Phage Therapy as a promising tool against antibiotic resistant bacteria

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Hebrew University

March 2018
Do we need more antibiotics?

New FDA-approved antibiotics

<table>
<thead>
<tr>
<th>Year Range</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>83-'87</td>
<td>16</td>
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<tr>
<td>88-'92</td>
<td>14</td>
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<tr>
<td>93-'97</td>
<td>10</td>
</tr>
<tr>
<td>98-2002</td>
<td>7</td>
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<tr>
<td>03-'07</td>
<td>5</td>
</tr>
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<td>08-'12</td>
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</table>

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.
“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!

**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

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 AEM. 2015
Dysbiosis in antibiotic treated CF patients
Phages are highly specific

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

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Origin</th>
<th>EFDG1</th>
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<tbody>
<tr>
<td><em>Enterococcus</em> strains</td>
<td></td>
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<tr>
<td><em>E. faecalis</em> (v583)</td>
<td>ATCC 700802</td>
<td>S</td>
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<td><em>E. faecalis</em> (aef01)</td>
<td>Clinically isolated from urine</td>
<td>S</td>
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<tr>
<td><em>E. faecalis</em> (aef03)</td>
<td>Clinically isolated from urine</td>
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<td><em>E. faecalis</em> (aef04)</td>
<td>Clinically isolated from venal blood flow</td>
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<td><em>E. faecalis</em> (aef05)</td>
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<td><em>E. faecium</em> (aefc06)</td>
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<td><em>E. faecium</em> (aefc08)</td>
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<td>S</td>
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<tr>
<td><em>E. faecium</em> (aefc09)</td>
<td>Clinically isolated from feces</td>
<td>S</td>
</tr>
<tr>
<td><em>E. faecium</em> (aefc10)</td>
<td>Clinically isolated from feces</td>
<td>S</td>
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<td><em>Staphylococcus</em> strains</td>
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<td>R</td>
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<td><em>S. aureus</em> (w0406)</td>
<td>Clinically isolated</td>
<td>R</td>
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<td><em>S. aureus</em> (Isa011)</td>
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<td>R</td>
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<tr>
<td>Other strains</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em> PA14</td>
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<td>R</td>
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<td><em>Pseudomonas aeruginosa</em> PA14 pqsA</td>
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<td>R</td>
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<tr>
<td><em>Streptococcus mutans</em> (Ism012)</td>
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<td><em>Streptococcus sobrinus</em> (Isb013)</td>
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<td>R</td>
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<tr>
<td><em>Fusobacterium nucleatum</em> (fs014)</td>
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<td><em>Porphyromonas gingivalis</em> (pg015)</td>
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<td>R</td>
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<td><em>Burkholderia cepacia</em> complex 25</td>
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<td><em>Burkholderia cepacia</em> complex 80</td>
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<tr>
<td><em>Klebsiella pneumonia</em> (b kp016)</td>
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<td>R</td>
</tr>
</tbody>
</table>

- Leron Khalifa and Daniel Gelman
No effect on the Microbiome

Towards microbiome engineering

E. faecalis
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.

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

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 ↗

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 OMK01 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, Tbilisy Georgia 2017
Some of the running projects in our lab
The Anti – *E. faecalis* phages project

**VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE)**

- **20,000** drug-resistant enterococcus infections
- **1,300** deaths from drug-resistant enterococcus infections
- **66,000** enterococcus infections per year

_Some enterococcus strains are resistant to vancomycin leaving few or no treatment options._
Ex-vivo dental models

Animal model: Root canal model in Rats

- Dr. Mor Shlezinger
- Prof. Nurit Beyth

- 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.

Sivan Alkalay and Sarit Sterenberg (Alpha)
Our phage are effective against virulent *B. anthracis* (Tested by Prof. D Elad, Veterinary Services)

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

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

Shira ben Porat and Daniel Gelman, “Zameret”

Our Shigella phages 1/5/2017
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

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

MDs
- Vered Molho (Hadassah)
- Shauli Beyth (Hadassah)

Biologists
- Michael Klutstein
- Ayelet Bernholtz

Phage Therapy
- Gregory Resche (Phagoburn)
- Mzia Kutateladze (Eliave, Tbilisi)

Pharmacology
- Micha Friedman

Support
- Beer
- Itai Gutman
- Amichai Saragovi

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

MA students
- Alexia Azoulai
- Hadar ben Zaken
- Elisheva Dorfman
- Tzemah Aouizerat
- Daniel Gelman
- Naama Gold
- Karen Adler
- Reut Kraitman
- Aya Shahin
- Mohanad Abed AlRahman

Other Students
- Ortal Yerushalmi

"Phage Hunters" (Alfa)
- Sarit, Noa, Eyal, Yael
- Boaz, Shilo, Elion

Lab Manager
- Shunit Glazer

The Hazan lab and friends