KLİMİK TÜRK KLİNİK MİKROBİYOLOJİ VE İNFEKSİYON HASTALIKLARI DERNEĞİ

Bilimle Sağlıkla 33 .Yıl

Antibiyotiklere Alternatif Faj Tedavisi

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İstanbul Üniversitesi İstanbul Tıp Fakültesi

İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji AD

Sunum Planı

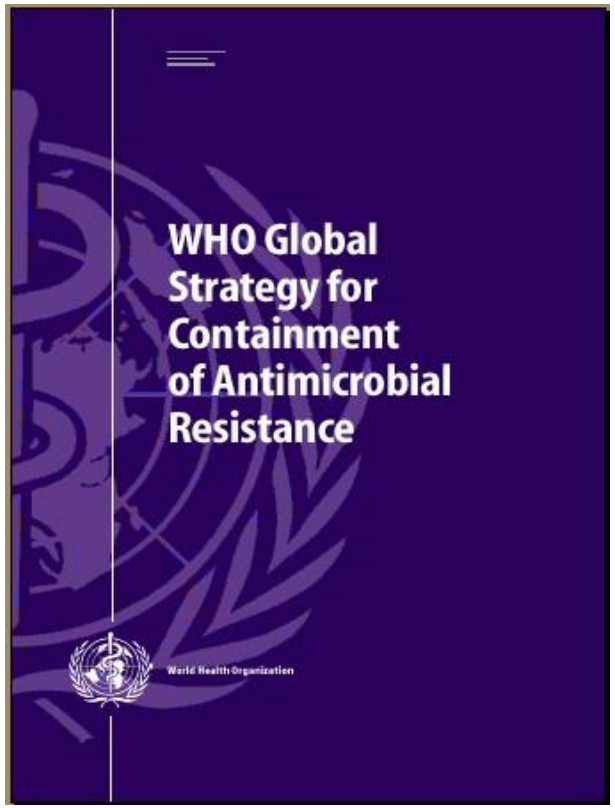
- Günümüzdeki durum
- Neden Bakteriyofaj?
- Tanım ve etki mekanizması
- Kullanım alanları
- Gelecekte bakteriyofaj tedavisi

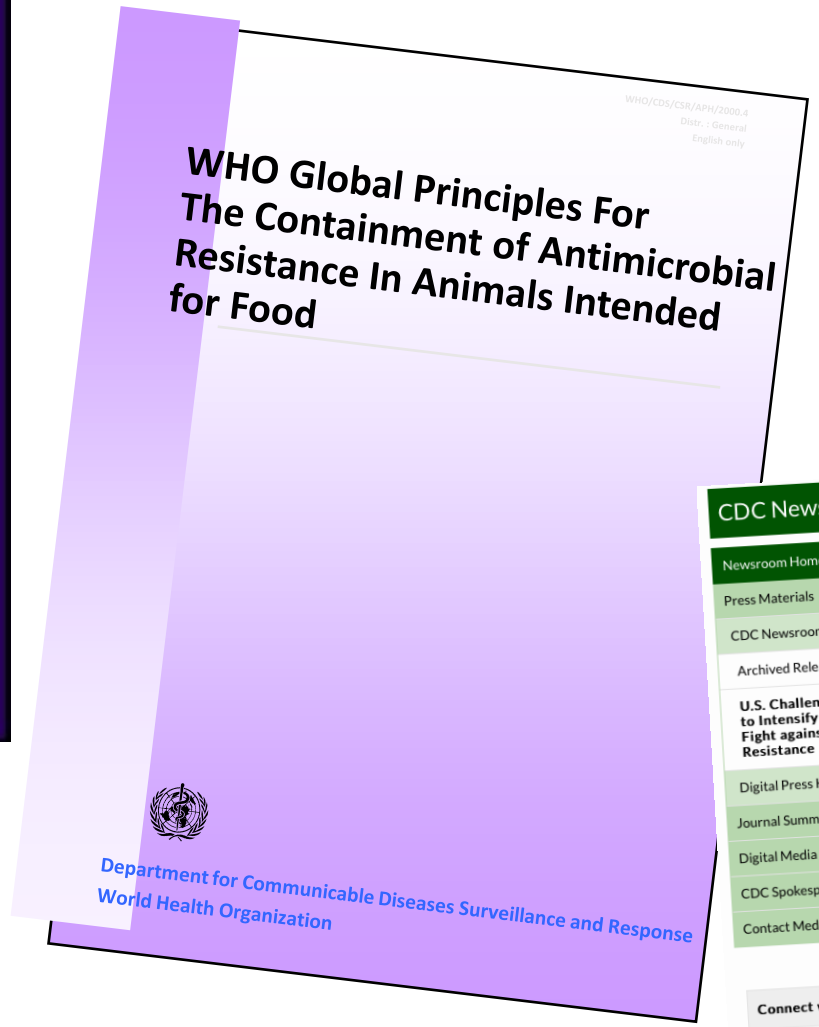
MDR Kaynaklı İnfeksiyonlar ne kadar Sorun?

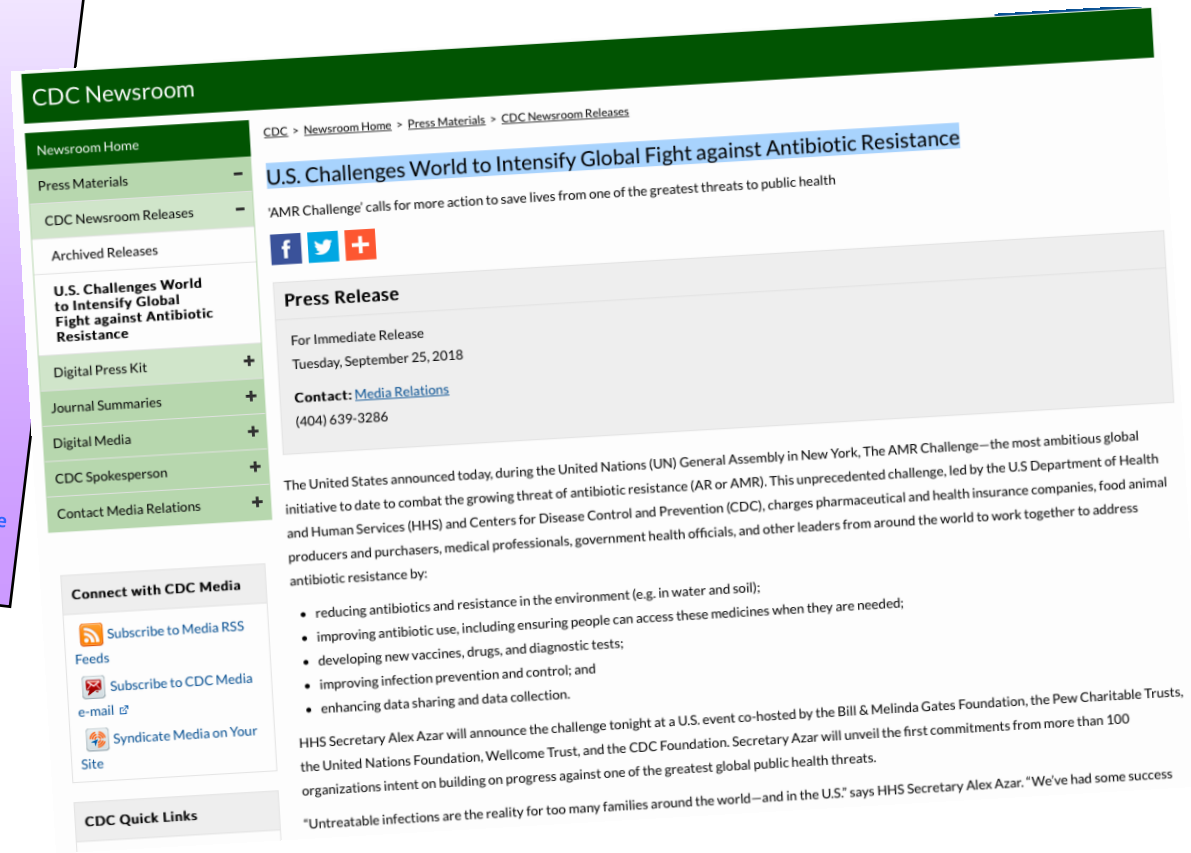
The Telegraph

The true cost of antibiotic resistance in Britain and around the world

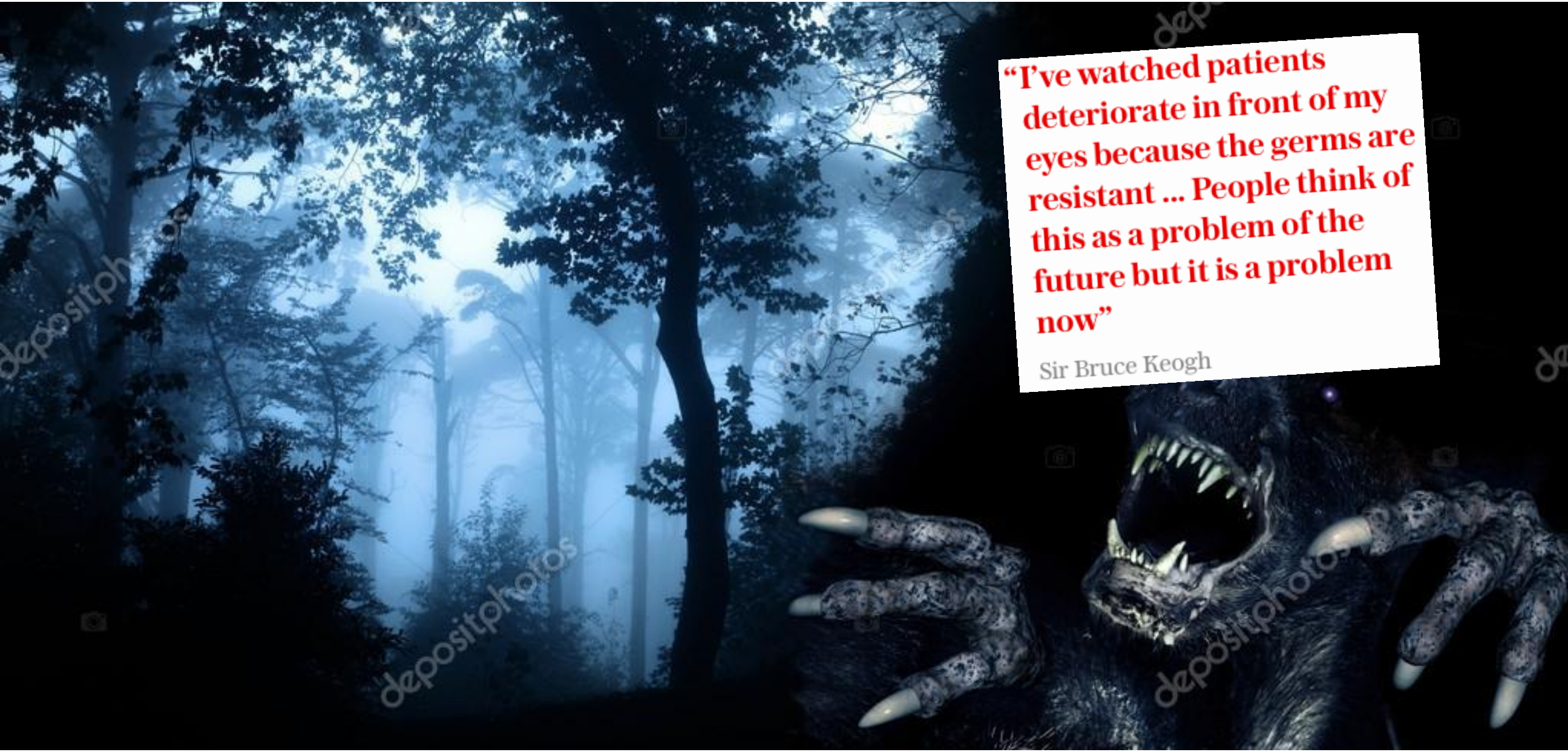
- Her yıl 700.000 insan daha fazla ölmekte.
- Yılda 5.000 Ölüm / UK (MDR)
- Beklenen ölüm oranının 2 katı
- Ölüm hızı? 1 insan/dakika
- 10 milyon insan / yıl (2050)







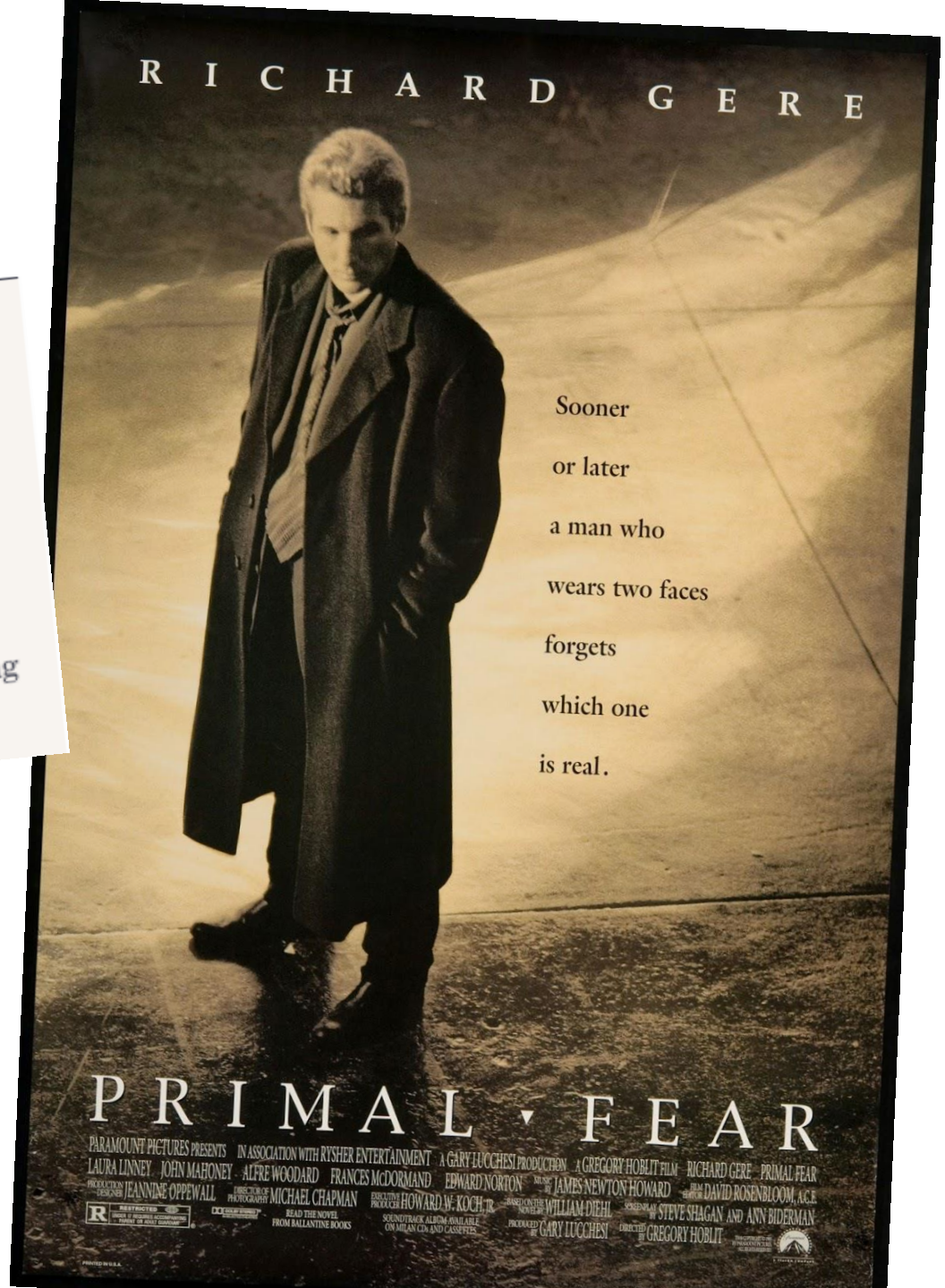
ABD'de her yıl en az 2 milyon insan infeksiyonlar nedeniyle, 23.000 insan da MDR bakteri infeksiyonları nedeniyle ölüyor.


"I've watched patients deteriorate in front of my eyes because the germs are resistant ... People think of this as a problem of the future but it is a problem now"
Sir Bruce Keogh

The Telegraph

Antibiotic resistance | Reasons for hope

- There are 28 new antibiotics in the late stages of clinical development - of which several are new and include new classes of antibiotic
- Access to vaccines has been expanded in recent years - many of these prevent illnesses, such as pneumococcal disease, which are treated with antibiotics
- Drug firms are waking up to the problem: pledging measures such as stopping pollution and withdrawing bonuses linked to sales of antibiotic
- Some biotechs are developing alternatives to antibiotics such as immunity-boosting probiotics and phage therapy which uses viruses to kill bacteria

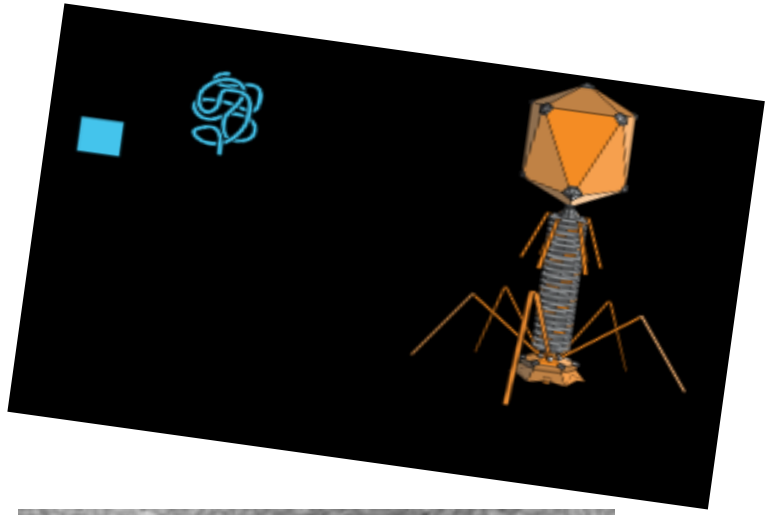


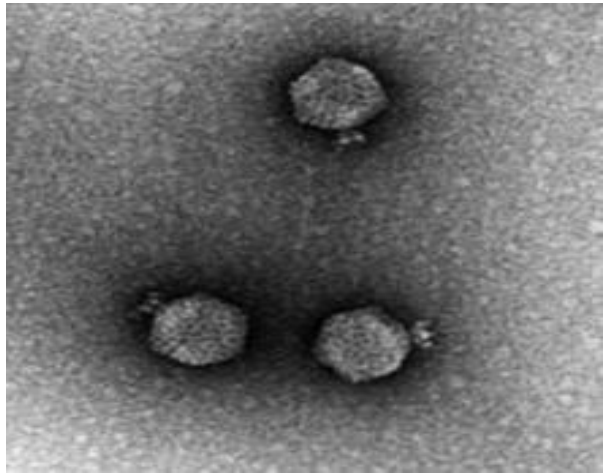
Yeni Arayışlar

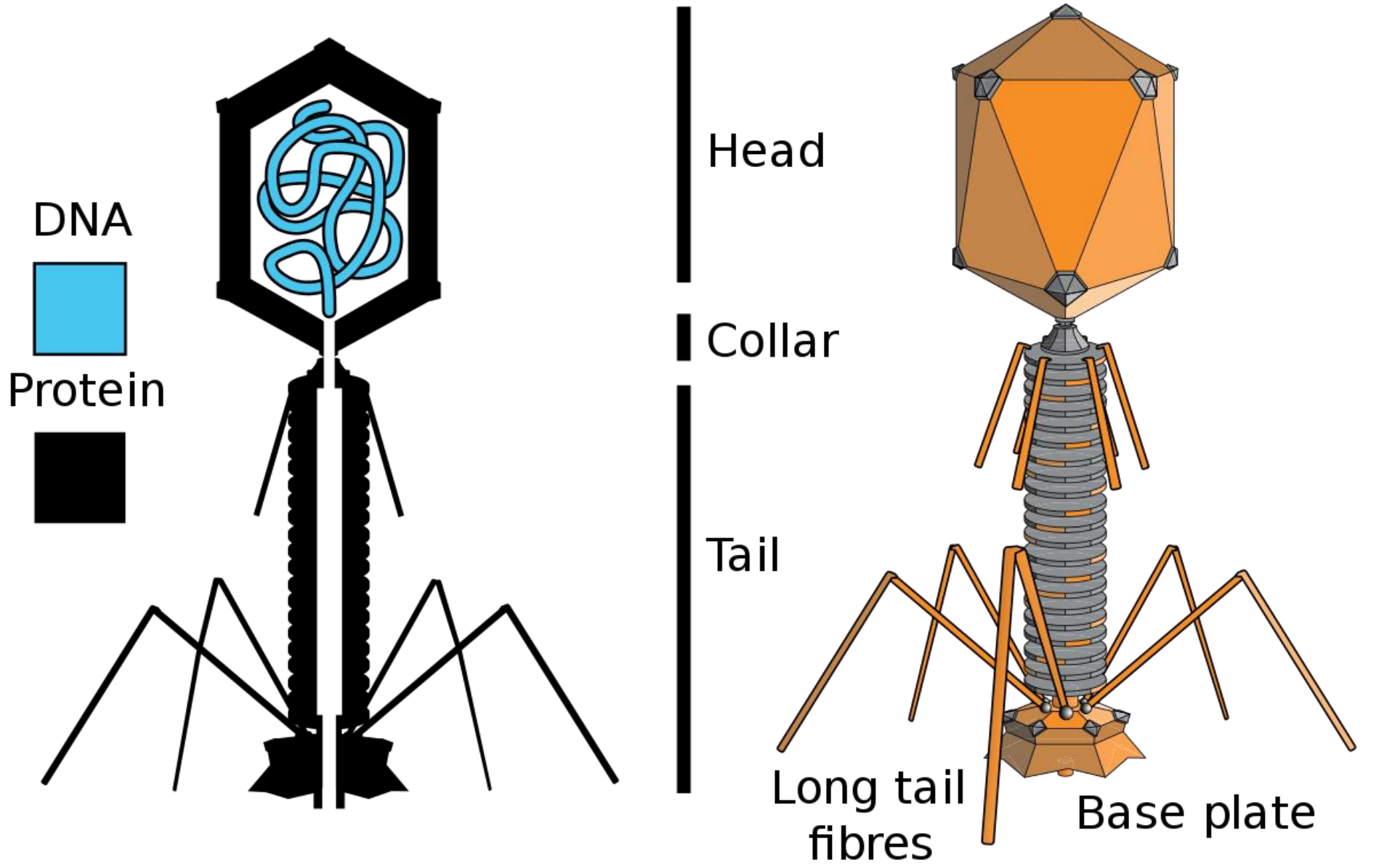
- Bakteriyosinler (lantibiyotikler, nisin)
- Esansiyel Yağlar
- Bakteriyofaj Tedavisi
- Quarum-Sensing inhibitörleri
- Antikorlar
- Nanoteröpatik ajanlar

Bakteriyofaj

- Bakteriyofaj kısaca faj olarak da bilinir
- Archaea ve bakterileri infekte edebilen virus
- Protein yapısında bir kapsül
- İçerisinde DNA veya RNA genomunu
- Bakterilerin olduğu her ortamda bulunur
- Yeryüzünde 10^{31} ’den daha fazla bakteriyofaj
- GRAS status by FDA in 2006



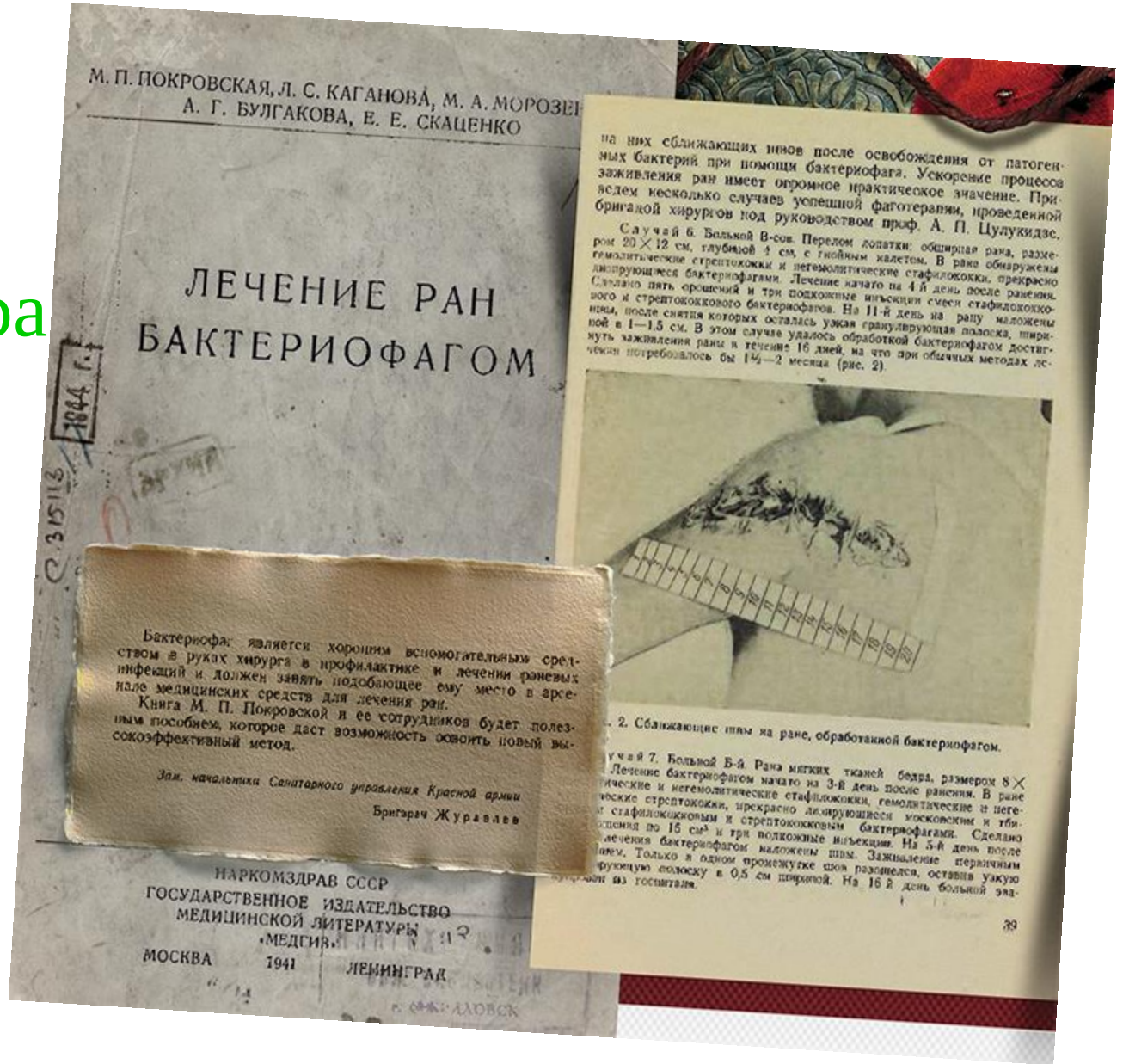



DNA
Protein
Head
Collar
Tail
Long tail fibres
Base plate
2D
3D

Bulunduğu Yerler

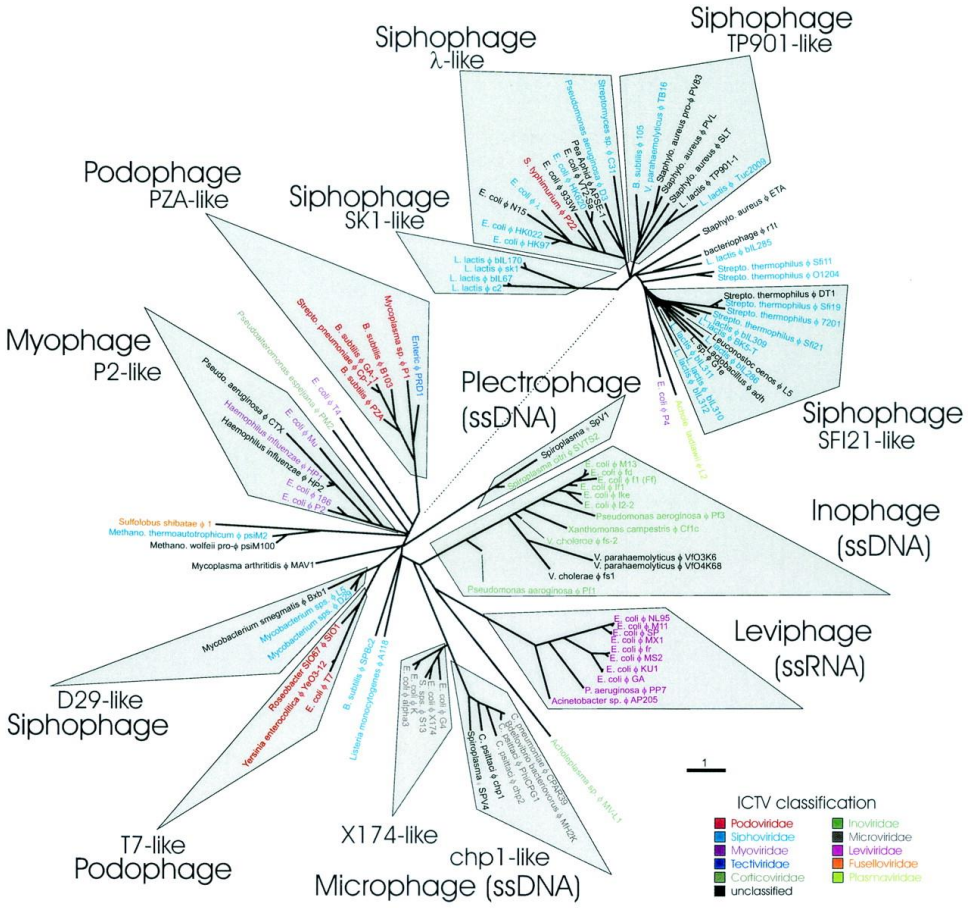
- Deniz suyu bakterilerinin %70'inde
- Tatlı su birikintilerinde
- Paspaslarda (9×10^8 viryon/mL)
- Yaygın olarak toprakta ve hayvanların sindirim sisteminde bulunur.

- Doksan yıldır antibiyotiklere alternatif olarak kullanılmakta
- Eski SCCB (Rusya), Orta Avrupa ve Fransa'da..
- Deri ve yumuşak doku infeksiyonlarının tedavisi
- Son dönemlerde MDR bakterilerin tedavisinde

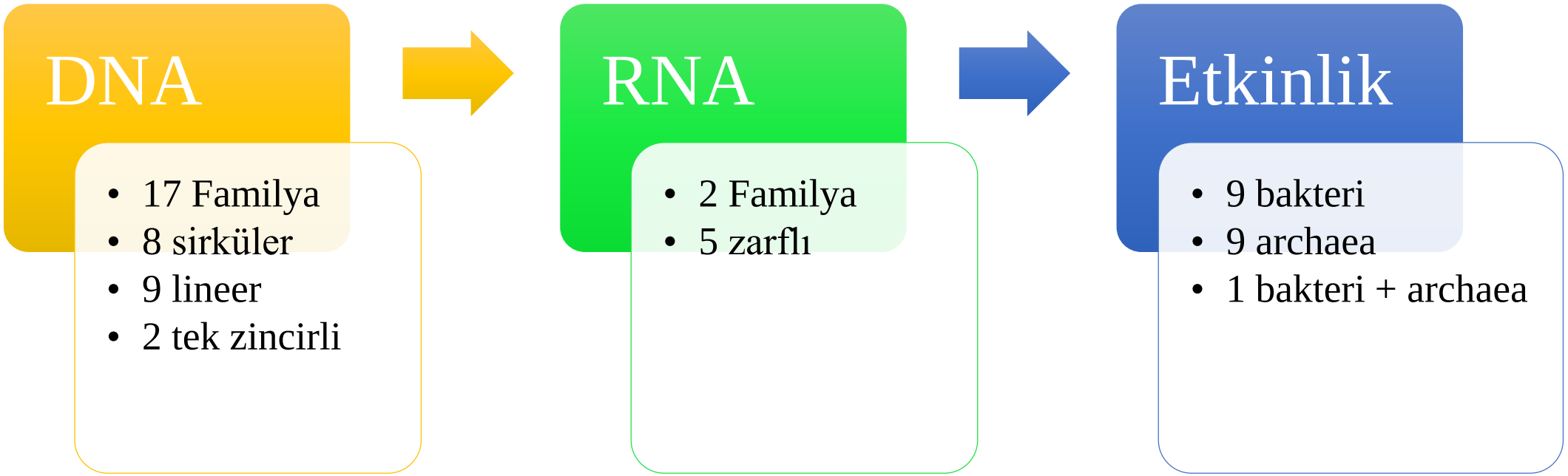


Sınıflama

- Farklı viryon yapıları, genomlar ve yaşam şekliyle
- International Committee on Taxonomy of Viruses (ICTV)
- Morfoloji ve nükleik asid yapısına göre 19 familya
- İki familya RNA içerir, 5 familya zarflı



Sınıflama


DNA
17 Familya
8 sirküler
9 lineer
2 tek zincirli
RNA
2 Familya
5 zarflı
Etkinlik
9 bakteri
9 archaea
1 bakteri + archaea

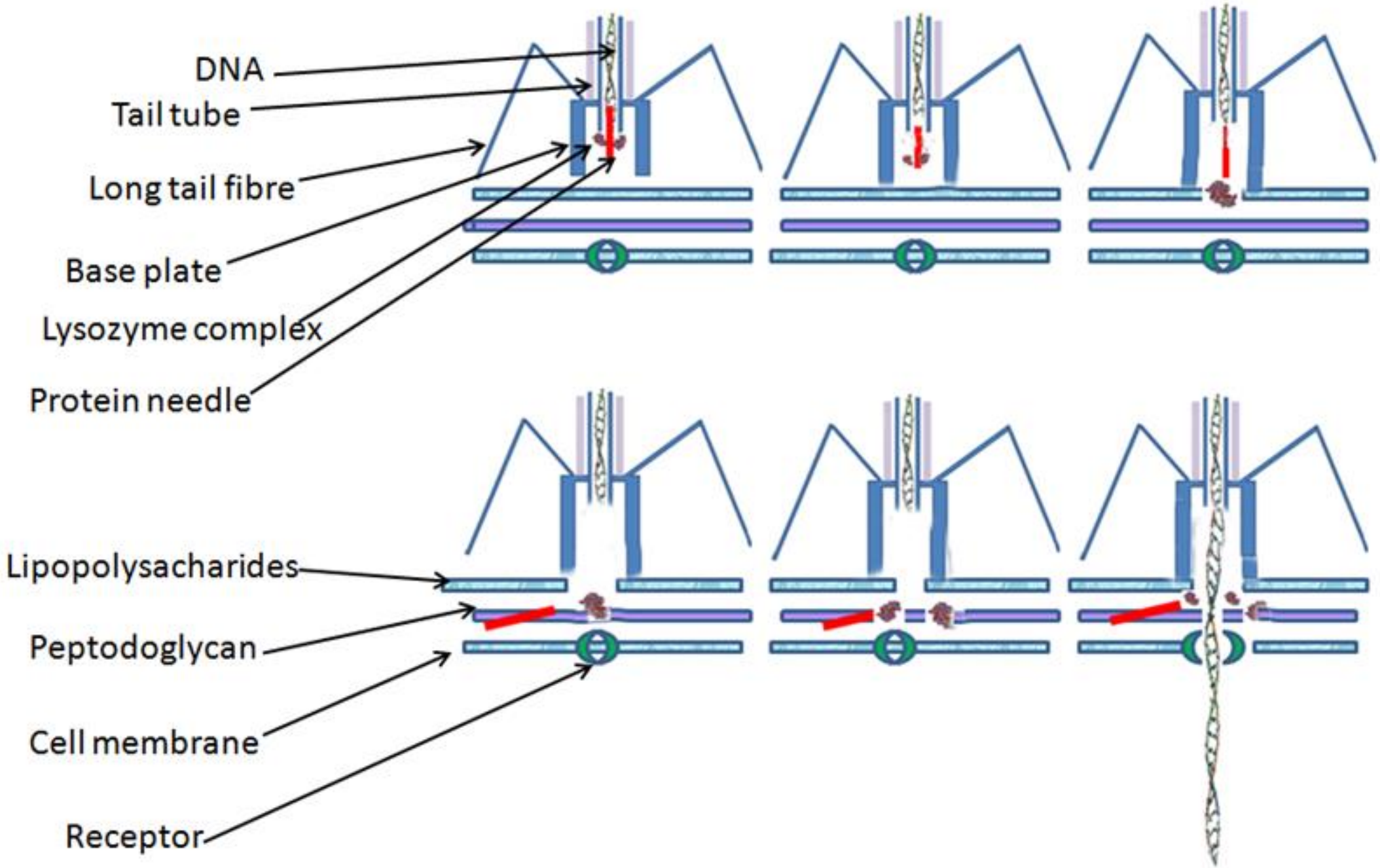
ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]				
Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	T7 phage, T3 phage, Φ29, P22, P37

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]				
Order	Family	Morphology	Nucleic acid	Examples
Ligamenvirales	*Lipothrixviridae*	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
	Ampullaviridae	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	Nonenveloped, rod-shaped	Circular dsDNA	
	Corticoviridae	Nonenveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Globuloviridae	Enveloped,	Linear	

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]				
Order	Family	Morphology	Nucleic acid	Examples
Unassigned	*Globuloviridae*	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	Nonenveloped, ovoid	Circular dsDNA	
	Inoviridae	Nonenveloped, filamentous	Circular ssDNA	M13
	Leviviridae	Nonenveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	Nonenveloped, isometric	Circular ssDNA	ΦX174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	Nonenveloped, isometric	Linear dsDNA	

Bağlanma ve Giriş

- Konak hücreye girmek için bakteri yüzeyindeki özgül reseptörler
  - Lipopolisakkaritler
  - Teikokik asitler
  - Proteinler
- Bir bakteriyofaj ancak bağlanabileceği spesifik reseptör taşıyan bakterileri infekte edebilir
- Faj virionları hareketsiz olduklarından solüsyonda rastlantısal olarak reseptör bağlantısı gerçekleşir

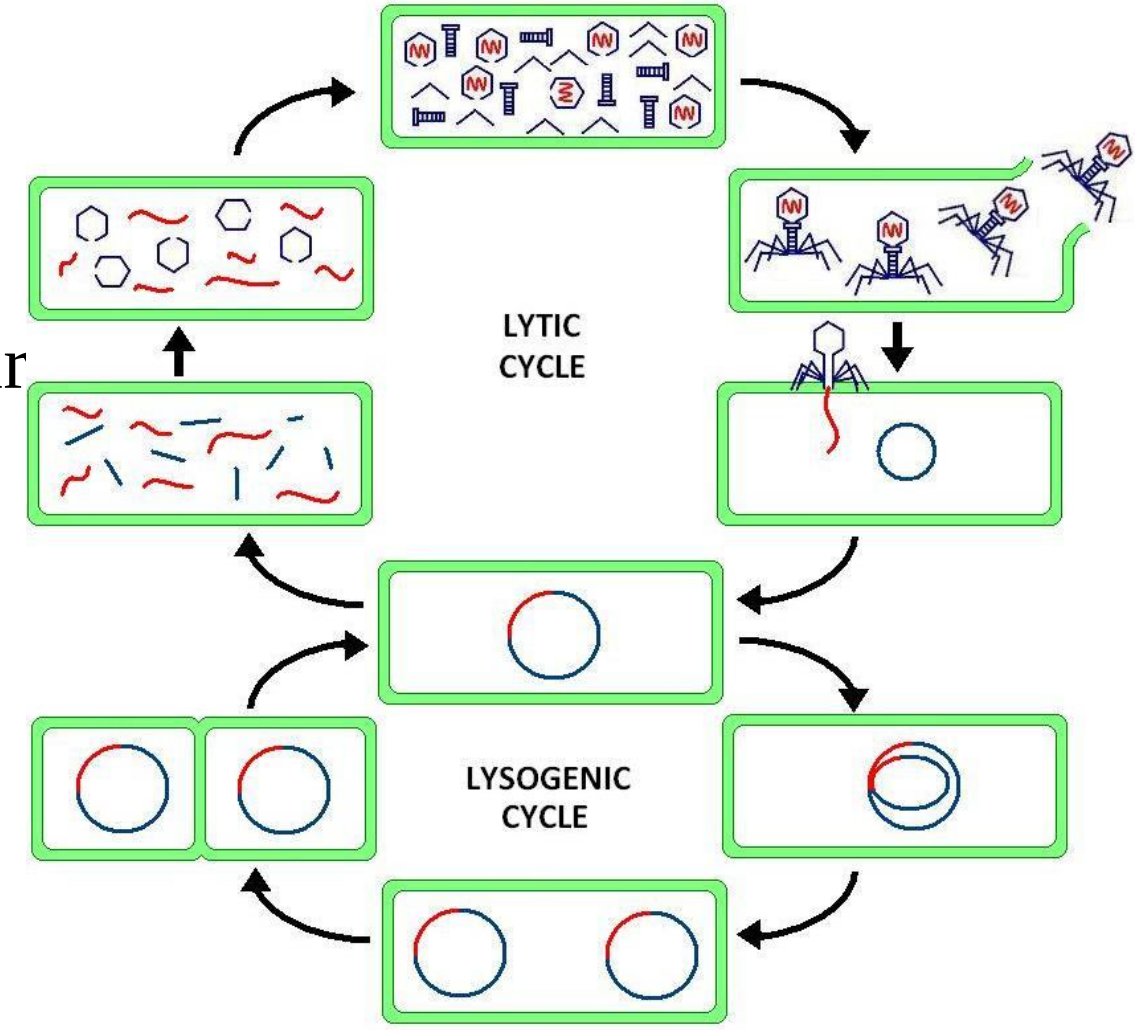

DNA
Tail tube
Long tail fibre
Base plate
Lysozyme complex
Protein needle
Lipopolysacharides
Peptodoglycan
Cell membrane
Receptor

Replikasyon

- Litik ve Lizojenik döngüye sahipler
- T4 faj gibi litik fajler bakteride çoğalıp hızla bakteriyi harap eder
- Daha sonra faj yeni bir bakteriyi infekte eder
- Litik fajlar tedavi için daha uygun özellik gösterir
- Lizis inhibisyonu, hücre dışı faj konsantrasyonu çok yüksekse hücre içi lizis yapan fajların dışarı çıkışı gecikir.

Lizojenik Faz

- Konak hücrenin hızla parçalanmasına neden olmaz
- Bunlara ılımlı faj (*temperate phage*) denir
- Konak DNA'sı ile integre olur ve zararsızdır (dormant)
- Konak DNA'sıyla birlikte eşlenir
- Plazmid gibi davranır



Lizojenik Faz

- Konak şartları bozulup besin azalınca faj aktiflenir
- Çoğalma süreci
- Konak hücre parçalanması
- Lizojenik döngü konak hücrenin çoğalmasına izin verir
- Böylece ana konak dışında yavru hücrelere entegre şekilde çoğalır
- *E.coli* lambda fajı buna bir örnektir

Filamentous Bacteriophage Promote Biofilm Assembly and Function

Patrick R. Secor,[1,*] Johanna M. Sweere,[2,3] Lia A. Michaels,[1] Andrey V. Malkovskiy,[2] Daniel Lazzareschi,[2] Ethan Katznelson,[2] Jayakumar Rajadas,[2] Michael E. Birnbaum,[3] Allison Arrigoni,[4] Kathleen R. Braun,[4] Stephen P. Evanko,[4] David A. Stevens,[5] Werner Kaminsky,[6] Pradeep K. Singh,[1] William C. Parks,[7,8] and Paul L. Bollyky[2,8]

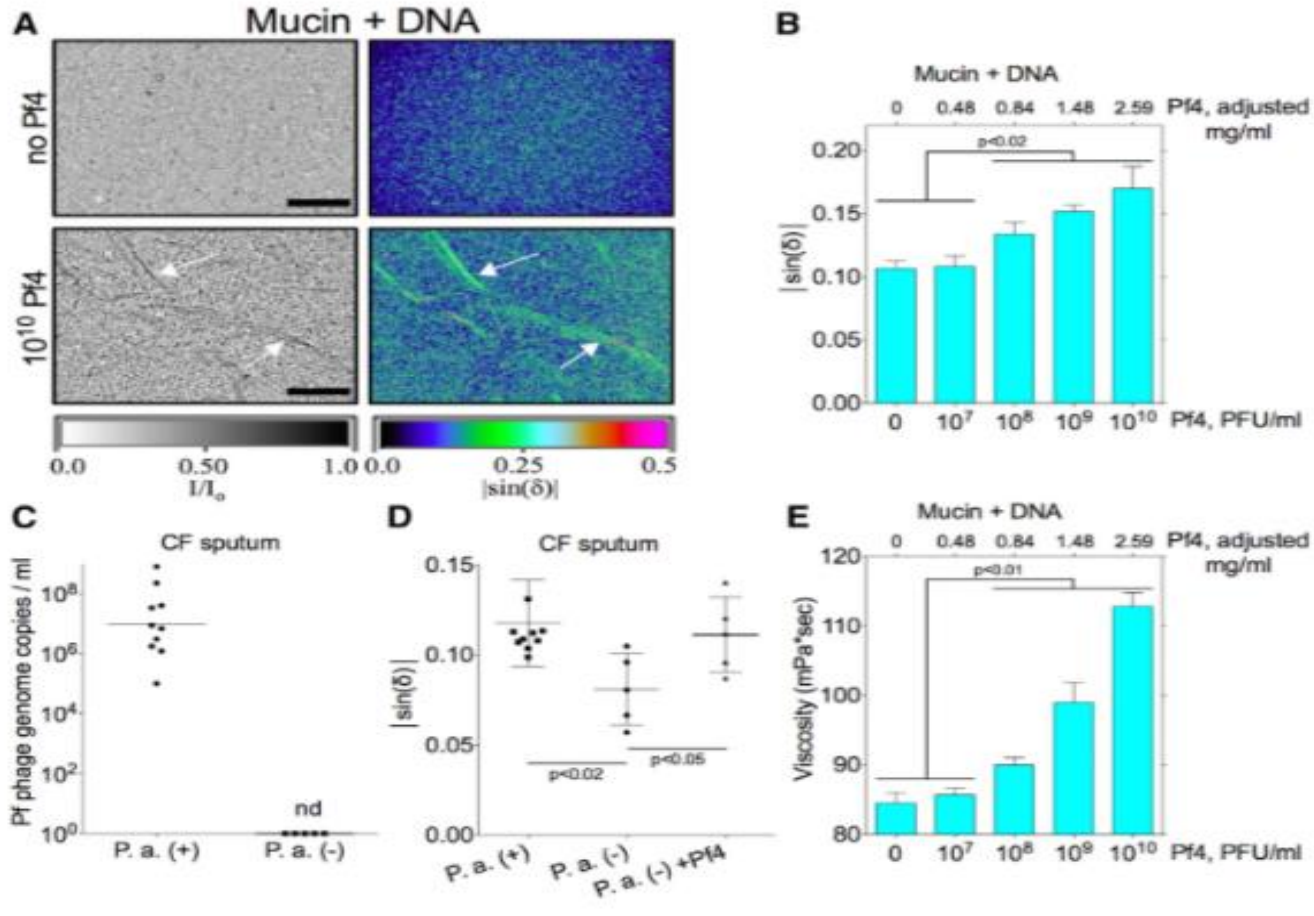


Figure 3. Pf4 Assembles Liquid Crystalline Structures in the Presence of Disease Relevant Polymers and Increases Viscosity

(A) Representative images of mixtures of mucin (8% solids) and DNA (HMW, 4 mg/ml) in the presence or absence of Pf4. Transmitted light is displayed as I/I_o where I represents intensity of light emerging from the sample and I_o represents intensity of incident light. Birefringence is displayed as $|\sin(\delta)|$. Arrows indicate filament assembly. Scale bars, 200 μm.

(B) Birefringence was quantified as $|\sin(\delta)|$ in mixtures of mucin and DNA described in (A) supplemented with increasing amounts of Pf4. Results are mean ± SD of three experiments.

(C) Pf phage were quantified by qPCR in sputum collected from CF patients infected by *P. aeruginosa* (P. a. (+), n = 10) or patients not infected by *P. aeruginosa* (P. a. (−), n = 5). Results are plotted as the geometric mean; nd, not detected.

(D) The birefringence ($|\sin(\delta)|$) of sputum samples described in (C) was quantified. The addition of 10^8 PFU/ml Pf4 to P. a. (−) sputum augmented birefringence. Results are mean ± SD.

(E) Changes in the viscosity (mPa•s) of mucin + DNA samples described in (A) were measured after supplementation with Pf4. Results are mean ± SD of four experiments.

See also Figure S4.

Filamentous Bacteriophage Promote Biofilm Assembly and Function

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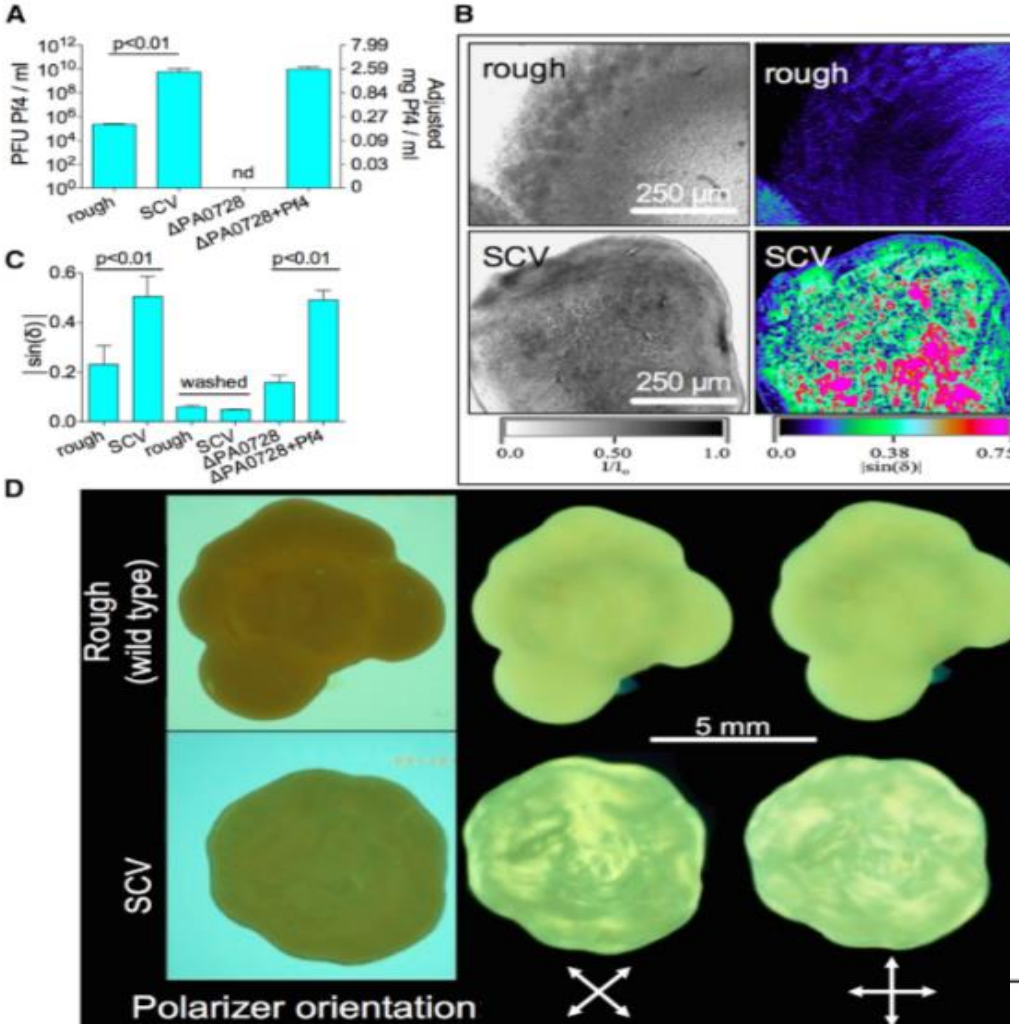
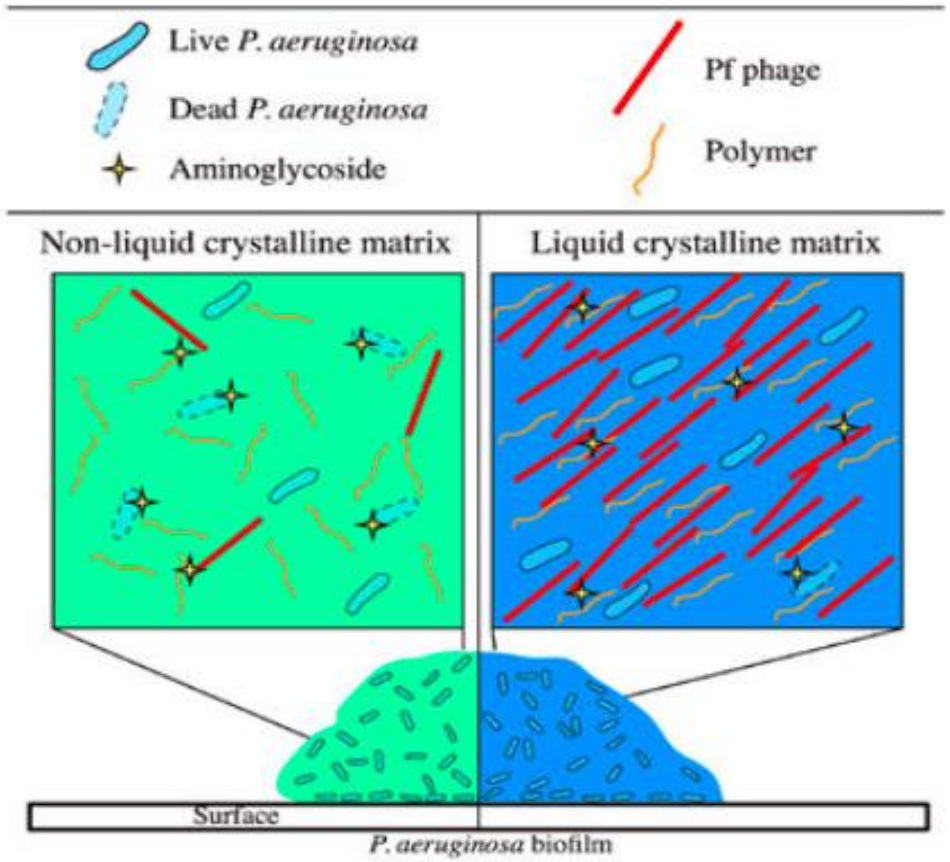
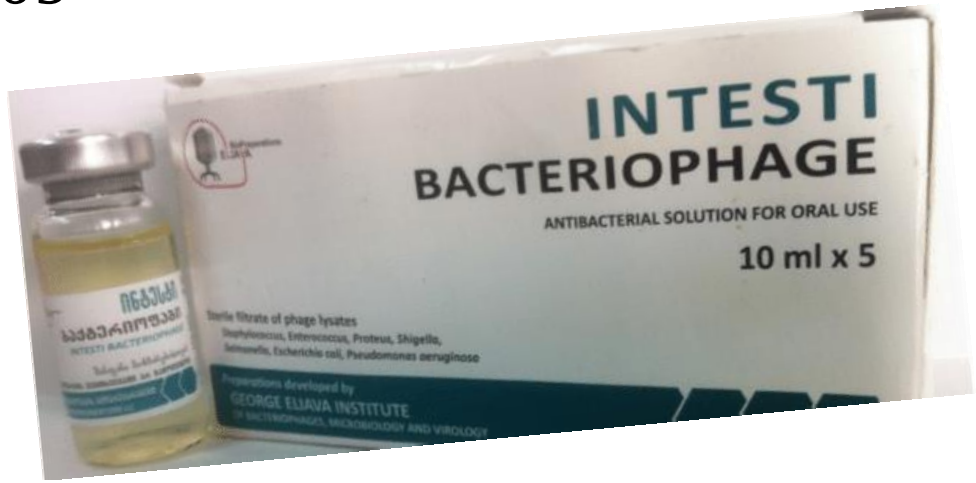




Klinik Kullanımı

- Soğuk savaş döneminde fajların kullanımı Gürcistan'da, Orta ve Doğu Avrupa ülkelerinde de devam etti.
- Randomize, çift kör ilk klinik çalışma 2009 yılında yapıldı.
- Bu çalışma ayağında infekte venöz ülseri bulunan bir hastada gerçekleştirildi.
- FDA bu çalışmayı Faz 1 çalışması olarak onayladı.



Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial

- **Objective:** This phase I trial set out to examine the safety of a bacteriophage-based preparation for difficult-to-treat wounds.
- **Method:** The intention-to-treat sample comprised 42 patients with chronic venous leg ulcers (VLUs); 39 patients completed the trial. The ulcers were treated for 12 weeks with either a saline control or bacteriophages targeted against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Follow-up continued until week 24.
- **Results:** No adverse events were attributed to the study product. No significant difference ($p>0.05$) was determined between the test and control groups for frequency of adverse events, rate of healing, or frequency of healing.
- **Conclusion:** This study found no safety concerns with the bacteriophage treatment. Efficacy of the preparation will need to be evaluated in a phase II efficacy study.
- **Declaration of interest:** One of the authors (AS) holds an equity interest in Intralytix. The other authors do not have any interest in commercial activities.

chronic venous leg ulcer; macrophage therapy; safety study

Managing the bacterial population of a wound presents significant challenges to the practitioner. Chronic wounds commonly contain biofilms.[1] However, the phenotype of biofilm communities is not fully responsive to antibiotics[2] and a growing number of wound pathogens are genotypically resistant to antimicrobials. Novel antimicrobial agents that can overcome these resistant bacteria need to be investigated so that better wound management regimens can be developed.

antimicrobial agents is available elsewhere.[5-7]

Although phage therapy is widely used and generally accepted as safe and beneficial in some parts of the world, very few controlled human trials have investigated it in the West.[5] Recent (scarce) reports of the use of phages to treat wound infections have focused on:

- Experimentally infected animals[8]
- Humans with chronic, suppurating, bacterial skin infections[9]
- Use of PhagoBioDerm in humans.[3,4]

D.D. Rhoads,[1] MT (ASCP), Laboratory Researcher;
R.D. Wolcott,[1] MD, Director;
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L.S. Ward, PhD, Director

A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy

Wright, A.*, Hawkins, C.H.†, Ånggård, E.E.† & Harper, D.R.†

*UCL Ear Institute and Royal National Throat, Nose and Ear Hospital, Grays Inn Road, London, and
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Accepted for publication 29 April 2009
Clin. Otolaryngol. 2009, **34**, 349–357

Objectives: To evaluate the efficacy and safety of a therapeutic bacteriophage preparation (Biophage-PA) targeting antibiotic-resistant *Pseudomonas aeruginosa* in chronic *otitis*.

Design: Randomised, double-blind, placebo-controlled Phase I/II clinical trial approved by UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review process.

Setting: A single specialist university hospital.

Participants: 24 patients with chronic otitis with a duration of several years (2–58). Each patient had, at the time of entry to the trial, an ear infection because of an antibiotic-resistant *P. aeruginosa* strain sensitive to one or more of the six phages present in Biophage-PA. Participants were randomised in two groups of 12 treated with either a single dose of Biophage-PA or placebo and followed up at 7, 21 and 42 days after treatment by the same otologist. Ears were thoroughly cleaned on each occasion and clinical and microbiological indicators measured.

Visual Analogue Scale (VAS). Patients reported discomfort, itchiness, wetness and smell also using a VAS. Bacterial levels of *P. aeruginosa* and phage counts from swabs were measured initially and at follow-up. At each visit patients were asked about side effects using a structured form. Digital otoscopic images were obtained on days 0 and 42 for illustrative purposes only.

Results: Relative to day 0, pooled patient- and physician-reported clinical indicators improved for the phage treated group relative to the placebo group. Variation from baseline levels was statistically significant for combined data from all clinic days only for the phage treated group. Variation from baseline levels was statistically significant for the majority of the patient assessed clinical indicators only for the phage treated group. *P. aeruginosa* counts were significantly lower only in the phage treated group. No treatment related adverse event was reported.

Conclusion: The first controlled clinical trial of a therapeutic bacteriophage preparation showed efficacy

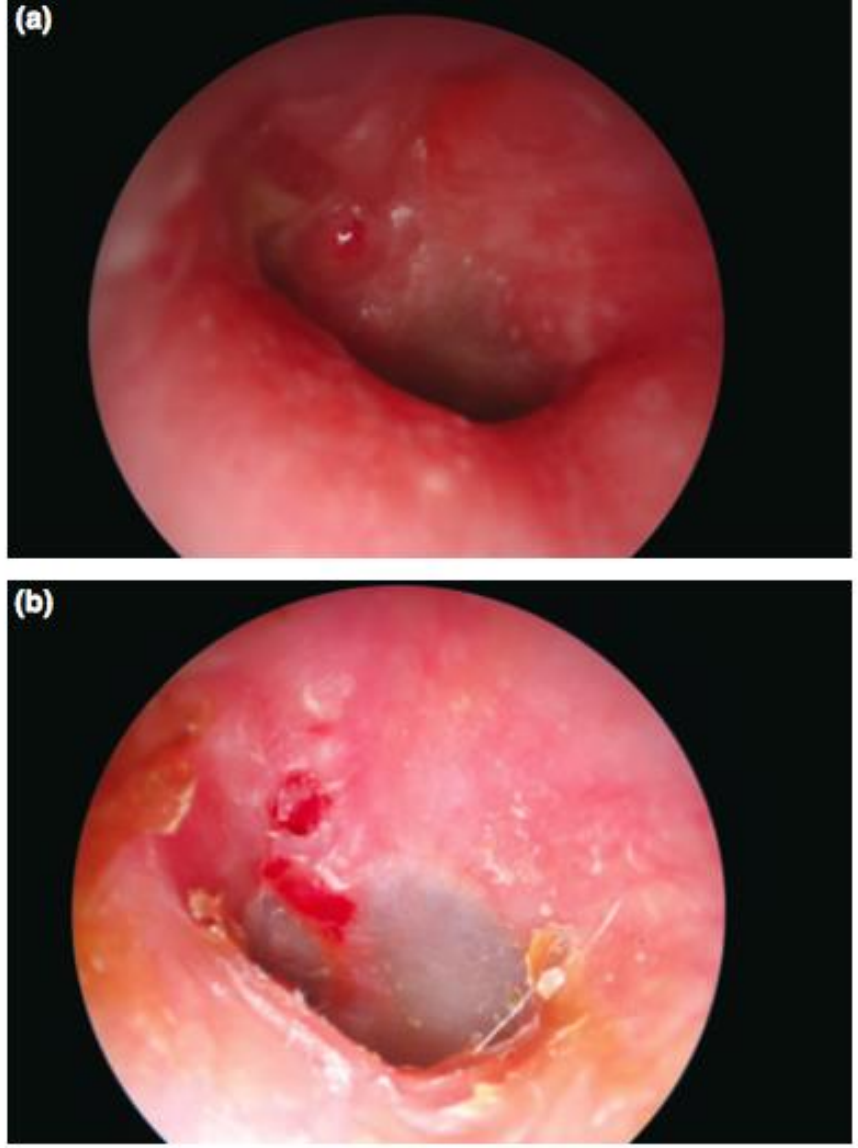
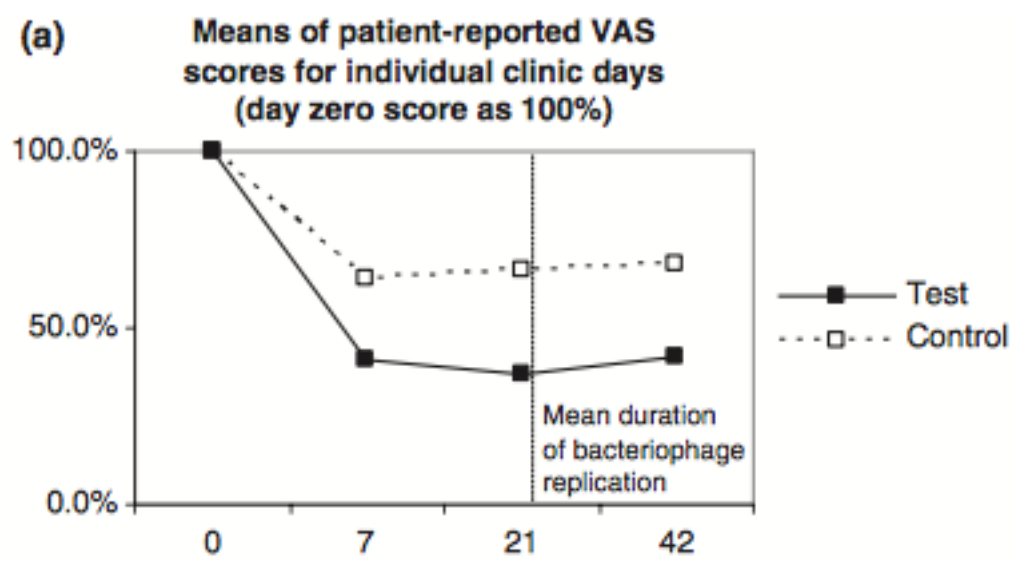
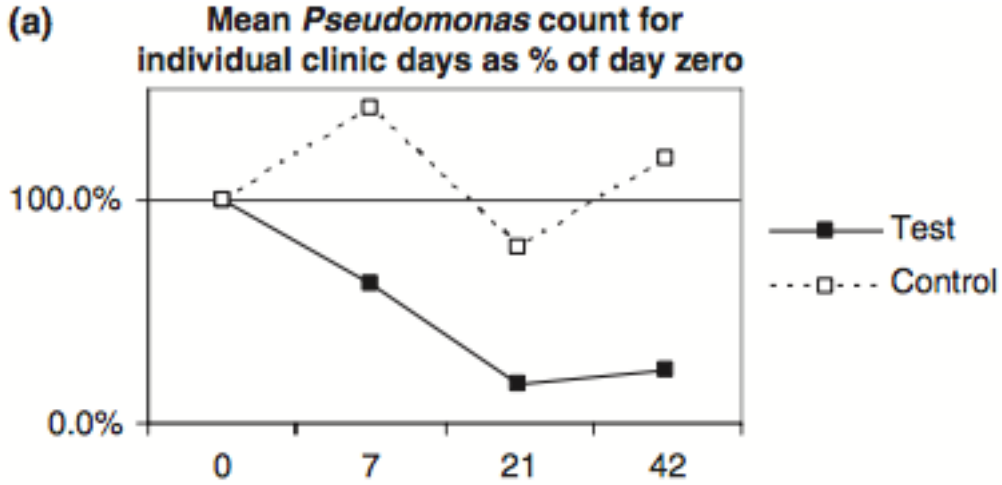


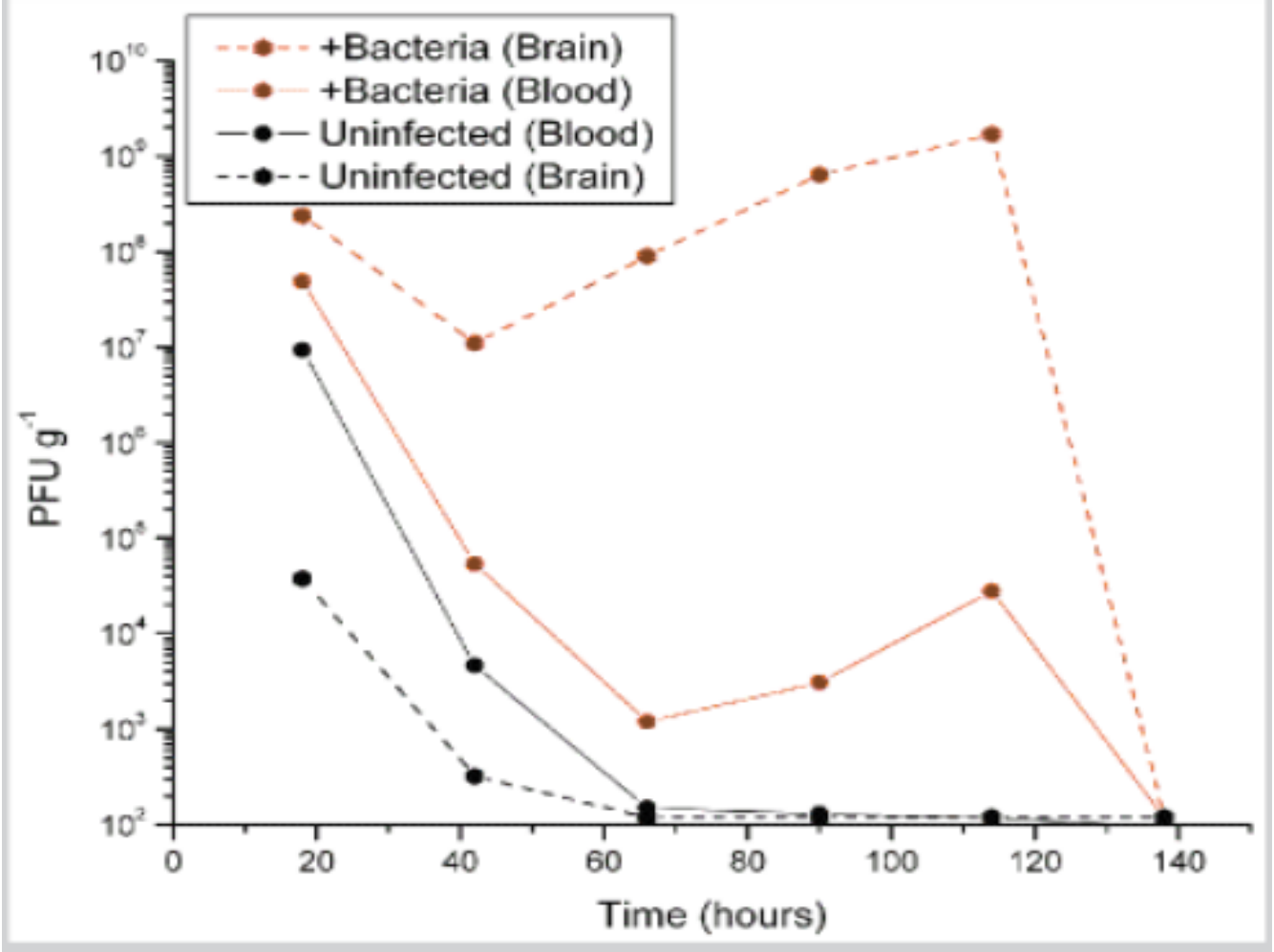
Fig. 5. Digital photography before (a) and after (b) in patient A0012. Patient A0012 had a longstanding resistant otitis externa. There was swelling of the deep canal skin and ulceration in the roof of the canal adjacent to the tympanic membrane. By day 42 this had resolved and the patient was essentially symptom free.





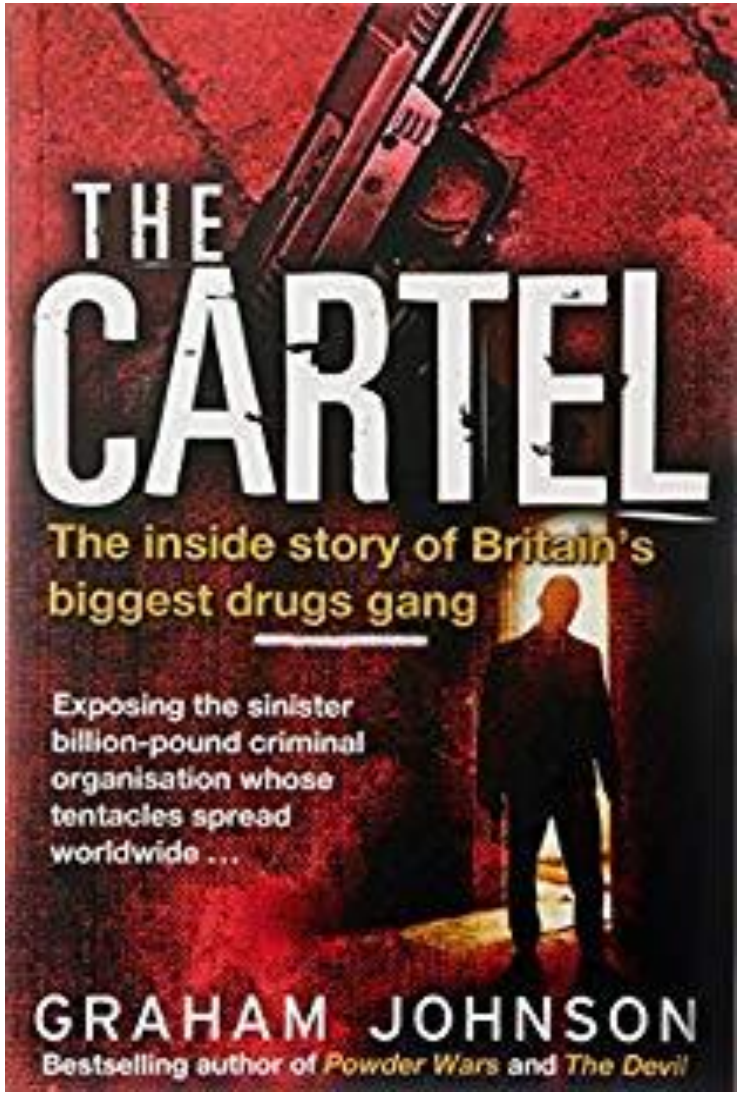
Bakteri Bulunmadığında Bakteriyofaj Sorun Teşkil Eder mi?

- Rene Dubos, 1943
- *S.aureus* (+)/(-) fareler
- İntraperitoneal spesifik bakteriyofaj
- Kan ve beyin dokusunda farklı konsantrasyon ve tam etki..



Klinik Kullanımı Neden Yaygınlaşmadı?

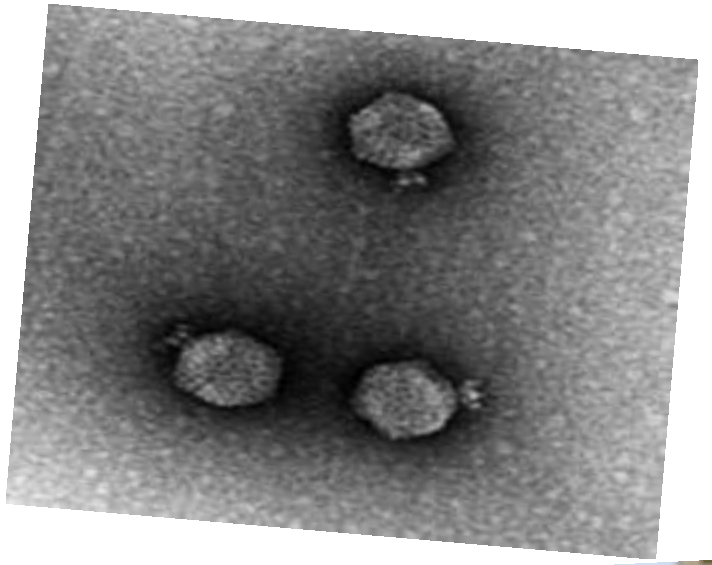
- Batıda kullanılmamasının birkaç nedeni var:
- Antibiyotikler kısa süre içerisinde bulunup, marketlerde yerini aldı. Bunların yapımı, depolanması ve kullanımı daha pratik ve kolaydı
- Fajla ilgili çeşitli medikal girişimler yapıldı, ancak bunların geçerliliği konusunda soru işaretleri hiç giderilemedi.
- Uluslararası alanda Eski Sovyetler Birliği'nde gerçekleştirilen ve Rusça ya da Gürcü diliyle yazılan makaleler yeterince anlaşılamadı ve geniş okuyucu kitlesine ulaşmadı.



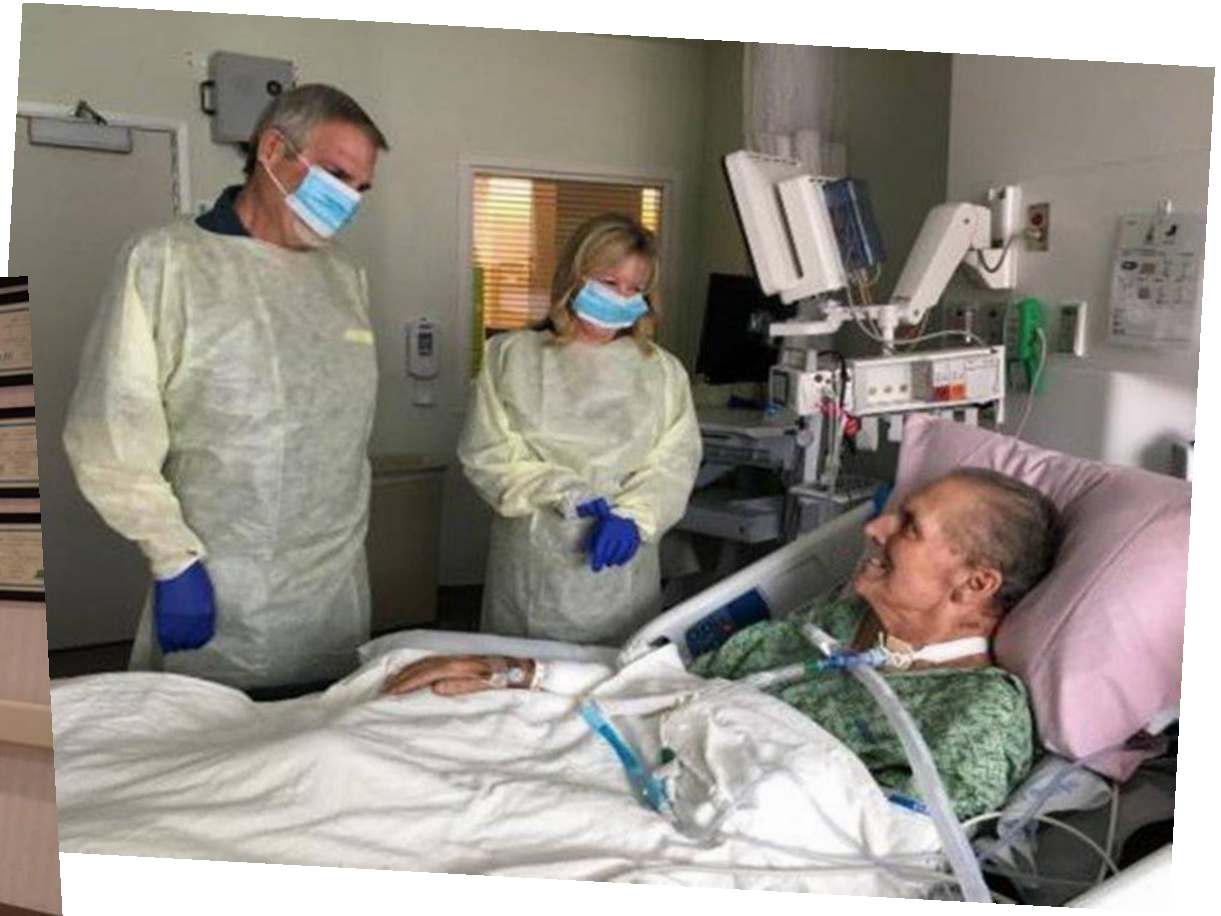
2015 University of California, San Diego

Professor Tom Patterson
Professor Steffanie Strathdee,

(MDR *Acinetobacter baumanii* bakteremi)



(MDR *Acinetobacter baumanii* bakteremi)



İntravenöz ve intraabdominal kavitenin faj tedavisine FDA onayı

Tedavinin üçüncü gününde tam yanıt

Bacteriophages: a promising approach to fighting antibiotic-resistant bacteria

By Laura H. Kahn, October 9, 2018

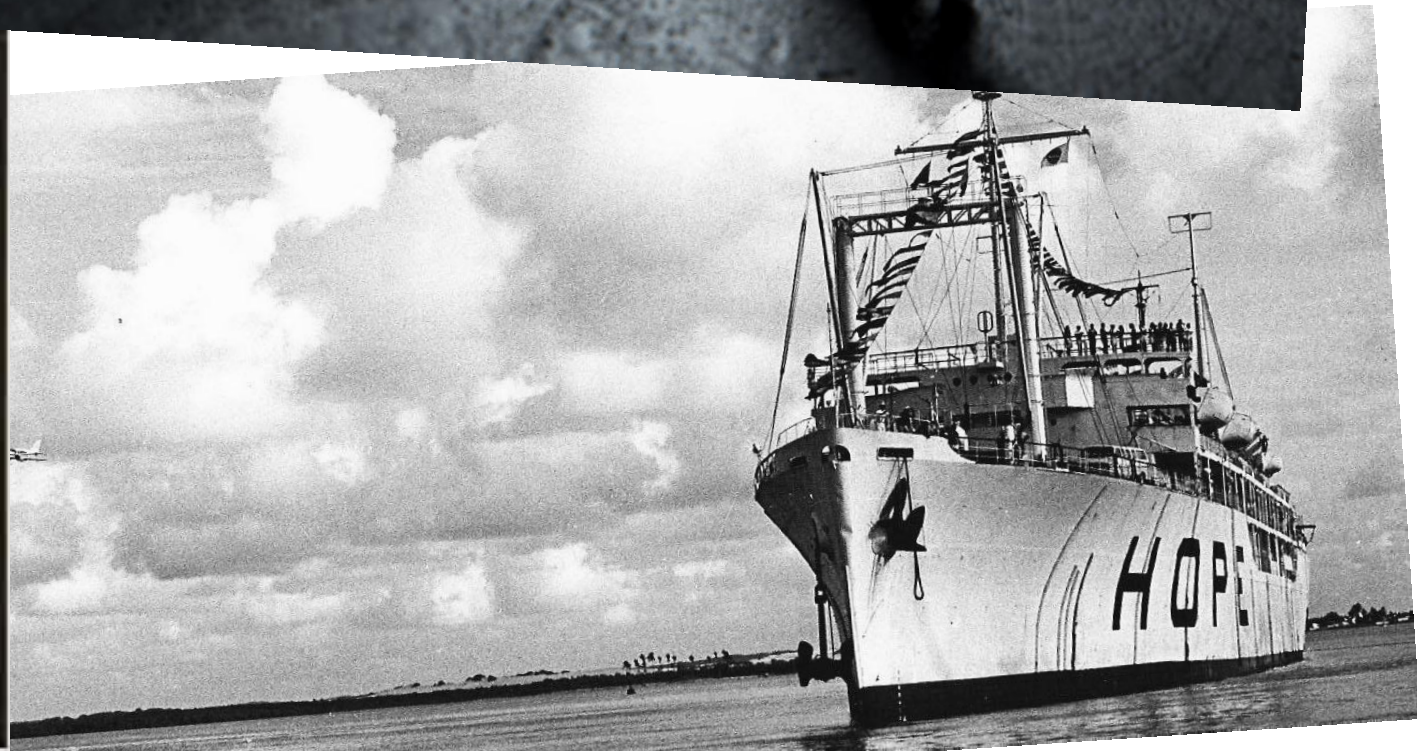
Professor Tom Patterson
Professor Steffanie Strathdee,

Hope in the Dark

Untold Histories, Wild Possibilities

REBECCA SOLNIT

WITH A NEW FOREWORD AND AFTERWORD



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Isolation, Characterization, and Antibacterial Activity of Bacteriophages Against Methicillin-Resistant *Staphylococcus aureus* in Pakistan

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Abstract

Background: In recent years, antibiotic resistance has been indicated as a paramount threat to public health. The use of bacteriophages appears to be a safer alternative for the control of bacterial infections.
Objectives: The present study aims to explore sewage water for the presence of indigenous bacteriophages, and to investigate their antibacterial potential against Methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: Bacterial isolates were first collected and identified from pus samples taken from the surgical and burn units using standard microbiological procedures. A cefoxitin disk screen test was then used and interpreted according to the clinical laboratory standards institute (CLSI) guidelines for the detection of MRSA. The sewage samples were processed and the phages enriched using *S. aureus* as a host organism. Turbid and clear plaques of different sizes were isolated using an overlay method, purified, and then enumerated by means of a dilution method.
Results: The phages exhibited good lytic activity against MRSA when tested in-vitro, and the highest activity was attained within three to six hours of phage infection. The isolated phage pq/48 was also found efficient in decreasing the bacterial count during an in-vivo trial in rabbits. A protein analysis using SDS-PAGE revealed 10 proteins of between 20 kDa and 155 kDa in size.
Conclusions: The overall results indicated that bacteriophages isolated from sewage exhibited excellent lytic activity against MRSA strains. In conclusion, bacteriophages can be further characterized and appear to be a promising candidate for phage therapy against MRSA in the future.

Keywords: MRSA, Bacteriophage, SDS-PAGE, Clear Plaques, In-Vitro Efficacy, In-Vivo Model

1. Background

The extensive use of antibiotics for nutritive and therapeutic purposes is linked to the progression of antibiotic resistance, which has been declared one of the most pressresistant *S. aureus* (MRSA) is more difficult to arrest due to its resistance to the antibiotics that are usually recommended. Not only is it resistant to beta-lactam antibiotics, it also shows resistance to fluoroquinoles, aminoglycosides, and glycopeptides (3). This remarkable potential

The Bacteriophage EF-P29 Efficiently Protects against Lethal Vancomycin-Resistant *Enterococcus faecalis* and Alleviates Gut Microbiota Imbalance in a Murine Bacteremia Model

Mengjun Cheng[1†], Jiaming Liang[2†], Yufeng Zhang[1], Liyuan Hu[1], Pengjuan Gong[1], Ruopeng Cai[1], Lei Zhang[1], Hao Zhang[1], Jinli Ge[1], Yalu Ji[1], Zhimin Guo[3], Xin Feng[1], Changjiang Sun[1], Yongjun Yang[1], Liancheng Lei[1], Wenyu Han[1,4] and Jingmin Gu[1]**

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Enterococcus faecalis is becoming an increasingly important opportunistic pathogen worldwide, especially because it can cause life-threatening nosocomial infections. Treating *E. faecalis* infections has become increasingly difficult because of the prevalence of multidrug-resistant *E. faecalis* strains. Because bacteriophages show specificity for their bacterial hosts, there has been a growth in interest in using phage therapies to combat the rising incidence of multidrug-resistant bacterial infections. In this study, we isolated a new lytic phage, EF-P29, which showed high efficiency and a broad host range against *E. faecalis* strains, including vancomycin-resistant strains. The EF-P29 genome contains 58,984 bp (39.97% G+C), including 101 open reading frames, and lacks known putative virulence factors, integration-related proteins or antibiotic resistance determinants. In murine experiments, the administration of a single intraperitoneal injection of EF-P29 (4×10^5 PFU) at 1 h after challenge was sufficient to protect all mice against bacteremia caused by infection with a vancomycin-resistant *E. faecalis* strain (2×10^9 CFU/mouse). *E. faecalis* colony counts were more quickly eliminated in the blood of EF-P29-protected mice than in unprotected mice. We also found that exogenous *E. faecalis* challenge resulted in enrichment of members of the genus *Enterococcus* (family *Enterococcaceae*) in the guts of the mice, suggesting that it can enter the gut and colonize there. The phage EF-P29 reduced the number of colonies of genus *Enterococcus* and alleviated the gut microbiota imbalance that was caused by *E. faecalis* challenge. These data indicate that the phage EF-P29 shows great potential

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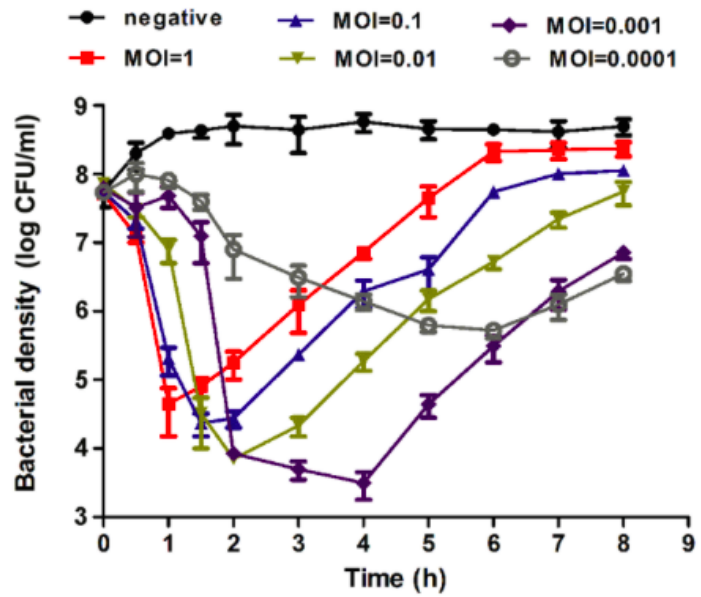

FIGURE 3 | Bactericidal activity of EF-P29 at different multiplicities of infection *in vitro*. The VREF 002 strain was lysed by phage EF-P29 in BHI medium at 37°C. CFU counts of the uninfected control culture and the parallel cultures that were infected with the phage at different MOI were measured over time. No phage was added in the negative control. The values represent the means and SD ($n = 3$).

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Critical Care

LETTER Open Access

Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury—a case report

Serge Jennes[1], Maia Merabishvili[2], Patrick Soentjens[3], Kim Win Pang[3], Thomas Rose[1], Elkana Keersebilck[1], Olivier Soete[1], Pierre-Michel François[1], Simona Teodorescu[1], Gunther Verween[2], Gilbert Verbeken[2], Daniel De Vos[2] and Jean-Paul Pirnay[2*]

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance, Colistin, Bacteraemia, Acute kidney injury, Intraven
Bacteriophage therapy

Sepsis from *Pseudomonas aeruginosa* bacteraemia may be fatal, especially when no appropriate therapy can be given.

In June 2016, a 61-year-old man was hospitalised for *Enterobacter cloacae* peritonitis and severe abdominal sepsis with disseminated intravascular coagulation, secondary to a diaphragmatic hernia with bowel strangulation. The patient had a prolonged hospital course complicated by gangrene of the peripheral extremities, resulting in the amputation of the lower limbs and the development of large necrotic pressure sores (Fig. 1).

Three months after admission, the patient was transferred to the Queen Astrid military hospital for surgical management of the pressure sores. Wound cultures on admission revealed colonisation with, amongst others, multidrug-resistant *P. aeruginosa*. One month after admission, the patient developed septicaemia with colistin-only-sensitive *P. aeruginosa*. Intravenous colistin therapy was started.

Ten days later, the patient developed acute kidney injury with rising serum creatinine and urea levels (Fig. 2), presumably sepsis- and drug-induced. Antibiotic therapy was discontinued to prevent further kidney damage. Unfortunately, on 12 November, *P. aeruginosa* septicaemia re-emerged with rapid heart rate, low blood pressure, fever and high C-reactive protein (CRP) leve
November, the patient went into a coma wit
(E2V2M5) on the Glasgow Coma Scale. Beca
risk of colistin nephrotoxicity and the famil
avoid intensive therapy interventions such a
tration, bacteriophage therapy was initiated
umbrella of Article 37 (Unproven Interv
Clinical Practice) of the Declaration of H
Fifty microlitres of purified bacteriopha
BFC1 [2], containing two bacteriophages t
in vitro activity against the patient's *P.*

Fig. 1 Large pressure sores in the sacral and spinal back areas. Situation on 17 November 2016

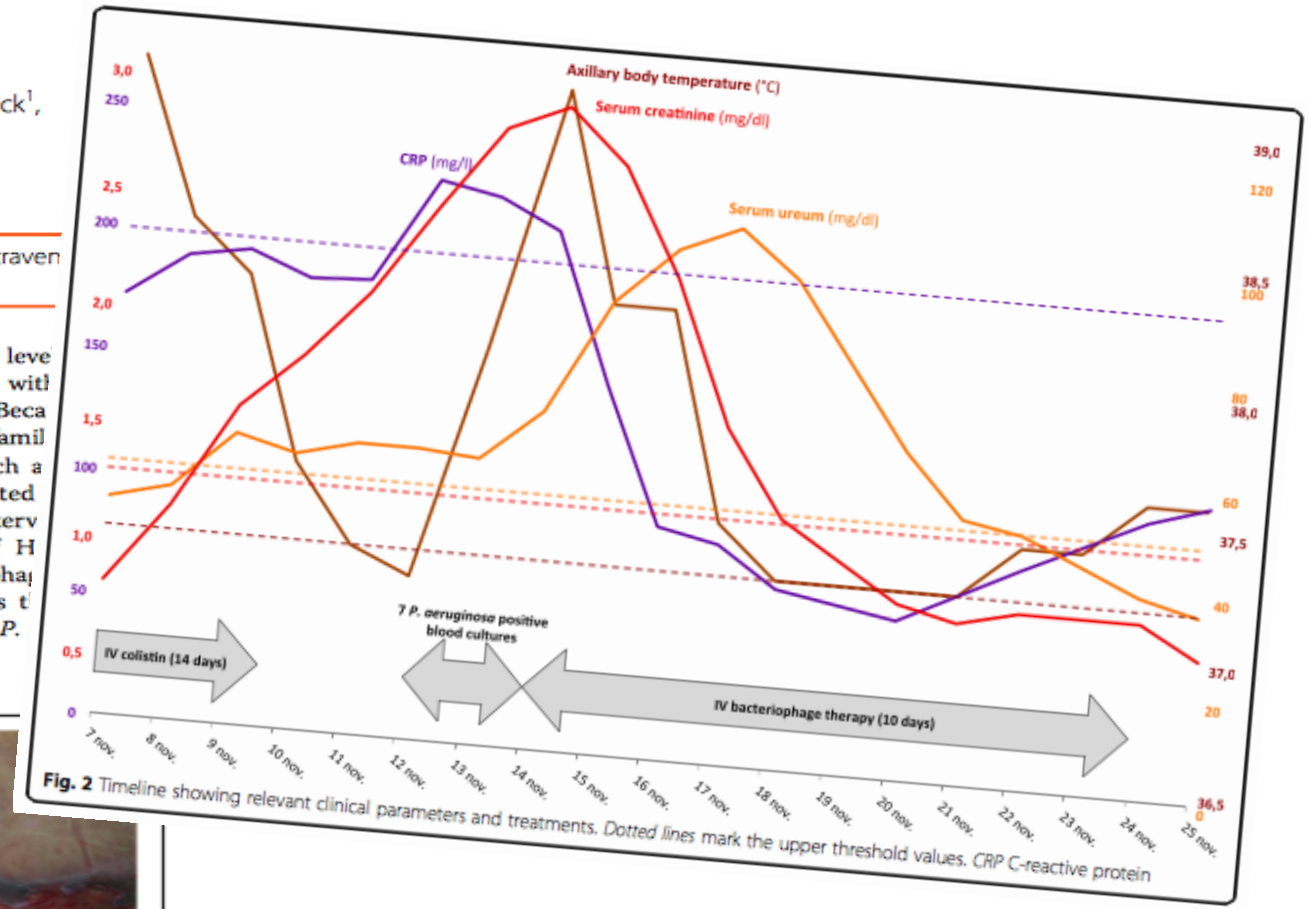

Fig. 2 Timeline showing relevant clinical parameters and treatments. *Dotted lines* mark the upper threshold values. *CRP* C-reactive protein

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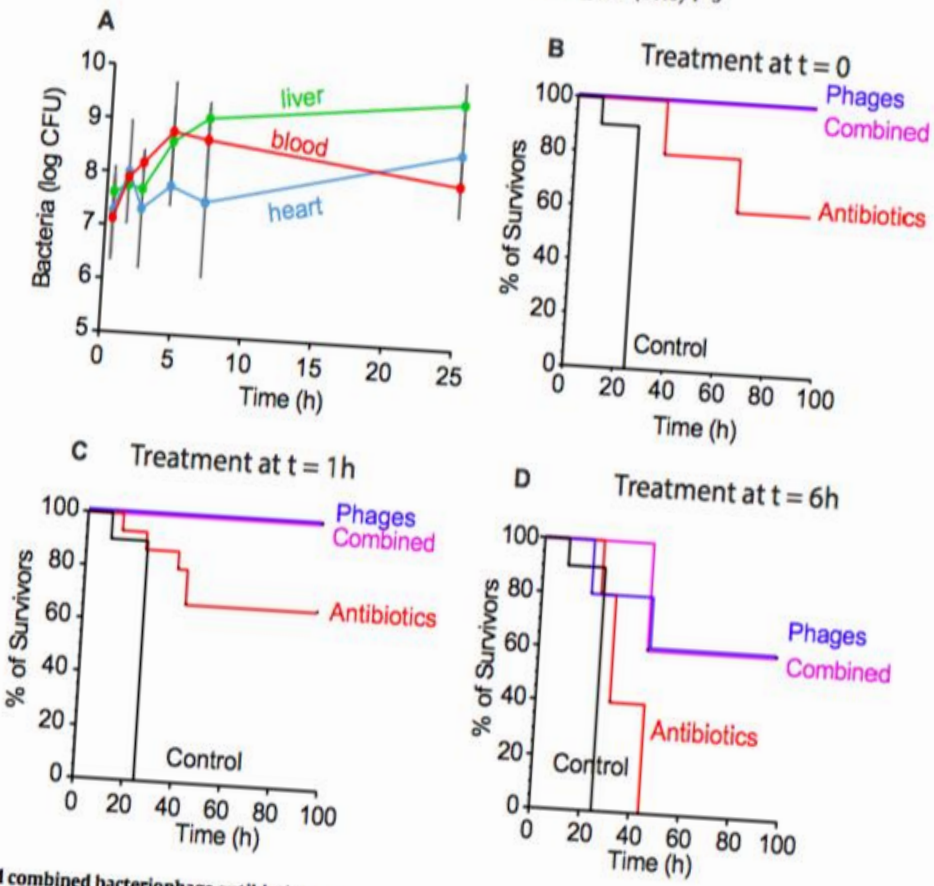

Fig. 1. Bacteriophage therapy and combined bacteriophage antibiotic treatment rescue mice suffering from fulminant septic peritonitis. Mice were induced with a peritonitis model by an intra-peritoneal injection of vancomycin-resistant *E. faecalis* (2×10^9 CFU). **(A) Establishment of bacterial dissemination.** Various tissues of mice sacrificed at several time points after the bacterial injection were analyzed for bacterial infiltration. Each time point represents two mice, except for t = 25 h, representing seven mice. The bacterial loads are represented by a logarithmic scale of CFU/g for the liver and heart samples, and by a logarithmic scale of CFU/ml for blood samples. The means and standard deviations are shown. **(B–D) Survival curves of mice induced with the Mouse Peritonitis Model.** The treated mice were divided into three groups: bacteriophage cocktail treatment (EFDG1-EFLK1, 5×10^7 PFU – blue), antibiotic treatment (12.5 mg/kg ampicillin-red), or combined bacteriophage-antibiotic treatment (-purple), with each of the treatments given in several time points relative to the bacterial injection. Mice induced with the peritonitis model with no further treatment (n = 10) are presented (-black). The survival rates (percentages) are shown in the graphs. The Mantel–Cox test was used to compare between the survival curves. **(B)** The mice were treated simultaneously to the bacterial injection (n = 5 in each treatment group). Significant differences were found between each of the treatments to the untreated mice ($p < 0.0004$). **(C)** The mice were treated 1 h after the bacterial injection (n = 15 in each treatment group). Significant differences were found between the bacteriophage treatment or the combined bacteriophage-antibiotic treatment to the antibiotic treatment ($p = 0.016$), and between the antibiotic treatment to the untreated mice ($p = 0.0003$). **(D)** The mice were treated 6 h after the bacterial injection (n = 5 in each treatment group). Significant differences were found between each of the treatments to the untreated mice ($p < 0.0185$), and between the combined bacteriophage-antibiotic treatment to the antibiotic treatment ($p = 0.0181$).

ELSEVIER

Research in Microbiology

Combined
against VR

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EFLK1
Mouse peritonitis

ng significant attention
e. Thus, clinically relevant
therapy given as rescue
cocktail consisting of two
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ction of the bacteriophage
by Vancomycin-Resistant
e and laboratory test re-
acteriophages with a sub-
tment success.

Bakteriyofajların Diğer Kullanım Amaçları

- Gıda Endüstrisi
- FDA ve USDA çeşitli bakteriyofajları onayladı (2007)
- LMP-102 (Intralytix) kümes hayvanları ve et ürünleri için
- LISTEX (by Microes) peynirlerde *Listeria monocytogenes* için
- Aynı bakteriyofaj 2007'de tüm gıda ürünleri için onaylandı
- Gıda güvenliğinde litik faj kullanımının yaygınlaşması

Tanı

- FDA 2011'de bakteriyofaj-kaynaklı in vitro tanı ürünü geliştirdi
- KeyPath MRSA/MSSA Kan kültürü testi
- Bakteriyofaj kokteyli kullanarak kan kültüründe MSSA ve MRSA tayini
- Test sonuçları 5 saatte alınıyor (2-3 gün yerine)
- Bu da FDA tarafından onaylanan ilk hızlı antiboyotik duyarlılık yöntemi

Rapid detection and identification of bacteria: SEnsing of Phage-Triggered Ion Cascade (SEPTIC)

M. Dobozi-King,[1] S. Seo, J.U. Kim, R. Young,[1] M. Cheng and L.B. Kish*

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Methods for the rapid detection and identification of bacteria are urgently needed. Here we describe a method that combines the specificity and avidity of bacteriophages with fluctuation analysis of electrical noise. The method is based on the massive transitory ion leakage that occurs at the moment of phage DNA injection into the host cell. The ion fluxes require only that the cells be physiologically viable (i.e. have energized membranes) and can occur within seconds after mixing the cells with sufficient concentrations of phage particles. To detect these fluxes, we have constructed a *nano-well*, a lateral, micrometer-sized capacitor of titanium electrodes with gap size of 150 nm, and used it to measure the electrical field fluctuations in microlitre (mm^3) samples containing phage and bacteria. In mixtures where the analyte bacteria were sensitive to the phage, large stochastic waves with various time and amplitude scales were observed, with power spectra approximately following a $1/f^2$ law from 1–10 Hz. Development of this SEPTIC (SEnsing of Phage-Triggered Ion Cascades) technology could provide rapid detection and identification of live pathogenic bacteria on the scale of minutes, with unparalleled specificity. The method has a potential ultimate sensitivity of 1 bacterium/microlitre (1 bacterium/mm^3).

Keywords: electronic noise, bacteriophage, biochip, fluctuations, nano-well, rapid bio-sensing

1. INTRODUCTION

Bacteriophages are the most numerous biological entities, estimated at 10^{31} in the biosphere, and are almost unimaginably diverse [1]. Phages exist with a wide range of host specificities, from narrow host range phages like

For two of the three main morphotypes of dsDNA phages, the myophages with contractile tails and the siphophages with flexible tails, the injection of DNA into the host cell follows rapidly and involves the transitory formation of a channel through which the phage DNA

- Technological Background
  - Bacteriophage Biology
  - Phage proteins by Hyglos
- Applications

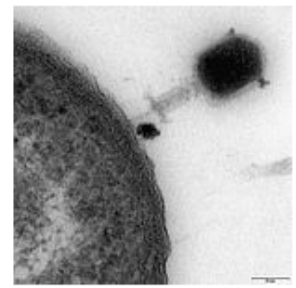
Technological Background - Phage-ligand Technology

Since the discovery of bacteriophages at the beginning of the 20th century, they have been widely used first in science and later in industry. Hyglos creatively combines long established knowledge with modern methods of protein engineering. This means using the power of nature to provide solutions for various needs of today. Hyglos' core competency is to exploit the principles of bacteriophage biology and the expert use of the proprietary phage-ligand technology for antimicrobial, diagnostic and research applications.

This technology enables the production of state-of-the-art molecules for the effective binding of bacteria and bacterial components and lysis of bacteria. Bacteriophages are viruses that have evolved for millions of years. They can recognize and infect bacteria everywhere they live, even under extreme conditions. Hyglos targets the highly specific interaction of naturally occurring bacteriophages with their host bacteria.

The technology makes use of molecules (phage proteins), which are identified from bacteriophages, characterized and recombinantly expressed for various applications. Such molecules are perfectly suitable for use in the fields of food and human diagnostics as well as the selective decolonization of pathogenic bacteria *e.g. Staphylococcus aureus*.

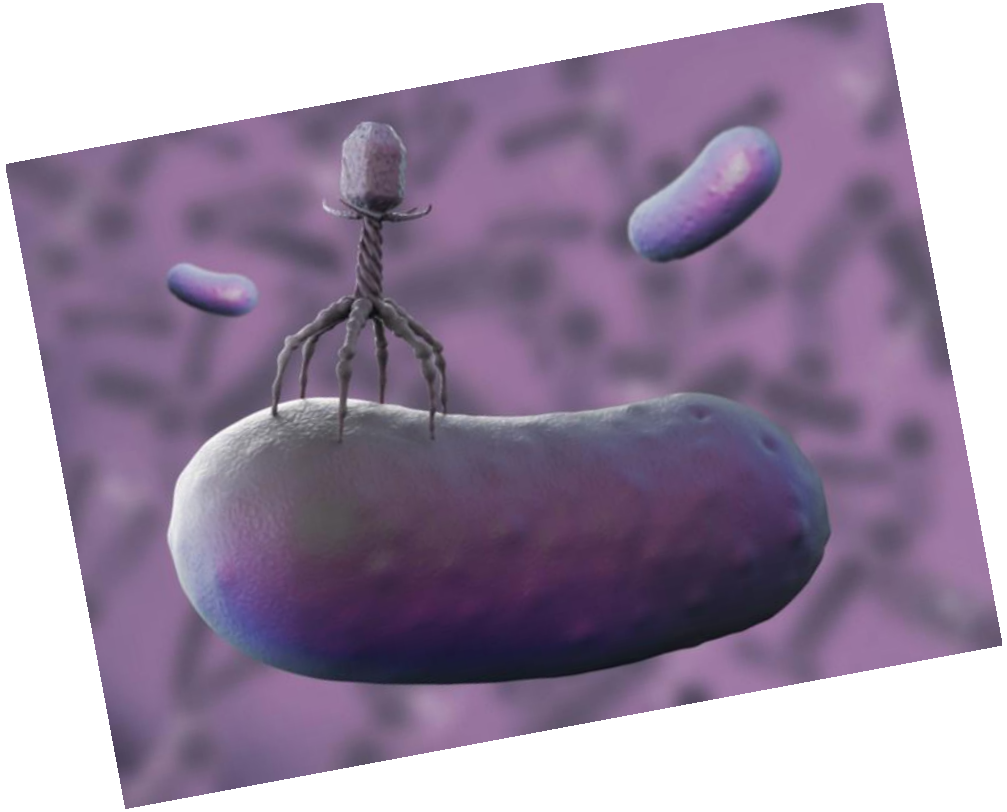
The image on the right seen through an electron microscope shows "the unfriendly take over" - a T4 bacteriophage binding to the surface of an *E. coli* bacteria cell.



Bacteriophage attacking a bacteria

Bakteriyofajların Tümü Yararlı mıdır?

- Inoviridae fajlarının pnömoni ve kistik fibrozlu hastalarda biyofilm tabakasını daha komplike hale getirerek, bakterilerin antibiyotiklerin etkisinden korunmalarını ve infeksiyonların kalıcı hale gelmelerini sağladığı gösterilmiştir.



Cell Host & Microbe
Article

Filamentous Bacteriophage Promote Biofilm Assembly and Function

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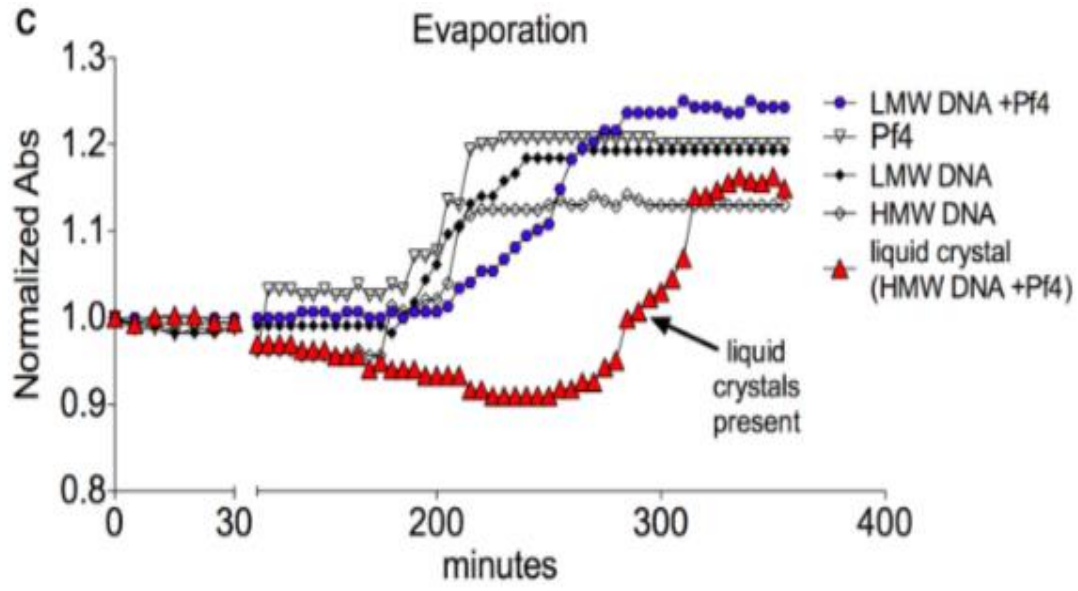

SUMMARY

Biofilms—communities of bacteria encased in a polymer-rich matrix—confer bacteria with the ability encased in a polymer-rich matrix. Bacteria within biofilms display increased tolerance to antibiotics and desiccation, allowing them to persist in host tissues and on medical device surfaces (Costerton et al., 1999).

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Review

Corynebacterium diphtheriae: Genome diversity, population structure and genotyping perspectives

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ABSTRACT

The epidemic re-emergence of diphtheria in Russia and the Newly Independent States (NIS) of the former Soviet Union in the 1990s demonstrated the continued threat of this thought to be rare disease. The bacteriophage encoded toxin is a main virulence factor of *Corynebacterium diphtheriae*, however, an analysis of the first complete genome sequence of *C. diphtheriae* revealed a recent acquisition of other pathogenicity factors including iron-uptake systems, adhesins and fimbrial proteins as indeed this extracellular pathogen has more possibilities for lateral gene transfer than, e.g., its close relative, mainly intracellular *Mycobacterium tuberculosis*.

C. diphtheriae appears to have a phylogeographical structure mainly represented by area-specific variants whose circulation is under strong influence of human host factors, including health control measures, first of all, vaccination, and social economic conditions. This framework core population ucture may be challenged by importation of the endemic and eventually toxigenic strains from new as thus leading to localized or large epidemics caused directly by imported strains or by teriophage-lysogenized indigenous strains converted into toxin production. A feature of *C. diphtheriae* existence with humans is its periodicity: following large epidemic in the 1990s, the present period is rked by increasing heterogeneity of the circulating populations whereas re-emergence of new igenic variants along with persistent circulation of invasive non-toxigenic strains appear alarming. To ntify and rapidly monitor subtle changes in the genome structure at an infraclonal level during and ween epidemics, portable and discriminatory typing methods of *C. diphtheriae* are still needed. In this w, CRISPRs and minisatellites are promising genomic markers for development of high-resolution ping schemes and databasing of *C. diphtheriae*.

Lysogenic conversion

Review

Corynebacterium diphtheriae: Genome diversity, population structure and genotyping perspectives

Igor Mokrousov *

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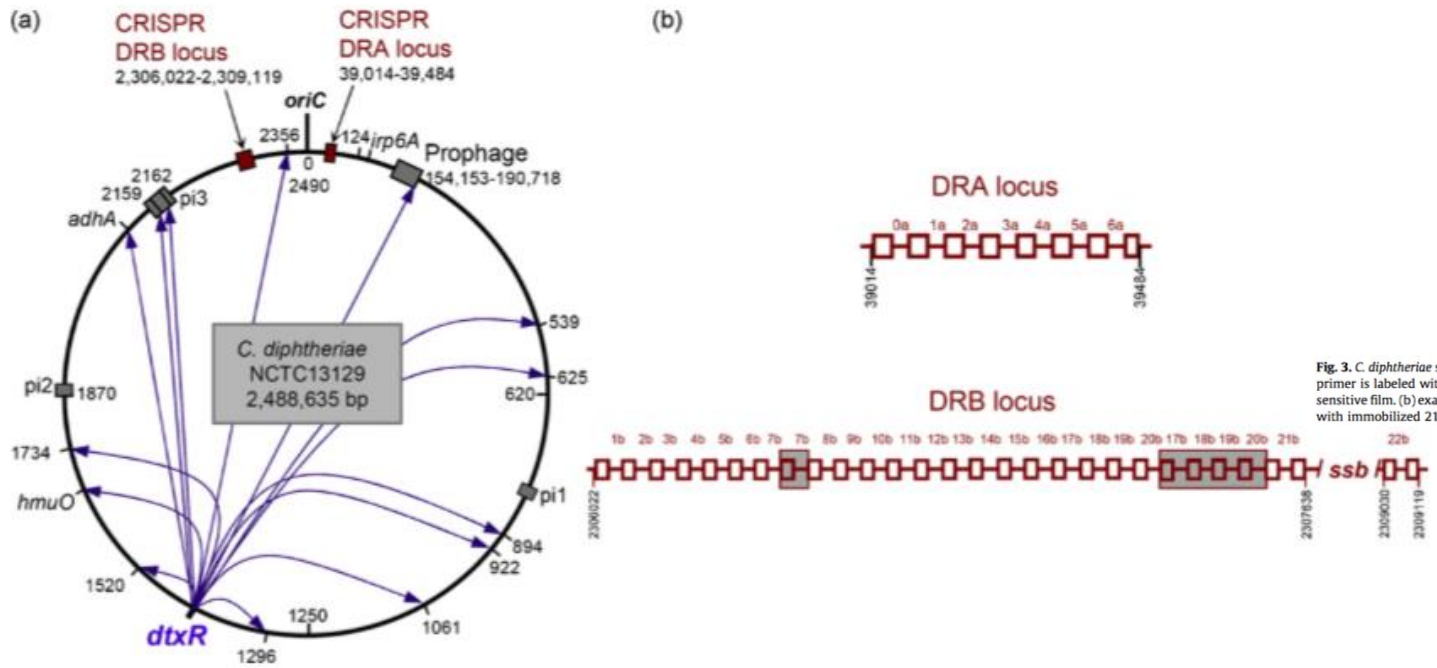

Fig. 1. (a) Genome map of the *Corynebacterium diphtheriae* strain NCTC13129 (Cerdeño-Tarraga et al., 2003) showing location of the transcriptional regulator gene *dtxR* and all DtxR-regulated genes (blue arrows) including the corynephage-encoded toxin (Canchaya et al., 2004) and location of CRISPR loci, DRA and DRB. (b) Schematic structure of the DRA and DRB CRISPR loci. Boxes are exact direct repeats, lines – variable spacers. Some DRB spacers are duplicated (shaded areas). Last repeat unit in DRA locus is truncated.

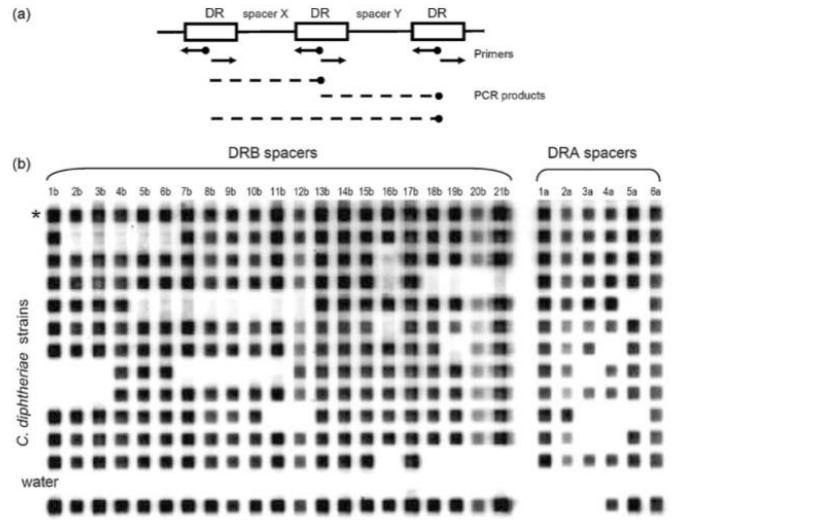

Fig. 3. *C. diphtheriae* spoligotyping. (a) PCR of a CRISPR locus with a locus-specific DR-sequence-defined one primer pair that amplifies all present variable spacers. Reverse primer is labeled with biotin (shown as black dot); this permits detection of hybridization signals via sterptovidine-peroxidase mediated chemiluminescence on a light-sensitive film. (b) examples of spoligoprofiles of the epidemic clone strains obtained by simultaneous hybridization of the co-amplified DRA and DRB spacers onto membrane with immobilized 21 DRB and 6 DRA spacers (Mokrousov et al., 2007); *designates the ancestral profile T1 with all 27 signals present.

Review

Corynebacterium diphtheriae: Genome diversity, population structure and genotyping perspectives

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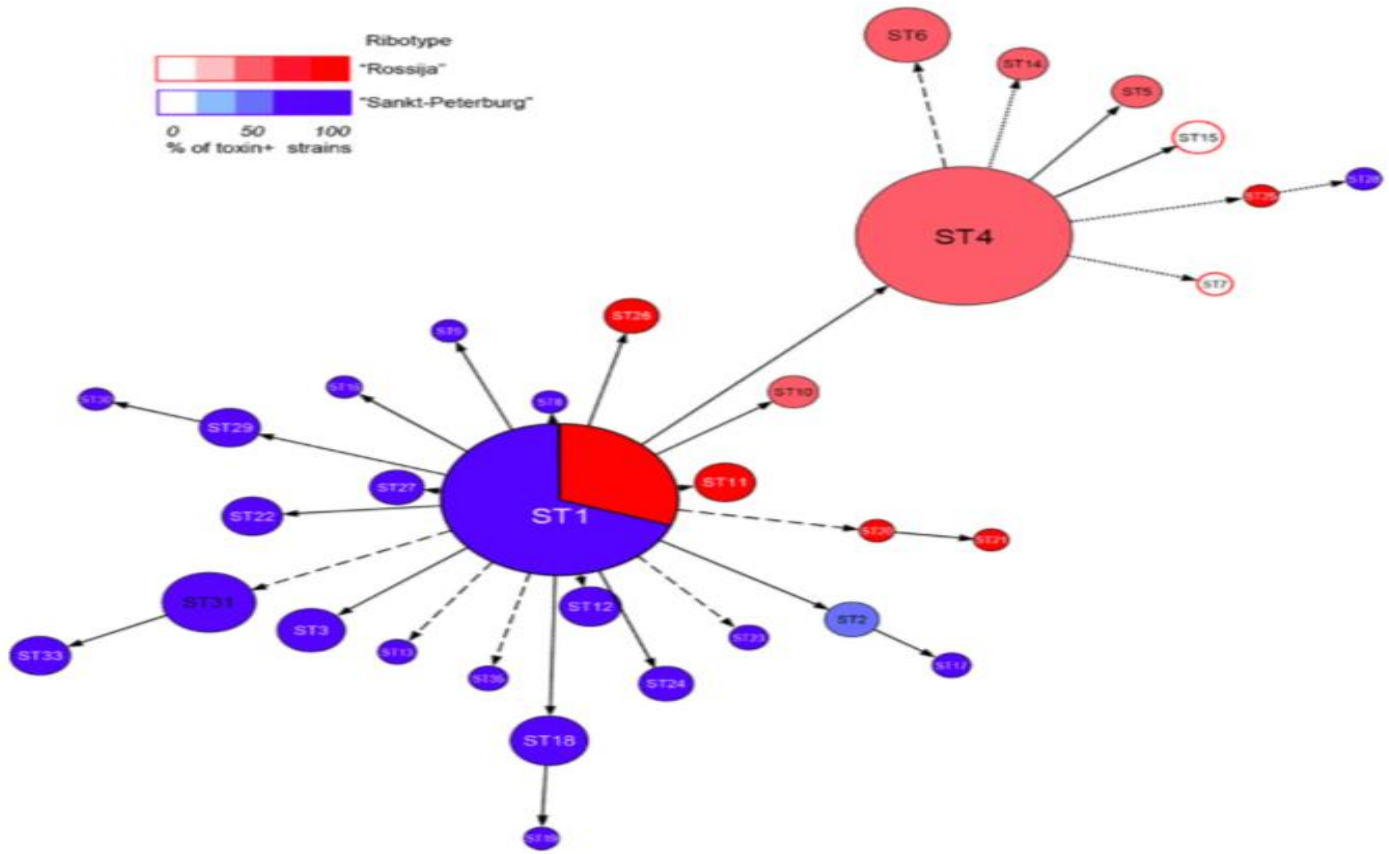

Fig. 4. Relationships of the *C. diphtheriae* CRISPR-(spoligo)types within the Russian epidemic clone visualized as a forest graph (DRB-locus based spoligoprofiles were taken from Mokrousov et al., 2005). Circle size is proportional to the number of strains. The "spoligoforest" burst output was generated with SpolTools program (http://www.emi.unsw.edu.au/spolTools) using Fruchterman–Reingold algorithm according to a deletion model (Reyes et al., submitted for publication; Tang et al., 2008). Solid edges are unique relationships between spoligotypes. Broken-line edges are chosen among multiple edges. Dotted lines have probability <0.5, while dashed lines have probability measure >0.5. For example, ambiguity of position of ST25 and ST28 is due to the absence of most spacers in their spoligoprofiles.

Bakteriyofajların Avantajları Nelerdir?

- Antibiyotikler gibi direnç gelişiminden etkilenmezler
- Bakteride direnç gelişse bile ona yönelik yeni faj sentezlenebilir
- Bakteriyi tamamen yok edene kadar etkisi devam eder
- Başka hücre ve bakterileri etkilemez
- Dar spektrumlu antibiyotik gibi davranır
- Yaşam süreleri sınırlı olup, hedef bakteri bittiğinde yok olurlar
- Bilinen bir yan etki oluşturmazlar
- Kullanılan diğer ilaçlarla etkileşmezler

Neden Fajlarda Direnç Gelişmez

- Milyonlarca yıldır olduğu gibi
- Bakterilerle birlikte evrimleşirler
- Etkili bir faj, özgül bakteriyi tamamen yok edene kadar infekte eder
- Bakterinin genetik yapısını kullanarak direnç gelişimine izin vermez
- Endolizin her durumda peptidoglikan tabakayı parçalamaktadır
- Bakteri yüzeyel determinantları değiştirse bile yeni virionlara hedef olur

Bakteriyofajların Dezavantajları Nelerdir?

- Polimikrobiyal infeksiyonlarda etkisi sınırlıdır
- İmmün sistem hücrelerinin hedefi olurlar
- Bu nedenle sistemik uygulamalarında başarı sınırlıdır
- Dirençli bakteriler için özel faj hazırlığı gerekir
- Yüz yılı aşkın süredir uygulanmasına karşın bilimsel veri sınırlıdır

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Environmental Microbiology

Two Novel Bacteriophages Improve Survival in *Galleria mellonella* Infection and Mouse Acute Pneumonia Models Infected with Extensively-drug-resistant *Pseudomonas aeruginosa*

Jongsoo Jeon, Dongeun Yong

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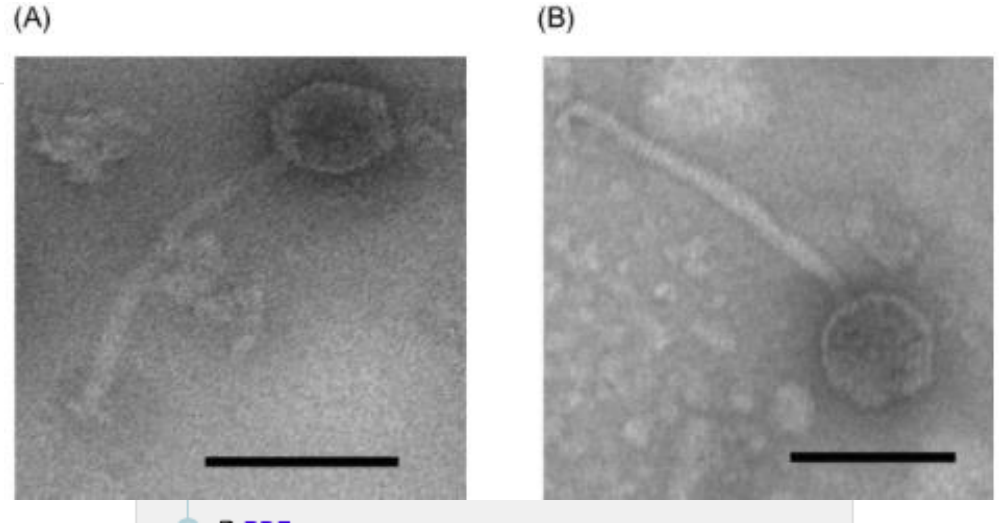
ABSTRACT

Extensively-drug-resistant *Pseudomonas aeruginosa* (XDR-PA) is a life-threatening pathogen that causes serious global problems. Here, we investigated two novel *P. aeruginosa* bacteriophages (phages), Bφ-R656 and Bφ-R1836, *in vitro*, *in silico,* and *in vivo* to evaluate the potential of phage therapy to control XDR-PA clinical strains. Bφ-R656 and Bφ-R1836 belong to the *Siphoviridae* family and exhibited broad host ranges which could lyse 18 (64%) and 14 (50%) of the 28 XDR-PA strains. In addition, the two phages showed strong bacteriolytic activity against XDR-PA host strains from pneumonia patients. The whole genomes of Bφ-R656 and



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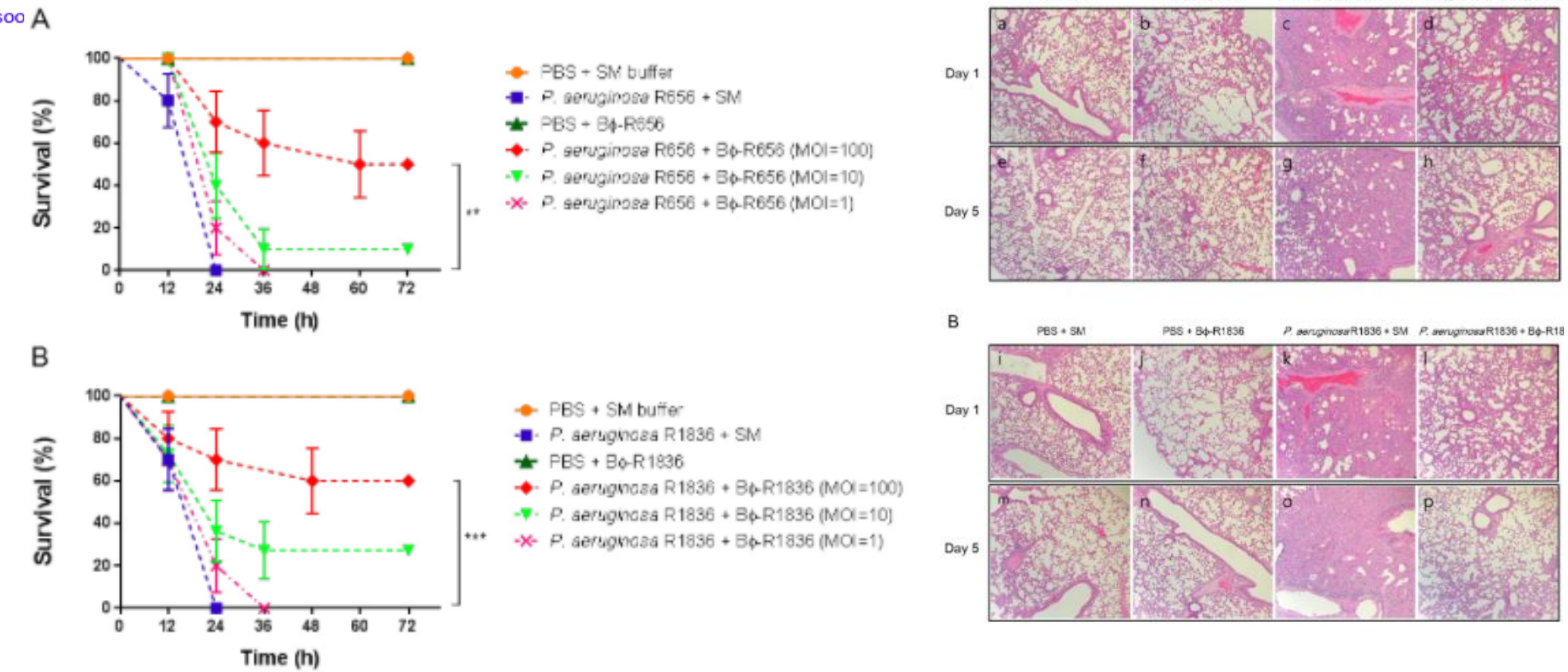
Two Novel Bacteriophages Improve Survival in *Galleria mellonella* Infection and Mouse Acute Pneumonia Models Infected with Extensively-drug-resistant *Pseudomonas aeruginosa*

Jongsoo



Fighting Pathogenic Bacteria on Two Fronts: Phages and Antibiotics as Combined Strategy

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With the emerging threat of infections caused by multidrug resistant bacteria, phages have been reconsidered as an alternative for treating infections caused by tenacious pathogens. However, instead of replacing antibiotics, the combination of both types of antimicrobials can be superior over the use of single agents. Enhanced bacterial suppression, more efficient penetration into biofilms, and lowered chances for the emergence of phage resistance are the likely advantages of the combined strategy. While a number of studies have provided experimental evidence in support of this concept, negative interference between phages and antibiotics have been reported as well. Neutral effects have also been observed, but in those cases, combined approaches may still be important for at least hampering the development of resistance. In any case, the choice of phage type and antibiotic as well as their mixing ratios must be given careful consideration when deciding for a dual antibacterial approach. The most frequently tested bacterium for a combined antibacterial treatment has been *Pseudomonas*

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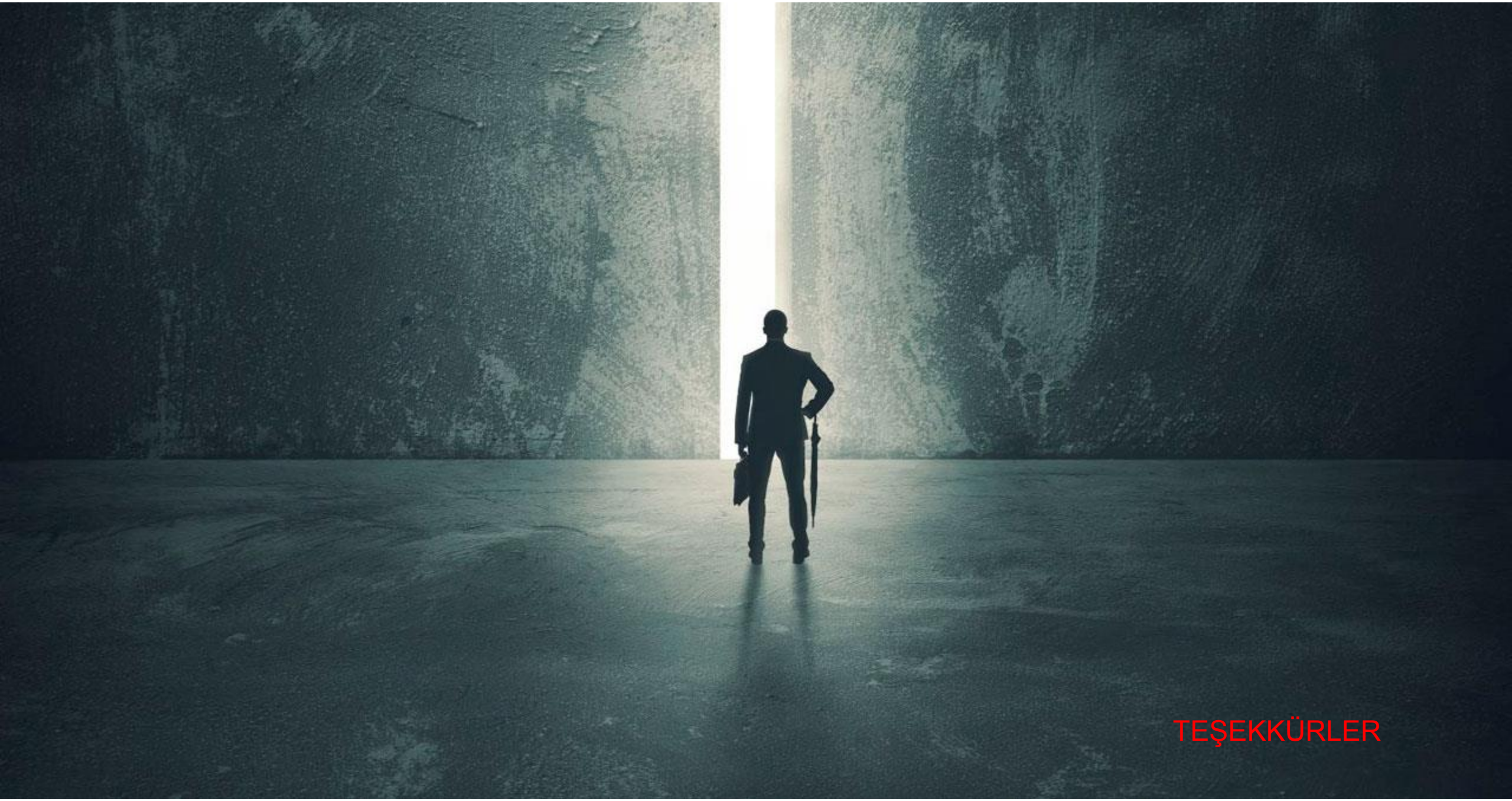
MDR Bakteri	Bakteriyofaj	Antibiyotik
Pseudomonas aeruginosa	NP1 (*Siphoviridae, NP1Virus*) ve NP3 (*Myoviridae*)	Seftazidim, Siprofloksasin, Tobramisin, Gentamisin
Klebsiella pneumoniae	KPO1K2 (*Podoviridae, T7-like virus*)	Siprofloksasin
Acinetobacter baumannii	vB_AbaM-KARL-1 (*Myoviridae*, *T4- like virus*)	Meropenem, Siprofloksasin, Kolistin
Staphylococcus aureus	SAP-26 (*Siphoviridae, Phietavirus*)	Vankomisin, Siprofloksasin, Amikasin
Escherichia coli	RB32 ve RB33 (*Myoviridae*) T4, T3 (*Podoviridae, T7virus*)	Sefotaksim Siprofloksasin
Burkholderia cepacia	KS12 (*Myoviridae*) and KS14 (*Myoviridae*, *P2-like virus*)	Minosiklin, siprofloksasin, meropenem

Sonuç Olarak Faj Tedavisi..

- MDR Bakteri infeksiyonlarının tedavisinde
- Biyofilm tabakasına etkinliği ile
- Antibiyotiklerle kombine kullanılabilmeleri ile
- Direnç geliştirmemesi
- Yan etkilerinin bulunmaması ile..

- İYİ BİR ALTERNATİF OLABİLECEK DURUMDADIR.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