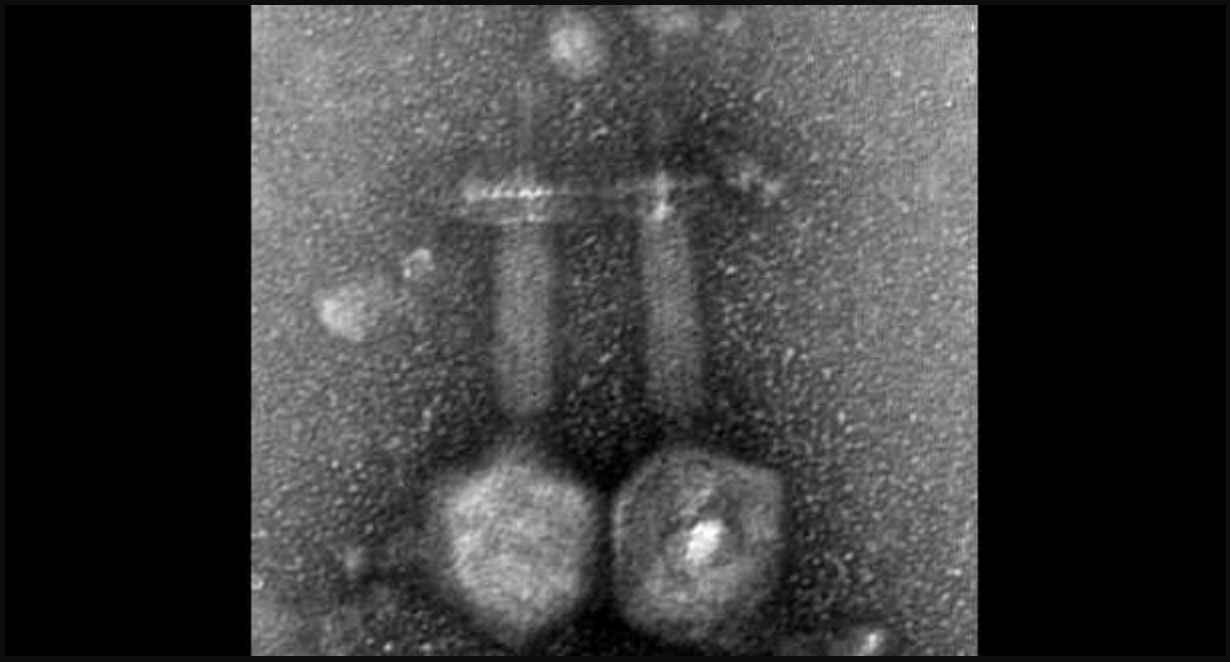


.Gelman. D

Khalifa, Leron, et al. *AEM* 81.8 (2015).

Why phages ?

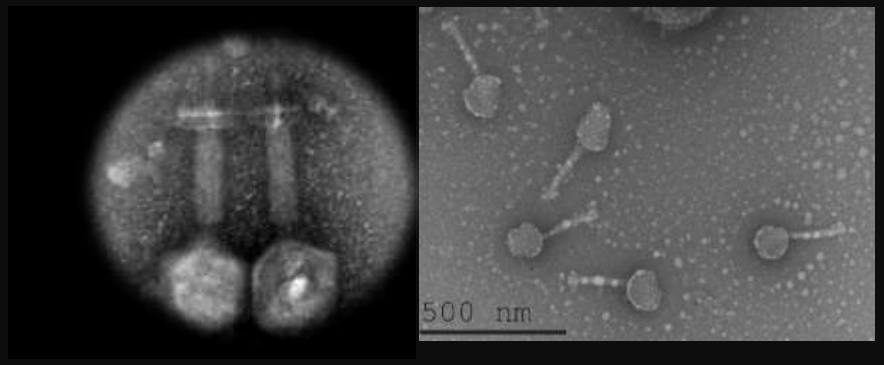
Because phage therapy works exactly where antibiotics fail

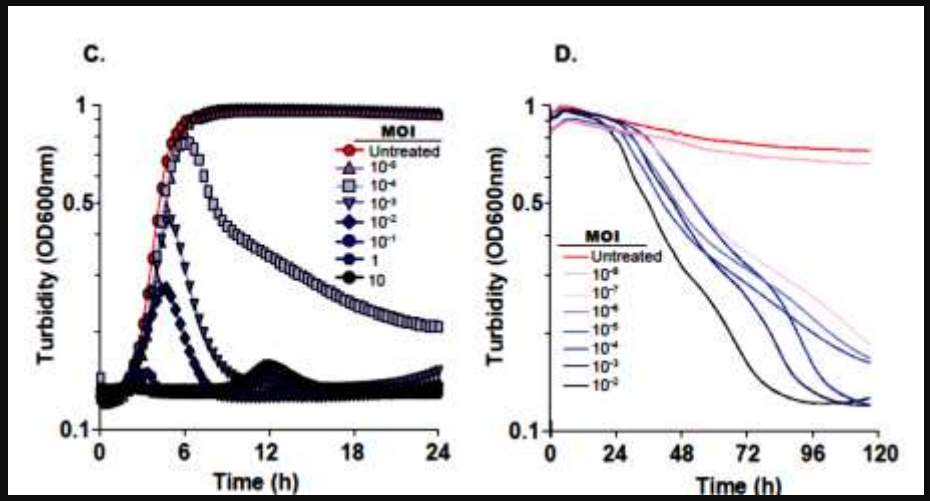


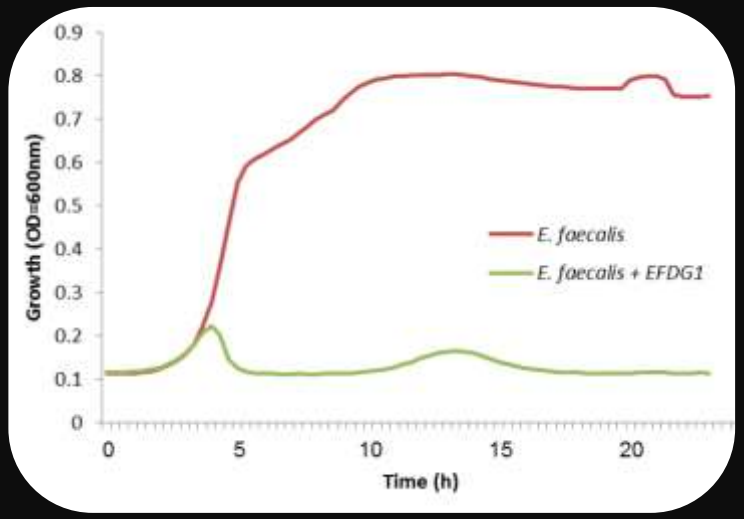
Resistance to phages

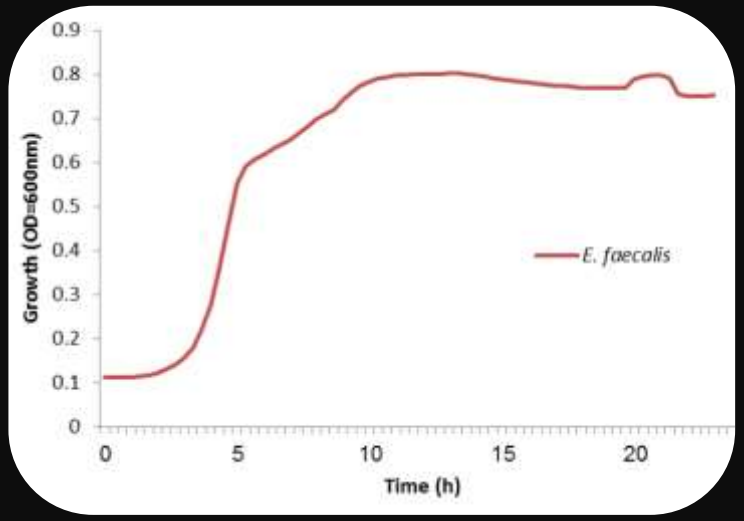
Not the end of the world !

1) Emergence of resistant bacteria









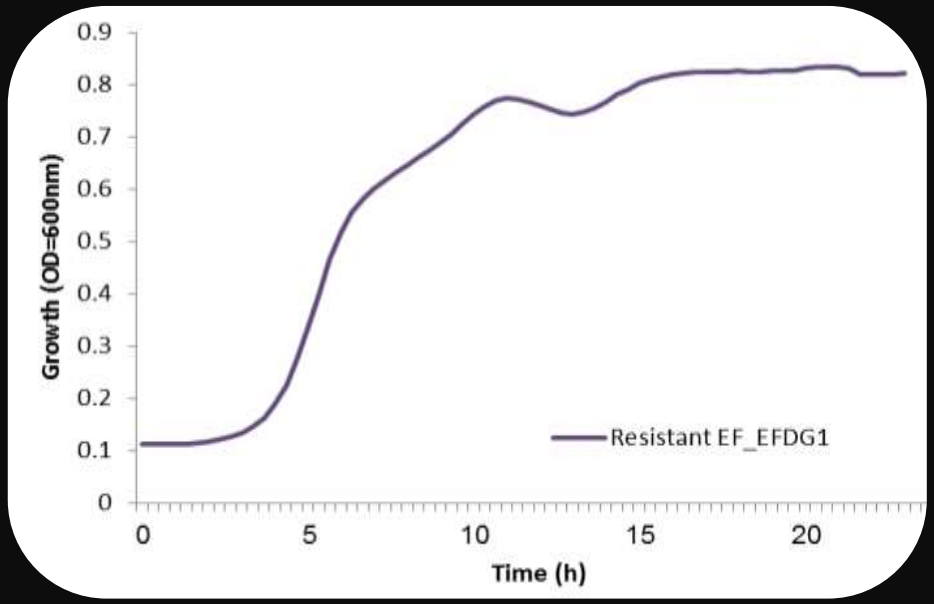
Resistance to phages

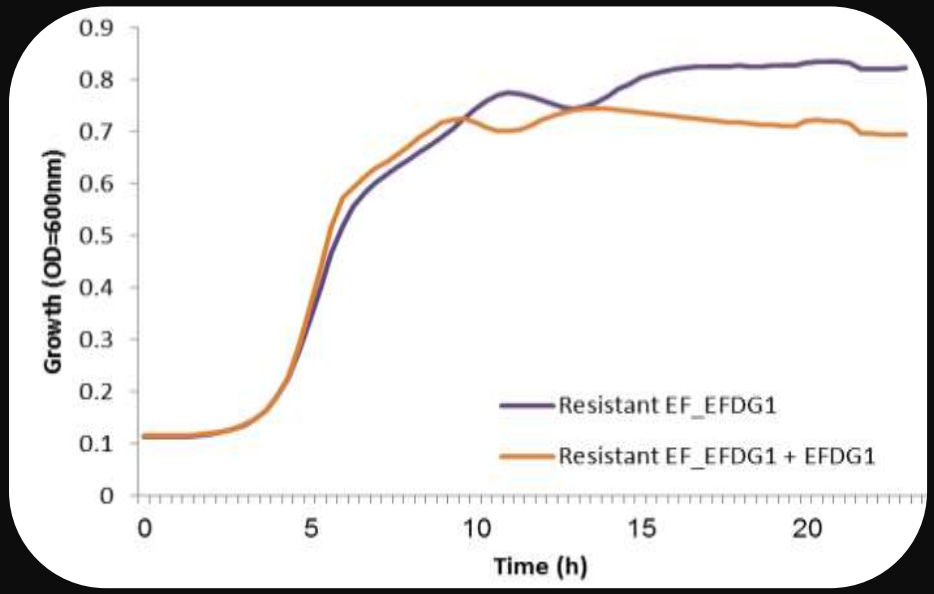
Not the end of the world !

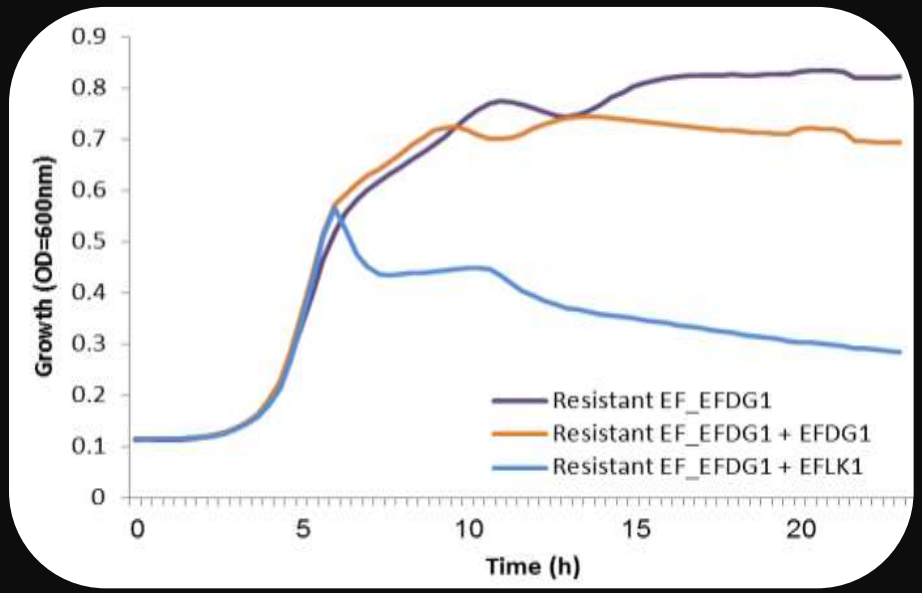
1) Emergence of resistant bacteria

More options:

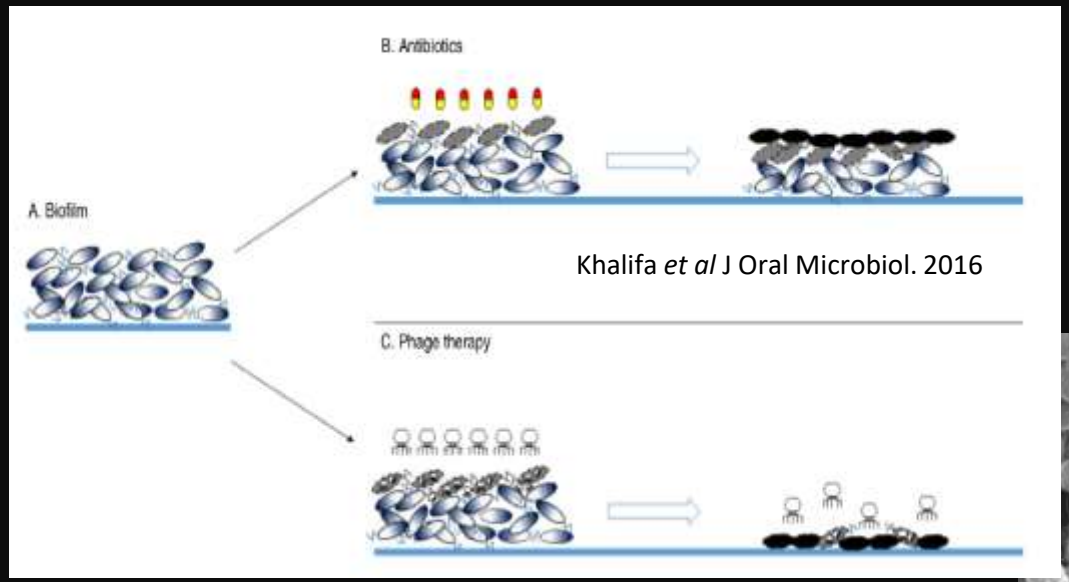
- Mutate the phages (Co-evolution)
- Engineer the phages



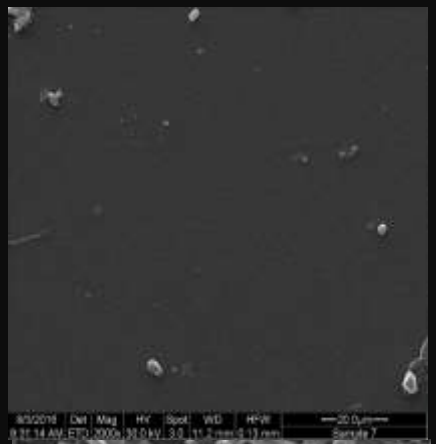


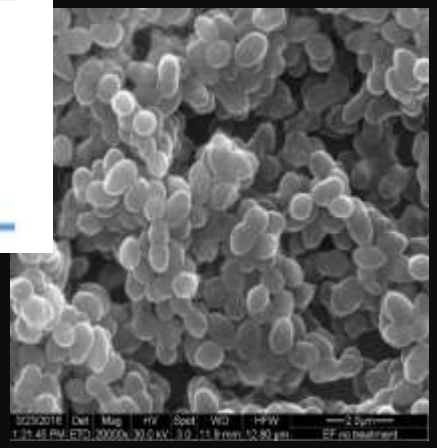


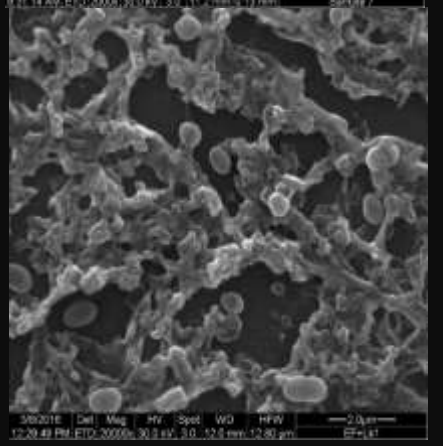
Phages are efficient against Biofilm

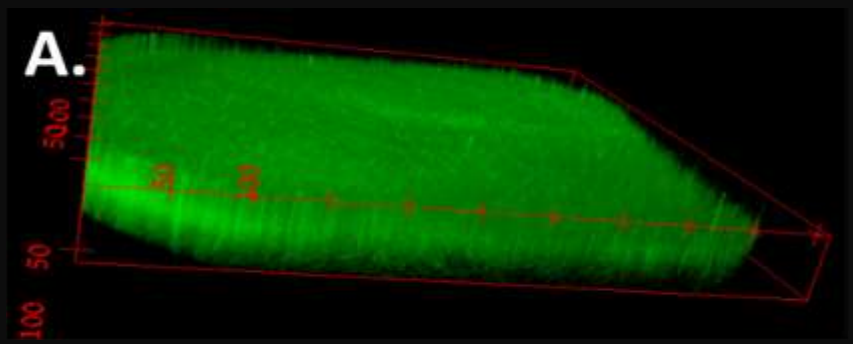


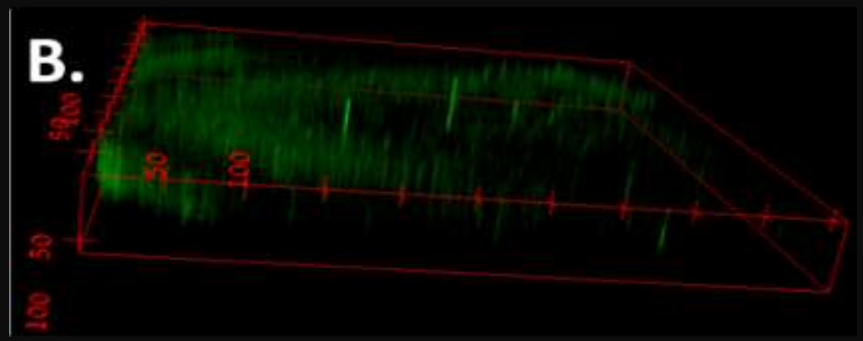
Khalifa *et al* J Oral Microbiol. 2016





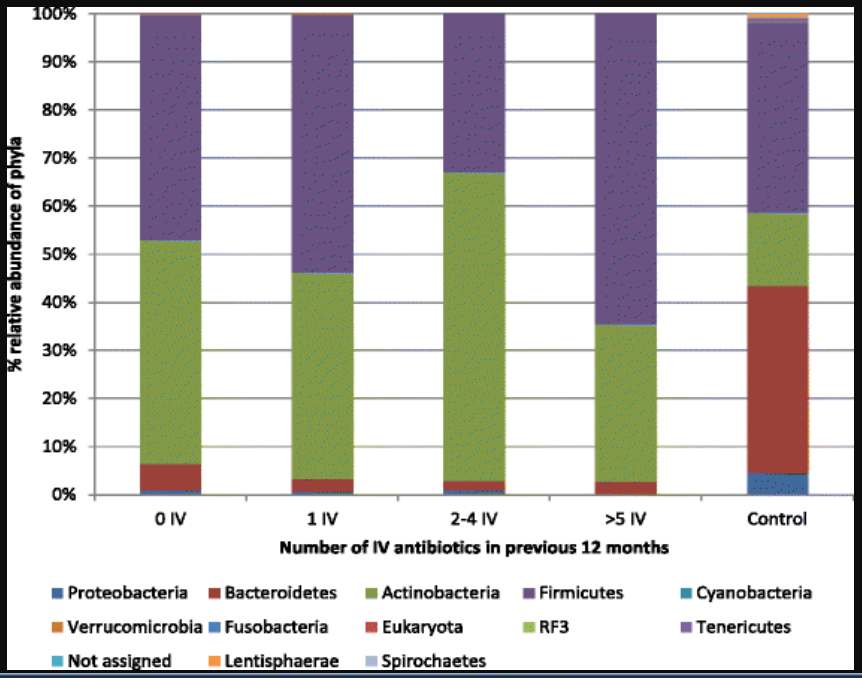






Khalifa *et al* AEM. 2015

Dysbiosis in antibiotic treated CF patients



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The altered gut microbiota in adults with cystic fibrosis

D.G. Burke, F. Fouhy, M. J. Harrison, M. C. Rea, P. D. Cotter, O. O'Sullivan, C. Stanton, C. Hill, F. Shanahan, B. J. Plant and R. P. Ross

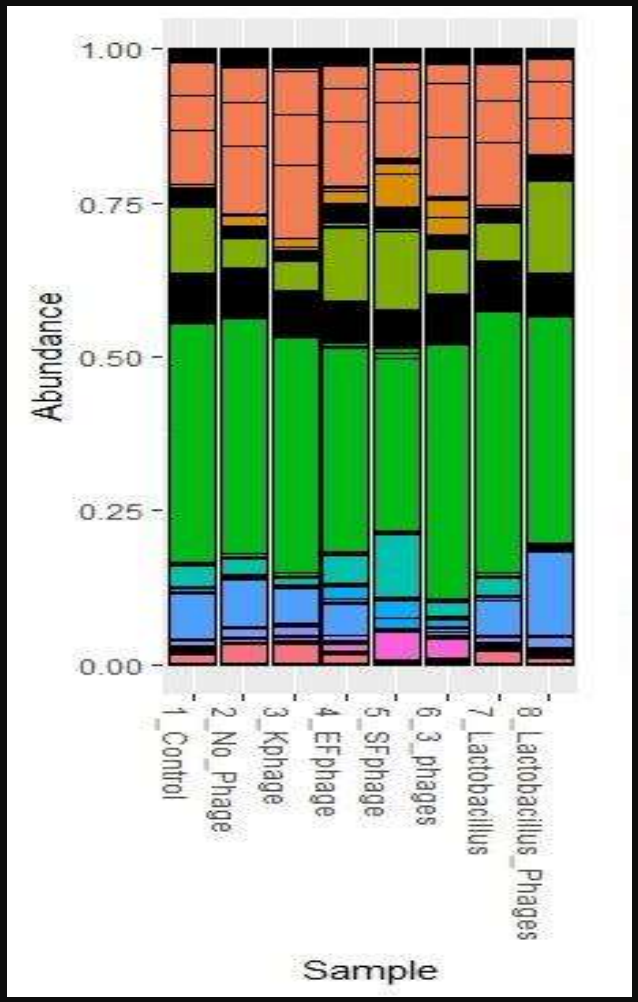
Phages are highly specific

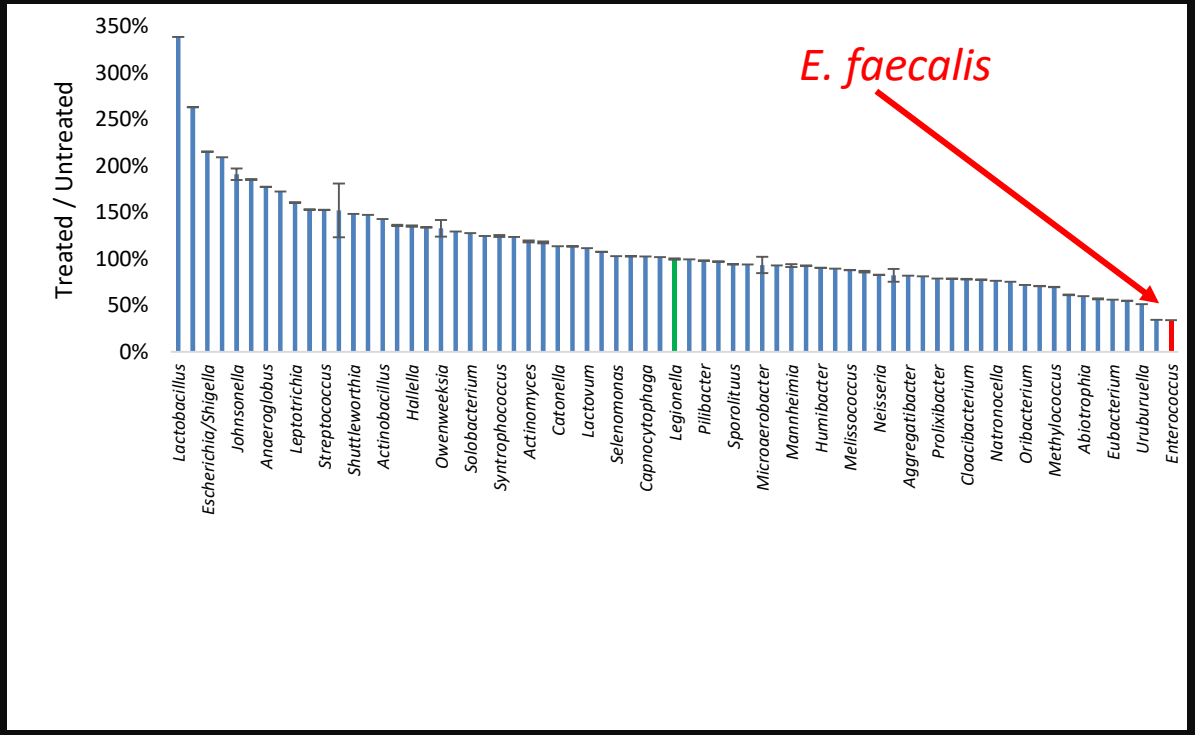
- In contrast to antibiotics that tend to cause microbiome dysbiosis, phages are very specific

Bacterial strain[a]	Origin[b]	EFDG1[c]
Enterococcus strains		
E. faecalis (*v583*)	ATCC 700802	S
E. faecalis (*aef01*)	Clinically isolated from urine	S
E. faecalis (*aef03*)	Clinically isolated from urine	S
E. faecalis (*aef04*)	Clinically isolated from venal blood flow	S
E. faecalis (*aef05*)	Clinically isolated from venal blood flow	S
E. faecalis (*cef02*)	Clinically isolated	S
E. faecium (*aefc06*)	Clinically isolated from venal blood flow	S
E. faecium (*aefc07*)	Clinically isolated from venal blood flow	S
E. faecium (*aefc08*)	Clinically isolated from venal blood flow	S
E. faecium (*aefc09*)	Clinically isolated from feces	S
E. faecium (*aefc10*)	Clinically isolated from feces	S
Staphylococcus strains		
S. aureus (*w6460*)	Clinically isolated	R
S. aureus (*w0406*)	Clinically isolated	R
S. aureus (*lsa011*)		R
Other strains		
Pseudomonas aeruginosa PA14		R
Pseudomonas aeruginosa PA14 *pqsA*		R
Streptococcus mutans (*lsm012*)		R
Streptococcus sobrinus (*lsb013*)		R
Fusobacterium nucleatum (*fs014*)		R
Porphyromonas gingivalis (*pg015*)		R
Burkholderia cepacia complex 25	Clinically isolated	R
Burkholderia cepacia complex 80	Clinically isolated	R
Klebsiella pneumonia (*bkp016*)		R

- Leron Khalifa and Daniel Gelman

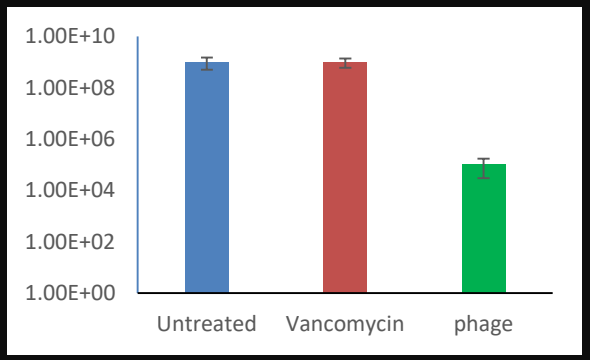
No effect on the Microbiome

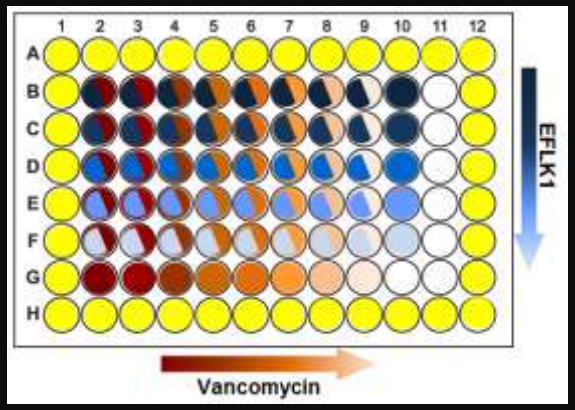


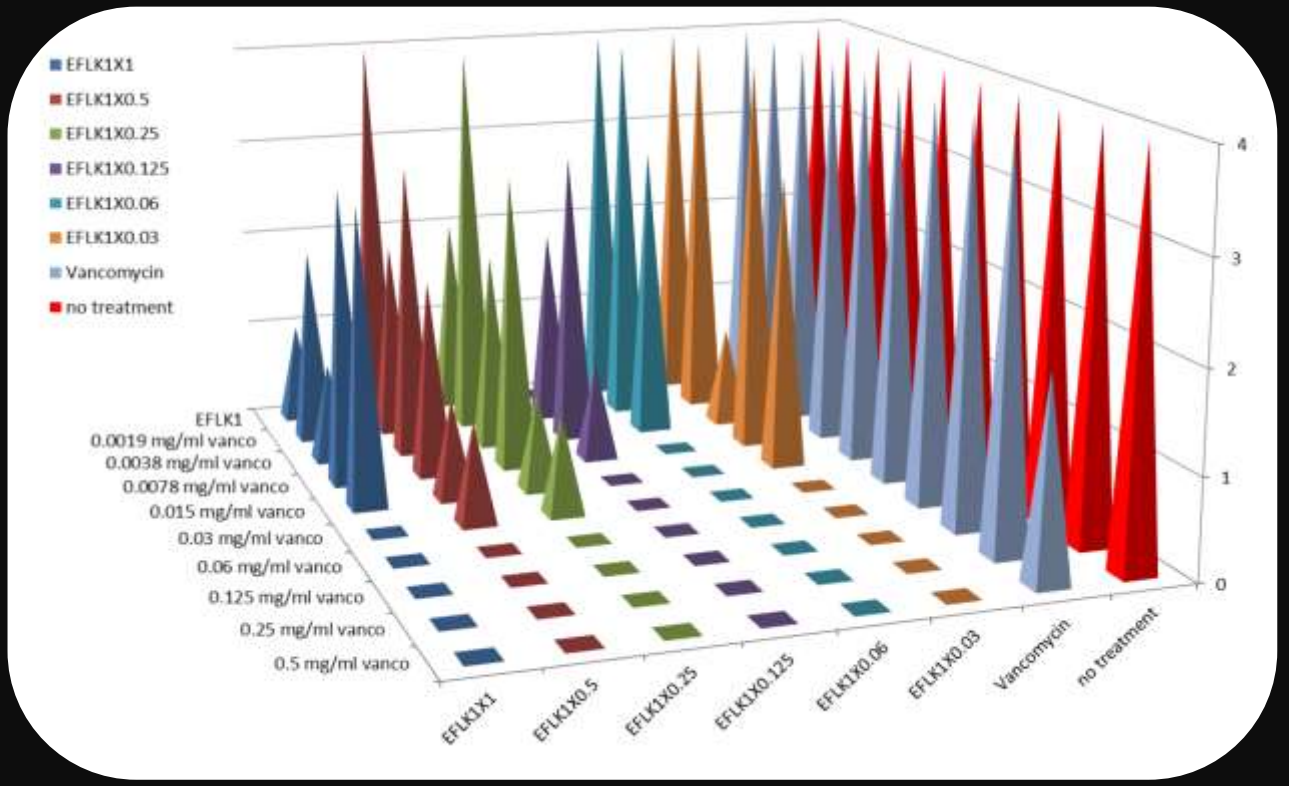


Towards microbiome engineering

Eradication of resistance and persisters by phages and antibiotics







- Both resistant and persisters were eradicated
- **Note** that we used here vanco on VRE!

- Shlezinger *et al*, *submitted*

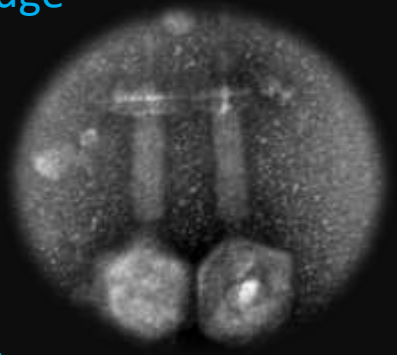
Phage therapy: more advantages

- ***"Personal medicine"***
- ***"Smart kinetics"***
- ***"Natural solution"***

known to our immune system

Phage therapy: cons

- Too Specific → Using cocktails, establishing phage banks, personal medicine.
- Immune response → Coating / passaging
- Might carry harmful genes → Sequencing / knowledge
- Lysogeny : Genetic engineering
- Problematic regulation: Under consideration in the FDA
- Resistance mechanisms (restriction enzymes, CRISPR): High MOI, large cocktails, use in conjugate with antibiotics



So, When should we use Phage Therapy?

- **In general, when antibiotics fail !**
- **Chronic diseases or time for "personalization"**
- **Ectopic treatments** (e.g. burns and wounds)
- **Multidrug resistant strains**
- **Biofilm**
- **Combination with antibiotics**

And when should phage therapy be avoided?

- In general, when antibiotics work !!
- Emergency situations (e.g. "Predator bacteria")

The case that made the difference

Novel Phage Therapy Saves Patient with Multidrug-Resistant Bacterial Infection

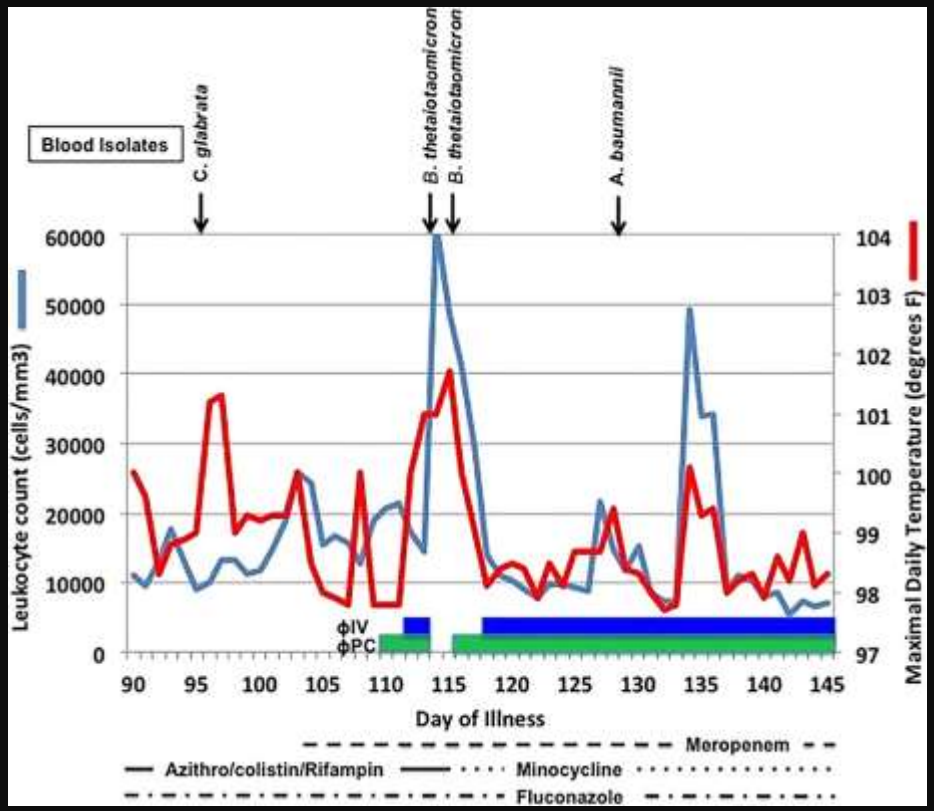
Intravenous viruses are used to target deadly bacterium; dramatic case suggests potential alternative to failing antibiotics

April 25, 2017 | Scott LaFee and Heather Buschman, PhD

Scientists and physicians at University of California San Diego School of Medicine, working with colleagues at the U.S. Navy Medical Research Center – Biological Defense Research Directorate (NMRC-BDRD), Texas A&M University, a San Diego-based biotech and elsewhere, have successfully used an experimental therapy involving bacteriophages — viruses that target and consume specific strains of bacteria — to treat a patient near death from a multidrug-resistant bacterium.

Phage Therapy Infected with a multidrug-resistant

The therapeutic approach, which has been submitted to a peer-reviewed journal, is scheduled to be featured in an oral presentation tomorrow at the Centennial Celebration of Bacteriophage Research at the Institute Pasteur in Paris by Biswajit Biswas, MD, one of the case study's co-authors and chief of the phage division in the Department Genomics and Bioinformatics at NMRC-BDRD. April 27 is Human Phage Therapy Day, designated to mark



The case that made the difference

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection

Robert T. Schooley,[a] Biswajit Biswas,[b,c] Jason J. Gill,[d,e] Adriana Hernandez-Morales,[f] Jacob Lancaster,[e] Lauren Lessor,[e] Jeremy J. Barr,[g,o] Sharon L. Reed,[a,h] Forest Rohwer,[g] Sean Benler,[g] Anca M. Segall,[g] Randy Taplitz,[a] Davey M. Smith,[a] Kim Kerr,[a] Monika Kumaraswamy,[a] Victor Nizet,[i,j] Leo Lin,[i] Melanie D. McCauley,[a] Steffanie A. Strathdee,[a] Constance A. Benson,[a] Robert K. Pope,[k] Brian M. Leroux,[k] Andrew C. Picel,[l] Alfred J. Mateczun,[b] Katherine E. Cilwa,[n] James M. Regeimbal,[b] Luis A. Estrella,[b] David M. Wolfe,[b] Matthew S. Henry,[b,c] Javier Quinones,[b,c] Scott Salka,[m] Kimberly A. Bishop-Lilly,[b,c] Ry Young,[e,f] Theron Hamilton[b]

Department of Medicine, University of California, San Diego, La Jolla, California, USA[a]; Biological Defense Research Directorate, Naval Medical Research Center, Frederick, Maryland, USA[b]; Henry M. Jackson Foundation, Bethesda, Maryland, USA[c]; Department of Animal Science, Texas A&M University, College Station, Texas, USA[d]; Center for Phage Technology, Texas A&M AgriLife Research and Texas A&M University, College Station, Texas, USA[e]; Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas, USA[f]; Department of Biology, San Diego State University, San Diego, California, USA[g]; Department of Pathology, University of California, San Diego, La Jolla, California, USA[h]; Department of Pediatrics, University of California, San Diego, La Jolla, California, USA[i]; Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego, La Jolla, California, USA[j]; National Biodefense Analysis and Countermeasures Center, Frederick, Maryland, USA[k]; Department of Radiology, University of California, San Diego, La Jolla, California, USA[l]; AmpliPhi Biosciences, San Diego, California, USA[m]; Advanced Surgical Imaging Program, Department of Regenerative Medicine, Naval Medical Research Center, Silver Spring, Maryland, USA[n]; Monash University, School of Biological Sciences, Melbourne, Australia[o]

Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*

Benjamin K Chan, Paul E Turner, Samuel Kim, Hamid R Mojibian, John A Elefteriades, Deepak Narayan

Evolution, Medicine, and Public Health, Volume 2018, Issue 1, 1 January 2018, Pages 60–66, https://doi.org/10.1093/emph/eoy005

Published: 08 March 2018 **Article history** ▾

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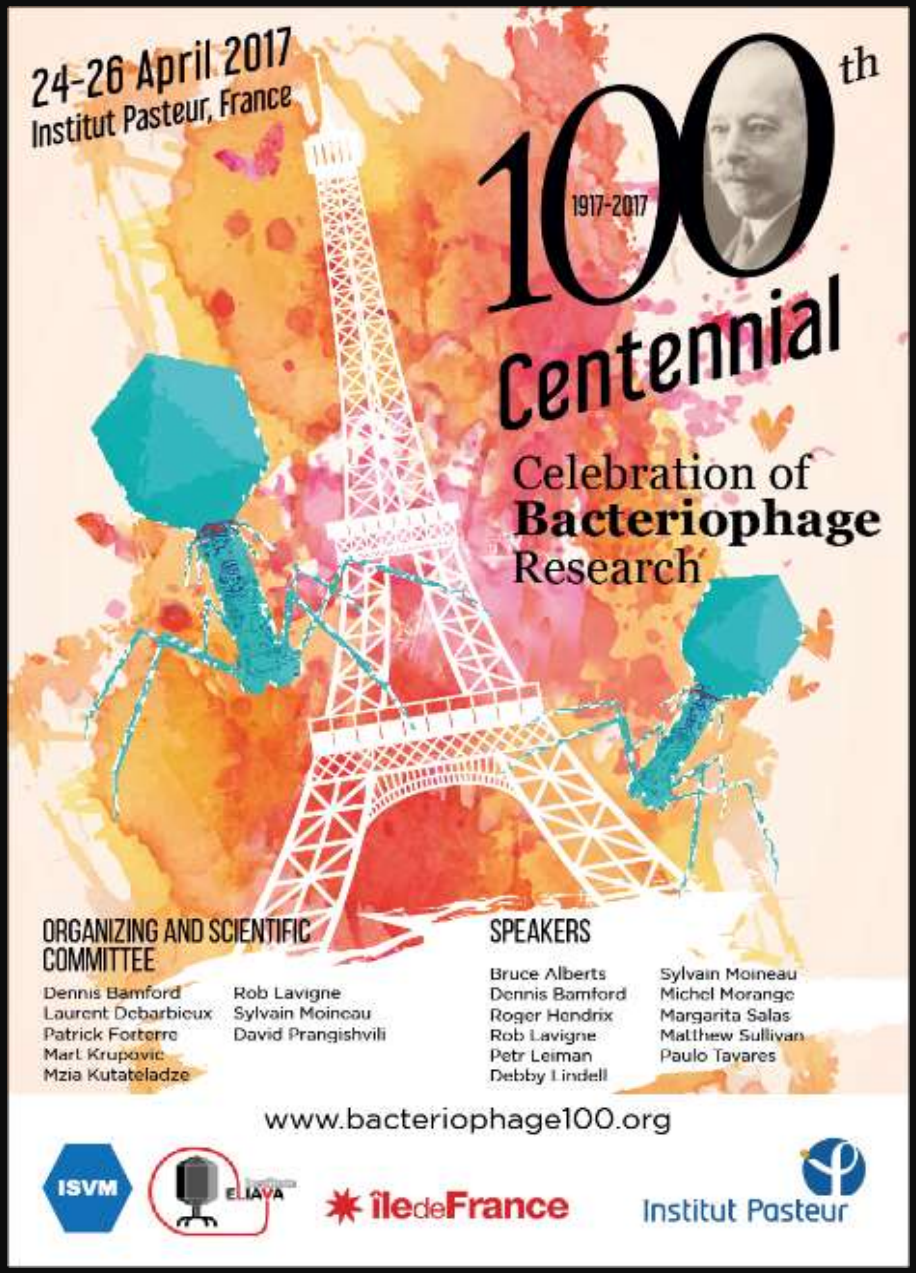
Abstract

Management of prosthetic vascular graft infections caused by *Pseudomonas aeruginosa* can be a significant challenge to clinicians. These infections often do not resolve with antibiotic therapy alone due to antibiotic resistance/tolerance by bacteria, poor ability of antibiotics to permeate/reduce biofilms and/or other factors. Bacteriophage OMKO1 binding to efflux pump proteins in *P. aeruginosa* was consistent with an evolutionary trade-off: wildtype bacteria were killed by phage whereas evolution of phage-resistance led to increased antibiotic sensitivity. However, phage clinical-use has not been demonstrated. Here, we

April 2017 – Paris: 100 years of phages



Meanwhile in Georgia :

more than 100 patients per year are being cured.

Many of them are medical tourists

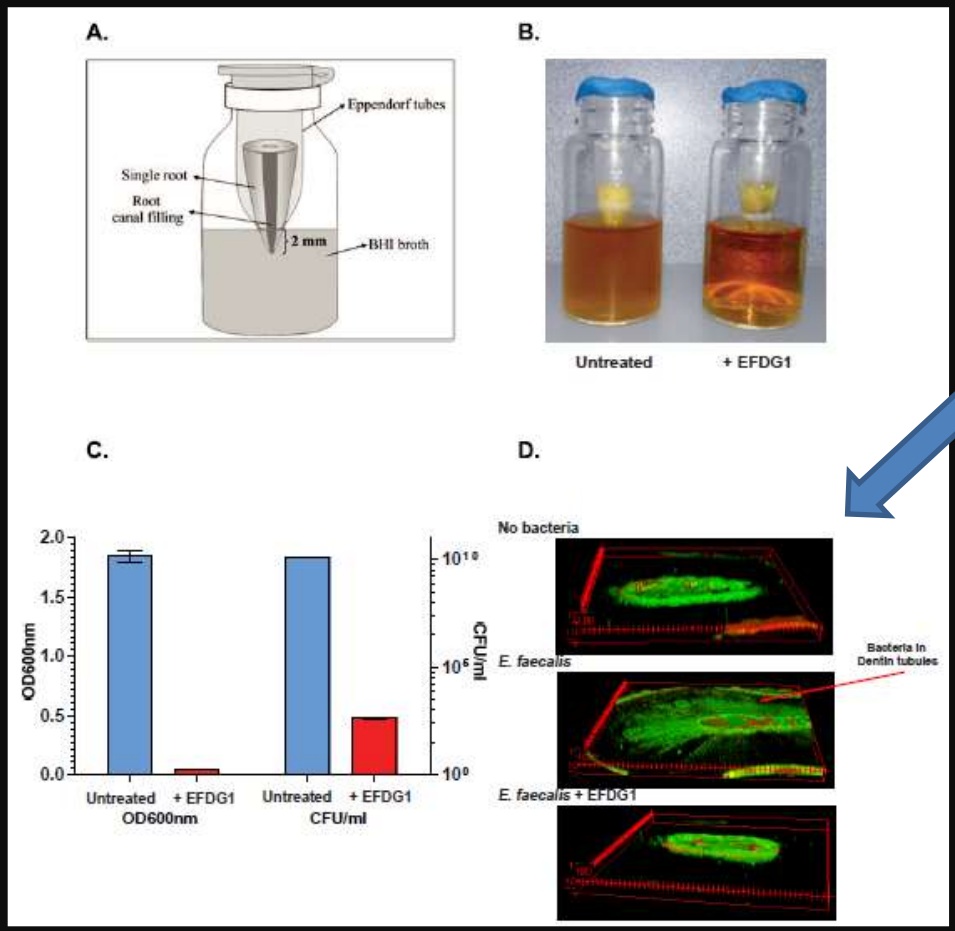
1935 – Current: Phage Therapy Centers in East Europe countries

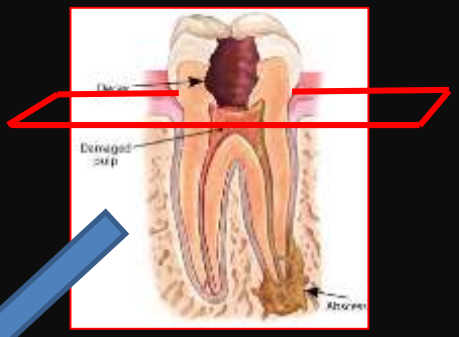
Eliave Institute,
Tiblisy Georgia
2017

Some of the running projects in our lab

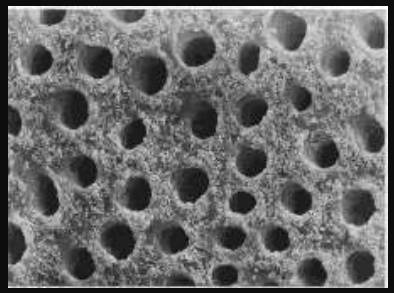
The Anti – *E. faecalis* phages project

Ex – vivo dental models



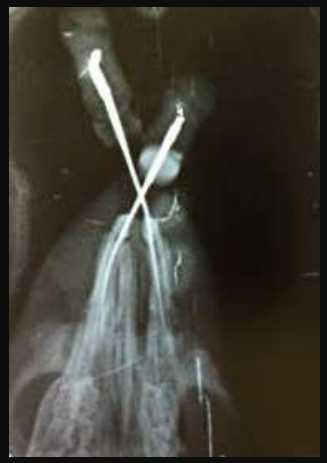


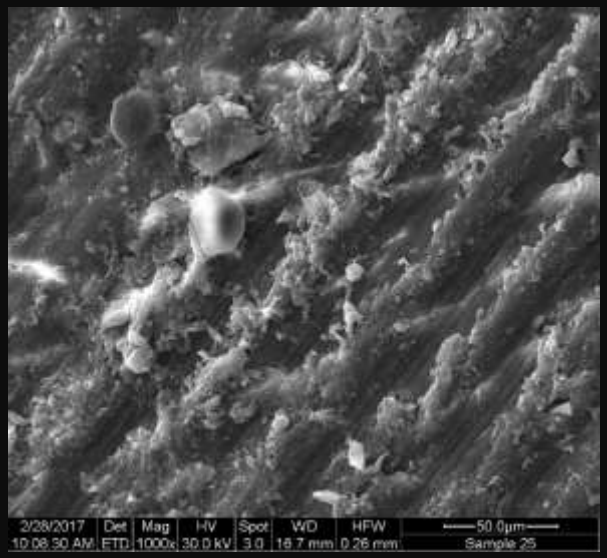
Dentin tubules



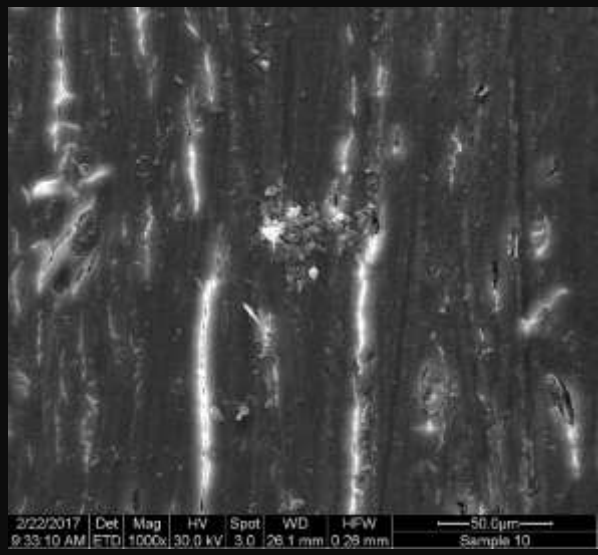
Khalifa et. al *Appl Environ Microbiol. 2015, 15;81(8):2696-2705.*

Animal model: Root canal model in Rats

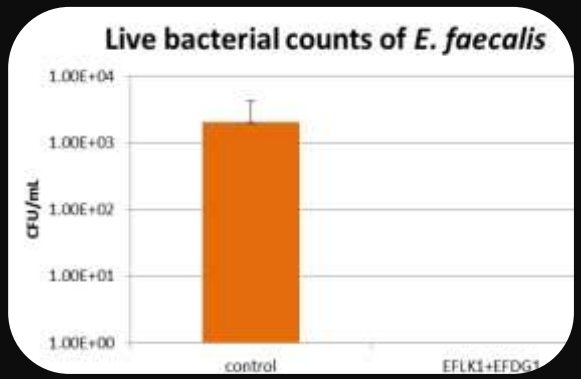




Untreated



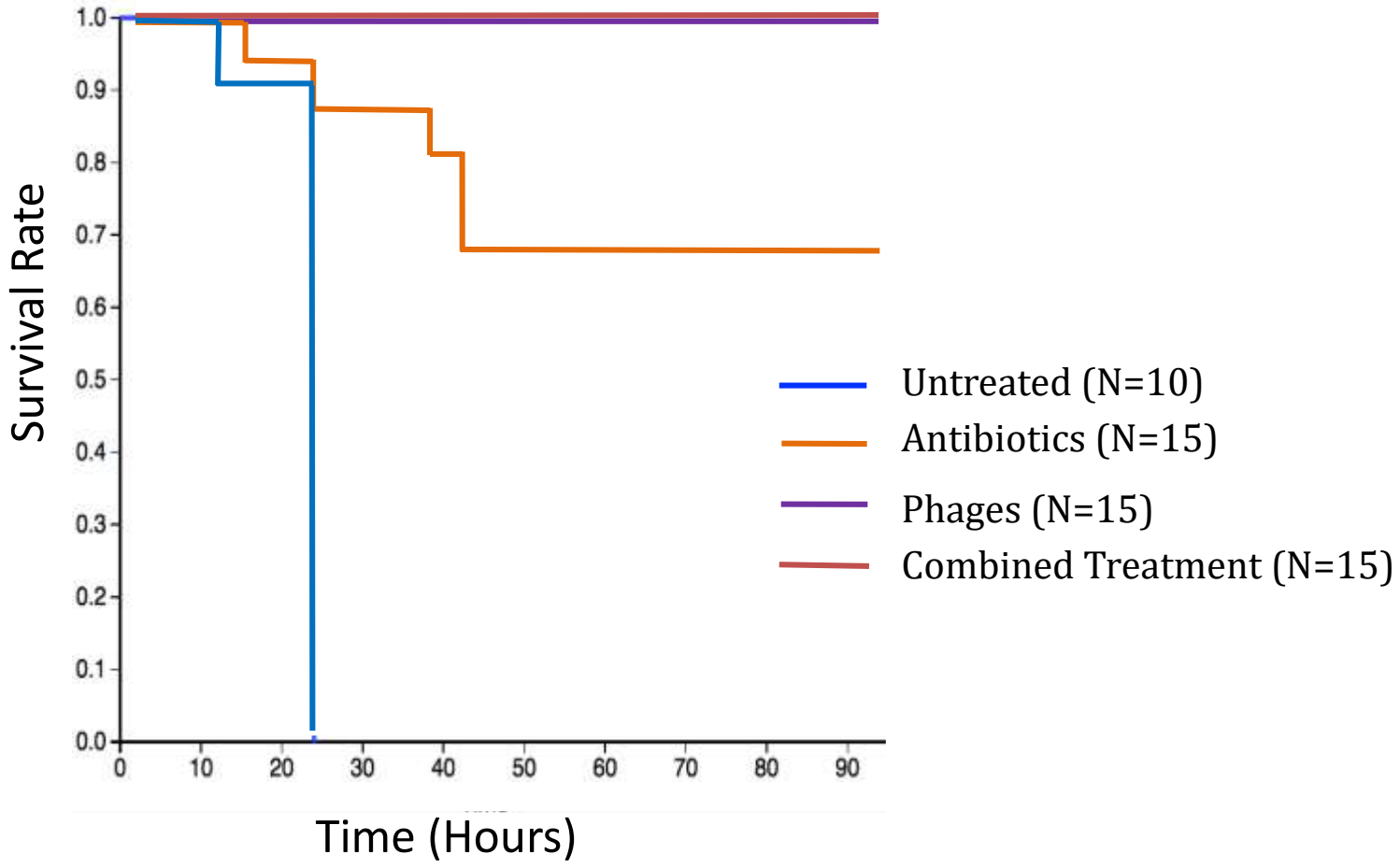
+ Phages



- 16S analysis is on the way

Mor Shlezinger in collaboration with Nurit Beyth

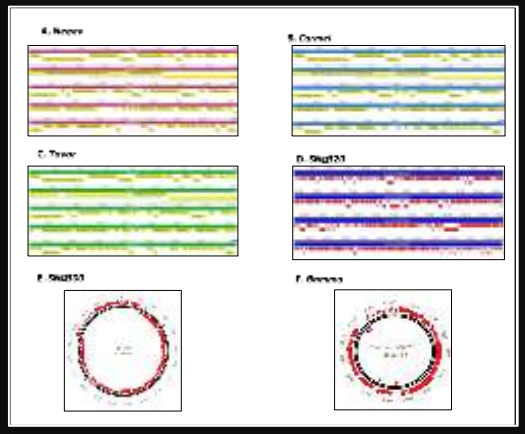
Phages saved the life of 100% of the animals in a peritonitis model

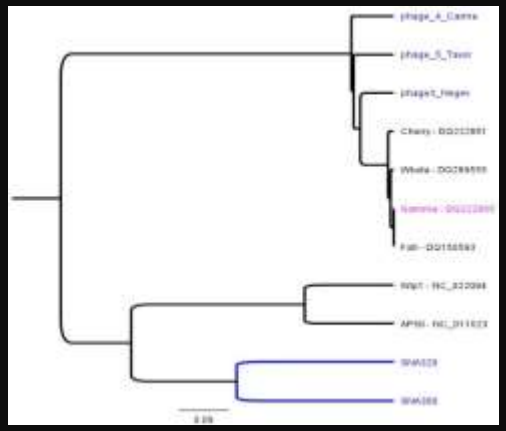


Gelman et al. (In Preparation)

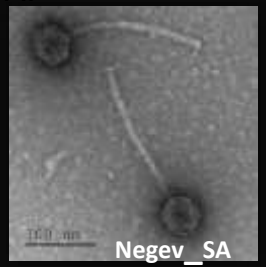
Phages against Anthrax

- It is expected that in a bioterrorism attack, antibiotic resistant bacteria will be used

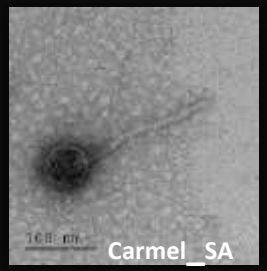




A.



B.



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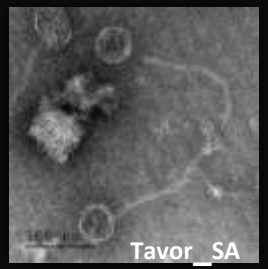

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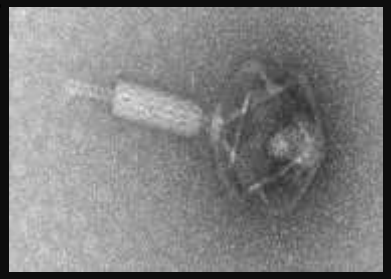

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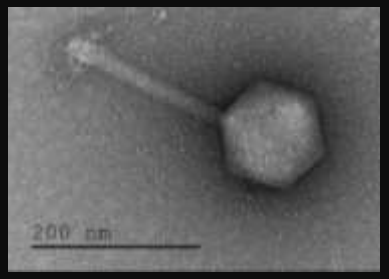
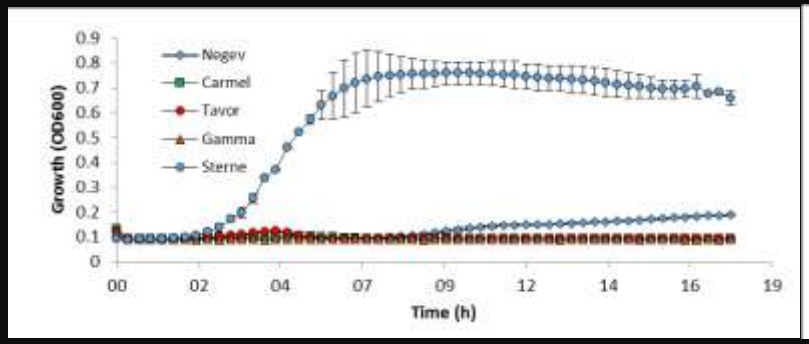
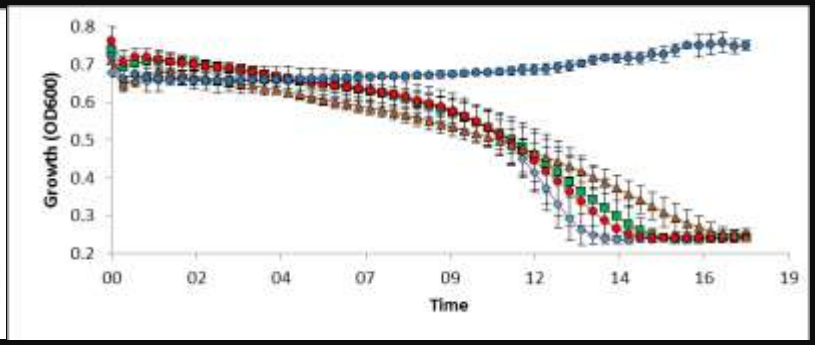


Sivan Alkalay and Sarit Sterenberg (Alpha)

Phages against Anthrax



Log phage



Stationary phage

B. Anthracis Strain	Year	Place	SNα300	SNα320	SA-Tavor	SA-Carmel	SA-Negev	Gamma*
Megido	2009	Megido	+++	+	+++	+++	+++	+++
151852	2012	Keshet	+++	-	+++	+++	+++	+++
151321	2012	Keshet	+++	+	+++	+++	+++	+++
Keshet	2012	Keshet	+++	-	+++	+++	+++	+++
151264	2012	Keshet	+++	+	+++	+++	+++	+++
152776	2012	Yehonatan	+++	+	+++	+++	+++	+++

* Brown, E. R., W. Cherry. 1955, J. Infect. Dis

Our phage are effective against virulent *B. anthracis*
(Tested by Prof. D Elad, Veterinary Services)

Sivan Alkalay and Sarit Sterenberg (Alpha)

Phages against diarrhea related bacteria

- One of the biggest problems of field units
- In “Zuk Eitan”, several combat unit were evacuated from the battle zone due to foodborne diseases
- The problem is not limited to the army

סלמונלה בגן בי-ם: 4 ילדים אושפזו

כ-30 ילדים במעון סבלו משלשולים, הקאות וחום גבוה וארבעה מהם אף אושפזו בביה"ח. משרד הבריאות אישר כי בדיקת מעבדה גילתה אצל אחד מהם את חיידק הסלמונלה וההורים טוענים שגם אצל ילדים נוספים התגלה החיידק. "הגן המשיך לפעול גם לאחר האיבחון ועדו ילדים חלו", אמרה אחת האמהות. "לא ייתכן שיסכנו כך את ילדינו"

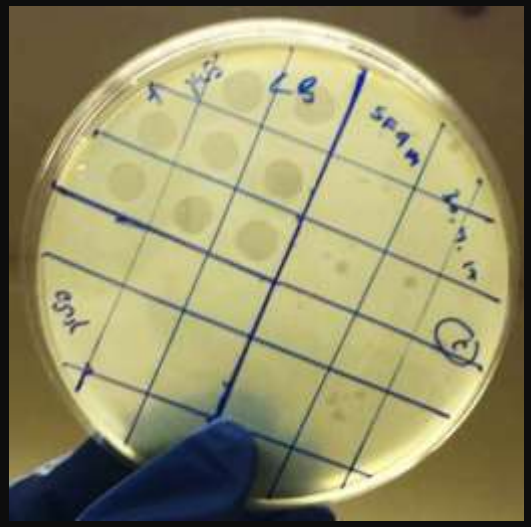
יואב אבן | חדשות 2 | פורסם 04/05/17 12:51

התפרצות שיגלה בישראל

2016	2015		2016	2015	
496	116	חיפה	302	76	אשקלון
0	0	נצרת	290	291	דרום
170	35	עכו	**1105**	**424**	**תל אביב**
170	6	עפולה	523	181	רחובות
17	7	כנרת	528	125	רמלה
3	23	צפת	542	165	פתח תקוה
1848	**607**	**מחוז ירושלים**	86	62	נתניה
6225	2143	סה"כ	145	25	חדרה

מקרי התפרצות מדווחים למשרד הבריאות בשנתיים החולפות

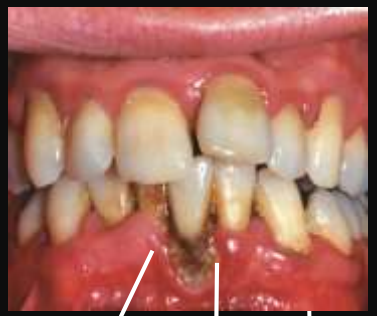
- We are developing “Anti food born diseases phage cocktail” –
  - *Shigella, Campilobacter, Salmonella and* more



Our *Shigella* phages
1/5/2017

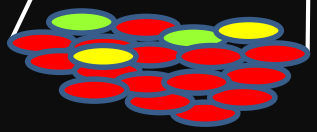
Shira ben Porat and Daniel Gelman, “Zameret”

Personal preventive dentistry

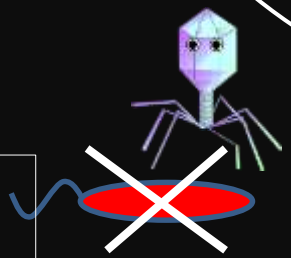


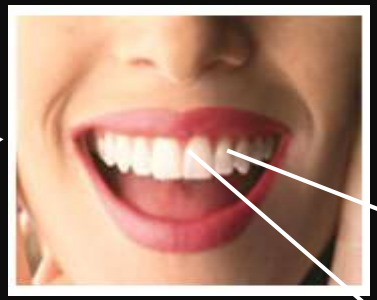
Oral Pro and Pre - biotic

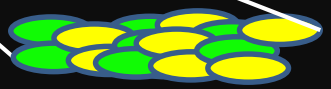
Streptococcus salivarius



Specific removal of unwanted bacteria







In collaboration with:

Prof. Nurit Beyth
Prof. Yael Houri Hadad
Dr. Asaf Wilensky
HUJI dental school

Prepare Phage for treatment

- *P. aeruginosa*
- *K. pneumonia CRE*
- *E. faecalis VRE*
- *A. baumanii*
- *P. acne (with Dr Vered Molho, Dermatology,* Hadassah)

In collaboration with:

Prof. Ran Nir-Paz

Infectious diseases, Hadassah

Summary

- We believe that phage therapy is a useful tool **when antibiotics fail**
- It seems that phage – antibiotic combinations have additive or synergistic effects
- We aim to use phages in personalized / precision manner
- Phages might be useful in many direction besides medicine

Thanks

Lab Manager
Shunit Glazer

PhD students
Leron Khalifa
Mor Shlezinger
Chaya Schoemann
Sivan Alkalai
Vanda Lerrer

MA students
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Elisheva Dorfman
Tzemah Aouizerat
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Karen Adler
Reut Kraitman
Aya Shahin
Mohanad Abed AlRahman

Other Students
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"Phage Hunters" (Alfa)
Sarit, Noa, Eyal, Yael
Boaz, Shilo, Eilon

Collaborators

Infectious diseases
- Ran Nir Paz (Hadassah)

Dentistry
- **Nurit Beyth (HU dental school)**
- **Yael Houri Hadad (HU dental school)**
- **Asaf Wilensky (HU dental school)**
- **Naama Keshet and Doron Afremian**

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- Shauli Beyth (Hadassah)

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- Michael Klutstein
- Ayelet Bernholtz

Phage Therapy
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- Mzia Kutateladze (Eliave, Tbilisi)

Pharmacology
- Micha Friedman

- **Beer**
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  - Amichai Saragovi

Support