KLİMİK TÜRK KLİNİK MİKROBİYOLOJİ VE İNFEKSİYON HASTALIKLARI DERNEĞİ

Bilimle Sağlıkla 30 Yıl

Antivirulan Tedavi ve Faj Tedavisi

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Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji AD

25.01.2017, Ankara

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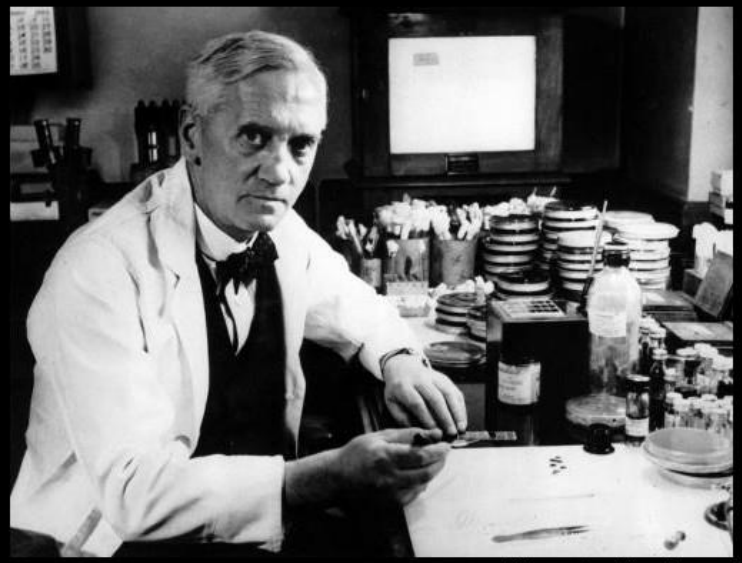
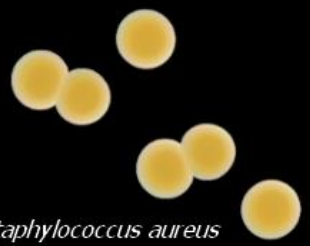
www.bacteriainphotos.com

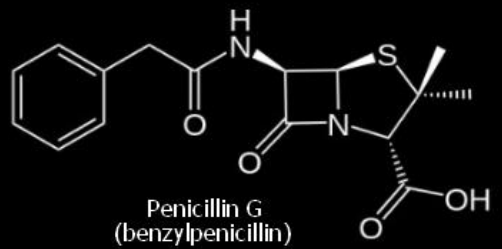
Penicillium chrysogenum
(P.notatum)



Alexander Fleming

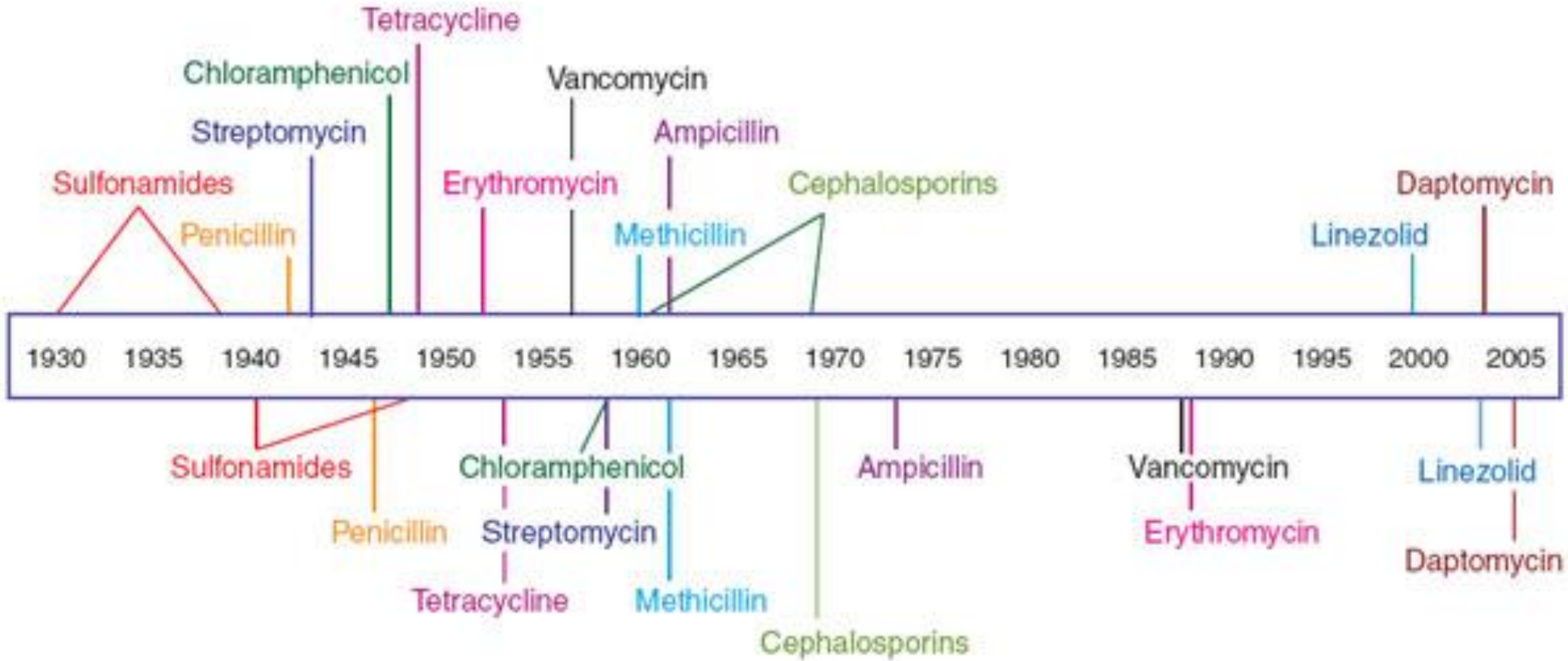


Staphylococcus aureus



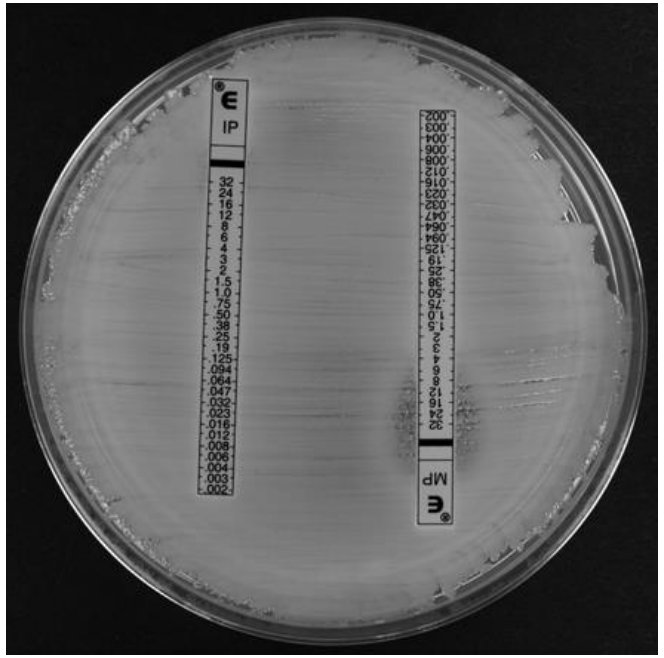
Penicillin G
(benzylpenicillin)

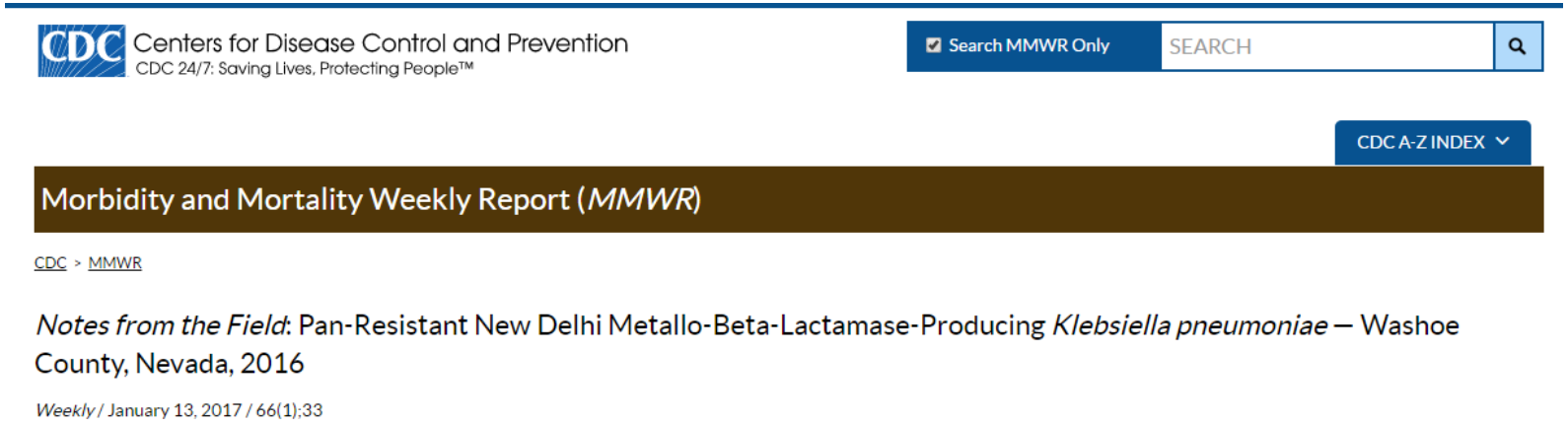
Antibiotic deployment



Antibiotic resistance observed

- Pan-rezistan Acinetobacter baumannii





- Antimicrobial susceptibility testing in the United States indicated that **the isolate was resistant to 26 antibiotics**, including all aminoglycosides and polymyxins tested, and intermediately resistant to tigecycline (a tetracycline derivative developed in response to emerging antibiotic resistance). Because of a high minimum inhibitory concentration (MIC) to colistin, the isolate was tested at CDC for the *mcr-1* gene, which confers plasma-mediated resistance to colistin; the results were negative. The isolate had a relatively low fosfomycin MIC of 16 µg/mL by ETEST.* However, fosfomycin is approved in the United States only as an oral treatment of uncomplicated cystitis; an intravenous formulation is available in other countries.

- The patient was a female Washoe County resident in her 70s who arrived in the United States in early August 2016 after an extended visit to India. She was admitted to the acute care hospital on August 18 with a primary diagnosis of systemic inflammatory response syndrome, likely resulting from an infected right hip seroma. **The patient developed septic shock and died.**

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Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *Klebsiella pneumoniae* clinical isolates to chlorhexidine

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ABSTRACT

Klebsiella pneumoniae is an opportunistic pathogen which is often difficult to treat due to its multidrug resistance (MDR). We have previously shown

Klorheksidine maruz kalan *Klebsiella pneumoniae* izolatlarında çapraz-direnç mekanizmasıyla **Kolistin** direnci de gelişiyor.

plasmid containing *phoPQ* from chlorhexidine adapted strains into wild-type *K. pneumoniae* resulted in elevated expression levels of *phoPQ*, *pmrD* and *pmrK* and increased resistance to colistin but not chlorhexidine. The potential risk of colistin resistance emerging in *K. pneumoniae* as a consequence of exposure to chlorhexidine has important clinical implications in infection prevention procedures.

- DSÖ:
  - Dünyada ölüme sebep olan en önemli 2. neden «antibiyotik dirençli mikroorganizmalar»

Trends in Biotechnology, July 2016, Vol. 34, No. 7

Alternatives to antibiotics—a pipeline portfolio review

Lloyd Czaplewski, Richard Bax, Martha Clokie, Mike Dawson, Heather Fairhead, Vincent A Fischetti, Simon Foster, Brendan F Gilmore, Robert E W Hancock, David Harper, Ian R Henderson, Kai Hilpert, Brian V Jones, Aras Kadioglu, David Knowles, Sigríður Ólafsdóttir, David Payne, Steve Projan, Sunil Shaunak, Jared Silverman, Christopher M Thomas, Trevor J Trust, Peter Warn, John H Rex

Antibiotics have saved countless lives and enabled the development of modern medicine over the past 70 years. However, it is clear that the success of antibiotics might only have been temporary and we now expect a long-term and perhaps never-ending challenge to find new therapies to combat antibiotic-resistant bacteria. A broader approach to address bacterial infection is needed. In this Review, we discuss alternatives to antibiotics, which we defined as non-compound approaches (products other than classic antibacterial agents) that target bacteria or any approaches that target the host. The most advanced approaches are antibodies, probiotics, and vaccines in phase 2 and phase 3 trials. This first wave of alternatives to antibiotics will probably best serve as adjunctive or preventive therapies, which suggests that conventional antibiotics are still needed. Funding of more than £1·5 billion is needed over 10 years to test and develop these alternatives to antibiotics. Investment needs to be partnered with translational expertise and targeted to support the validation of these approaches in phase 2 trials, which would be a catalyst for active engagement and investment by the pharmaceutical and biotechnology industry. Only a sustained, concerted, and coordinated international effort will provide the solutions needed for the future.

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Chemical Biology Ventures, Abingdon, Oxfordshire, UK (L Czaplewski PhD); **Abgentis, Edgbaston, Birmingham, UK** (L Czaplewski); **Persica Pharmaceuticals, Canterbury, Kent, UK** (L Czaplewski); **Transcrip Partners Reading,**

Introduction

Given the rise of antibacterial resistance and the challenges of conventional antibacterial agent discovery and development that have led to a very small pipeline of new therapies, it would be prudent to consider the potential of non-conventional approaches.[1,2] This Review—written by 24 scientists from academia and industry, commissioned by the Wellcome Trust, and jointly funded by the Department of Health (England)—considers the prospects for alternatives to antibiotics. Although there have been technical reviews of individual alternative approaches,[3]

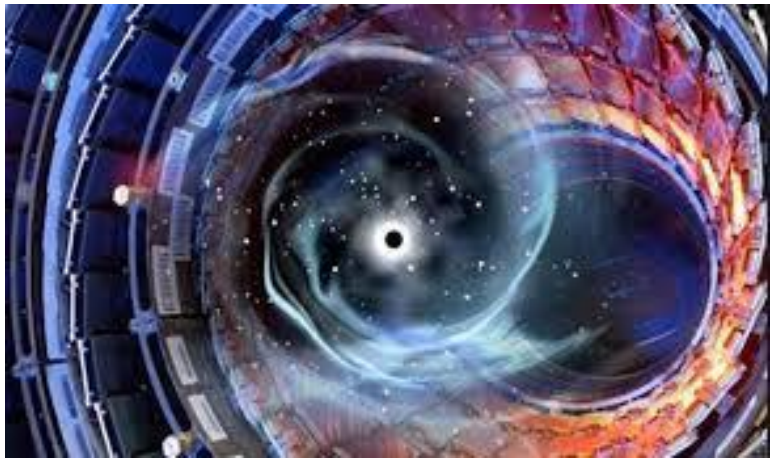
	Comment	Probable spectrum of activity and initial use
Tier 1 approaches (translational funding to clinical evaluation at phase 2)		
Antibodies[4,6-13]	Antibodies that bind to and inactivate a pathogen, its virulence factors, or its toxins were widely considered one of the alternative approaches most likely to have major clinical impact. Antibodies were considered a low-risk area with strong science basis, history of safe use, and a high degree of technical feasibility	Prevent Gram-positive and Gram-negative infection; possibly adjunct use
Probiotics[14-18]	Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host organism. Defined mixtures of bacteria or the use of non-toxigenic spores of *Clostridium difficile* will probably provide therapeutic and prophylactic therapies that will improve current clinical practice for the treatment of *C difficile*-associated diarrhoea and antibiotic-associated diarrhoea. Basic research to understand the mechanism of action of probiotics in different settings and how they might be used in combination with antibiotics and other alternatives to antibiotics (eg, bacteriophages) could enable their wider use in other indications	Prevent or treat *C difficile*-associated diarrhoea or antibiotic-associated diarrhoea
Lysins[19-27]	Phage lysins are enzymes used by bacteriophages to destroy the cell wall of a target bacterium and are potential replacements for antibiotics because of their direct antibacterial action, and as adjuncts because they act to reduce bacterial burden, weaken biofilms, or both. Emphasis on lysins active against Gram-negative pathogens would be beneficial	Treat Gram-positive infection
Wild-type bacteriophages[28-32]	Wild-type bacteriophages that infect and kill bacteria have the potential to replace antibiotics for some indications. Bacteriophage could be used in small doses because they replicate when their host bacterium is present. During treatment of an infection they might also evolve to infect the strains causing the disease. This replication and evolution makes them unique in pharmaceutical product development. More product than was dosed will be present in the patient and that product can change over time; what is sampled after dosing is not exactly what was given to the patient	Treat Gram-positive and Gram-negative infection
Engineered bacteriophages[33-36]	The ability to genetically engineer phages with new properties for therapeutic use is potentially advantageous. Many of the challenges associated with mixtures of wild-type phages, such as breadth of strain coverage, development of resistance, and rapid elimination after systemic administration, could be addressed. In-dose selection could be an advantage of this approach, exposure to the larger doses of non-replicating phage required to treat infection might be a drawback	Treat Gram-positive and Gram-negative infection
Immune stimulation[37-45]	Successful antimicrobial therapy depends on an appropriate immune response. Immune stimulation has been proposed as a potential adjunct approach in conjunction with antibiotic therapy. Repurposing of phenyl butyrate and vitamin D to enhance expression of innate antimicrobial peptides seems feasible Oral bacterial extracts are registered and used in clinic to reduce the incidence of respiratory tract infections in some at-risk groups in some regions. If successful, additional clinical trials to substantiate their efficacy in other populations would encourage wider use. The mechanisms by which these extracts might work are unclear but might involve TLRs—eg, TLR2 and TLR9. Targeted interventions could be devised once these mechanisms are understood The working group focused on assessment of repurposed drugs for immune stimulation rather than assessment of early translational research in this specialty. Generally, there was insufficient target validation for bacterial infection, a high potential for side-effects, variable responses and polymorphisms in patient populations, and responses specific to bacterial species and strain. The clinical development path for host-targeted therapies will probably use non-human primates during product development	Prevent or provide adjunct therapy for Gram-positive and Gram-negative infection
Vaccines[46-60]	The long established investment in vaccines for new targets should continue given their potential to substantially reduce the incidence of infection and, therefore, the need for antibiotics. In view of the ageing human population, we need better knowledge of the potential for vaccination in the elderly and how best to dose immune compromised individuals	Prevention, Gram-positive more than Gram-negative infection

Tier 2 approaches (strong support for funding while monitoring for breakthrough insights regarding systemic therapy)		
Antimicrobial peptides[61-72]	The advantages of antimicrobial peptides are their broad spectrum activity, which includes most major Gram-positive and Gram-negative bacteria, their bactericidal and rapid action, low target-based resistance, and low immunogenicity. Detailed scientific literature and early clinical trials have not yet led to a therapeutic breakthrough for systemic treatments. Studies will be needed that aim to establish why they have largely not been used systemically (eg, toxicity, cost, lability to proteases, etc) and how to overcome these deficiencies (eg, formulation, redesign or use of non-natural aminoacids, etc). In some instances topical application (eg, by aerosol) might supplement systemic therapy. The reasons why projects were stopped are not in the public domain. Public–private partnerships that fund and test the potential of antimicrobial peptides in well designed clinical trials and publish the outcomes will be necessary to inform future investment into this approach	Treat or adjunct for Gram-positive and Gram-negative infection
Host defence peptides and innate defence peptides[37,73-76]	Host defence peptides (small, natural peptides) and innate defence regulators (small, synthetic peptides) have indirect antimicrobial effects. They primarily act by increasing expression of anti-inflammatory chemokines and cytokines, and reducing the expression of proinflammatory cytokines. Additional resources are needed to accelerate their preclinical assessment and progression into clinical trials to provide validation of the approach. Targeting host responses could carry an increased risk of side-effects and make it more difficult to distinguish and understand immunological differences between rodents and humans at the population level	Adjunct for Gram-positive and Gram-negative infection
Antibiofilm peptides[77,78]	Peptides that specifically inhibit bacterial biofilm formation have been identified and are in preclinical development. Their use as adjunctive therapy could improve outcomes	Adjunct for Gram-positive and Gram-negative infections

	Target	Product name, reference	Phase as of January–March, 2015	Earliest anticipated registration
Antibodies				
Merck	*Clostridium difficile*	Bezlotoxumab[117,118]	Phase 3 ongoing	2017
MedImmune	*Staphylococcus aureus*	MEDI4893[6,118]	Phase 2 ongoing	2021
Aridis	*Pseudomonas aeruginosa*	AR-101[119]	Phase 2a complete	2021
Aridis	*S aureus*	AR-301[119]	Phase 2a ready	2022
MedImmune	*P aeruginosa*	MEDI3902[9]	Phase 1 ongoing	2023
XBiotech	*S aureus*	514G3[13]	Phase 1 ongoing	2023
Aridis	*P aeruginosa*	Aerucin[10]	IND ready	2025
Combined	..	..	..	..
Probiotics				
Seres	*C difficile*	SER-109[120]	Phase 3 ready	2018
Rebiotix	*C difficile*	RBX2660[121]	Phase 2 ongoing	2019
Shire (Viropharma)	*C difficile*	VP20621[122]	Phase 2 ready	2022
Combined	..	..	..	..

Lysins				
Intron Biotechnology	*S aureus*	SAL200[27]	Phase 1 ongoing	2022
ContraFect	*S aureus*	CF-301[19]	Phase 1 ongoing	2022
Combined	..	..	..	..
Bacteriophages				
Wild-type bacteriophages				
AmpliPhi	*C difficile*	AmpliPhage-004[123]	Pre-phase 1	2023
AmpliPhi	*P aeruginosa*	AmpliPhage-001[123]	Pre-phase 1	2023
Engineered bacteriophages				
Phico Therapeutics	*P aeruginosa*	PT-3.1[34]	Pre-phase 1	2023
Combined	..	..	..	..
Immune stimulation				
Akthelia	*C difficile*	Phenylbutyrate/vitamin D[38,40]	Phase 2 ready	2021
Various	Various	Bacterial extracts[43]	Phase 1 ready	2022
Combined	..	..	..	..
Vaccines				
Sanofi Pasteur	*C difficile*	*C difficile* toxoid vaccine[124]	Phase 3	2019
Valneva	*P aeruginosa*	IC43[125,126]	Phase 2 and Phase 3 ongoing	2019
Valneva	*C difficile*	IC84[126]	Phase 2 ongoing	2021
Pfizer	*S aureus*	SA4Ag[127]	Phase 2 ready	2021
Combined	..	..	..	..

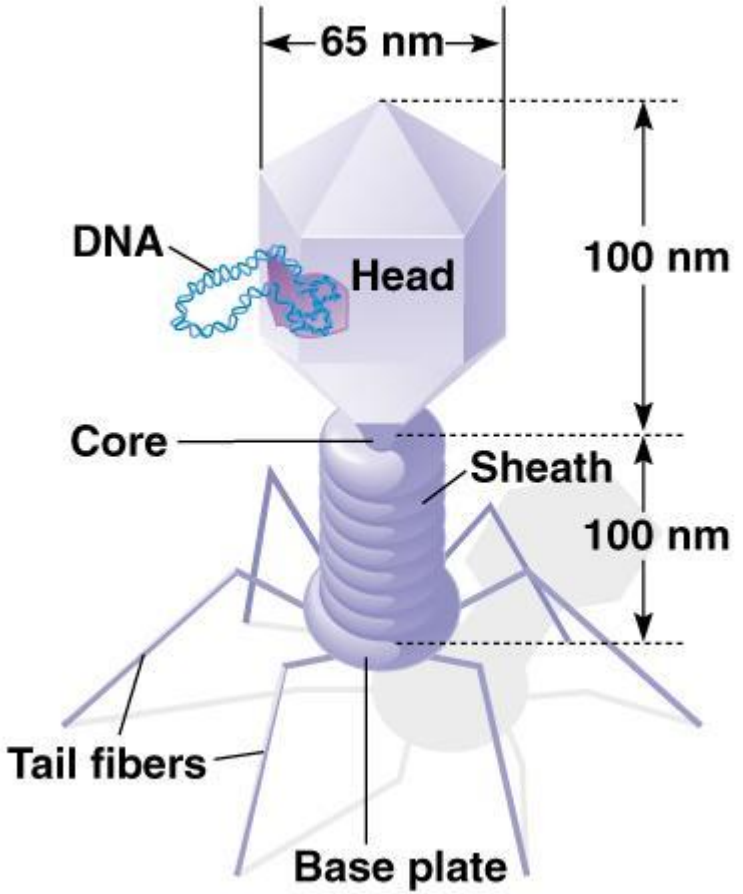
	Target	Product name, reference	Phase as of January–March, 2015	Earliest anticipated registration
(Continued from previous page)				
Antimicrobial peptides				
Roche	*P aeruginosa*	POL7080[128]	Phase 2 ongoing	2022
Novacta Biosystems	*C difficile*	NVB302[129]	Phase 1 ongoing	2022
Adenium	*S aureus*	AP-138[64]	Pre-phase 1	2023
Adenium	Urinary tract infection	AP-139[64]	Pre-phase 1	2023
Adenium	*C difficile*	AP-114[64]	Pre-phase 1	2023
Combined	..	..	..	..
Other peptides				
Various	Gram-negative and Gram-positive	..	Preclinical	2027



Büyük Hadran çarpıştırıcısı (CERN)
6 milyar sterlin

Uluslararası uzay istasyonu
96 milyar sterlin

Önümüzdeki 10 yıl içinde;
antibiyotik alternatifi tedavi geliştirme
araştırmaları için öngörülen harcama
1,5 milyar sterlin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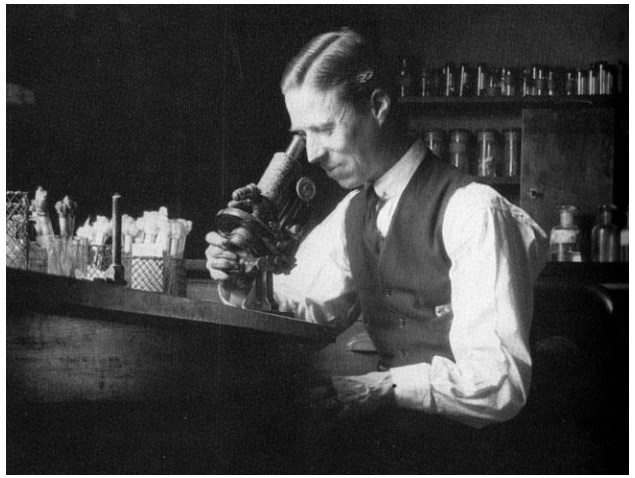
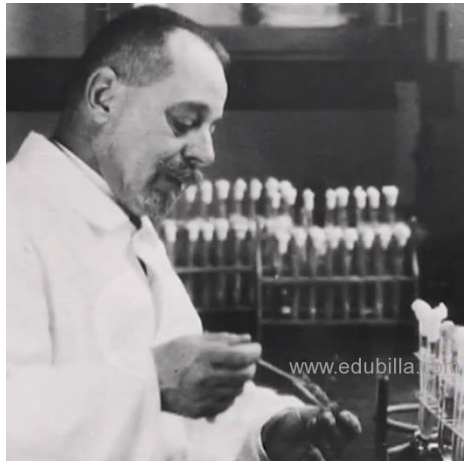
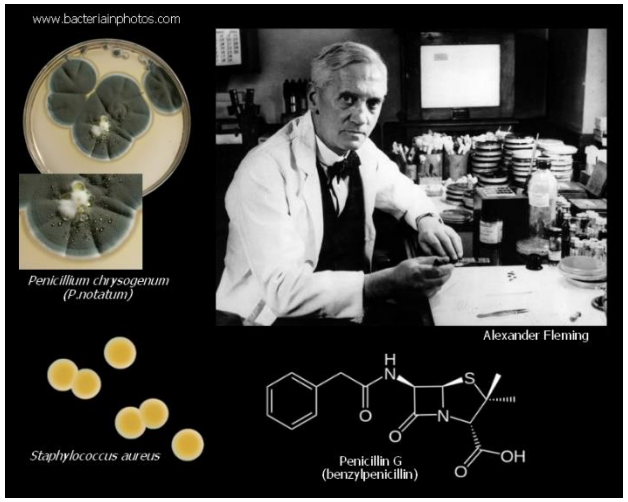
Tail fibers
Base plate



FREDERICK WILLIAM TWORT
1877-1950
İngiltere

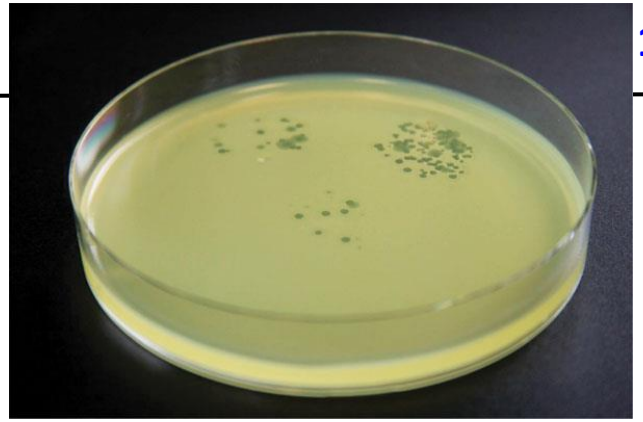


FÉLIX D'HÉRELLE
1873-1949
Kanada-Fransa

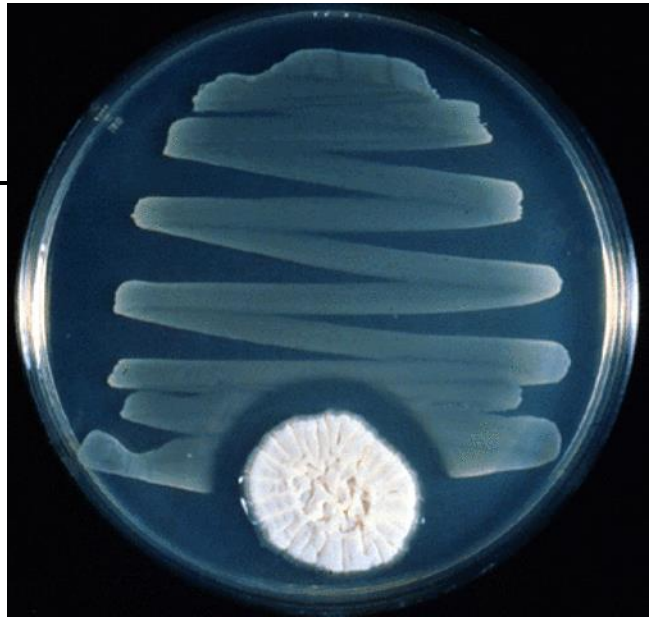


Alexander Fleming
1881-1955
İngiltere

1915

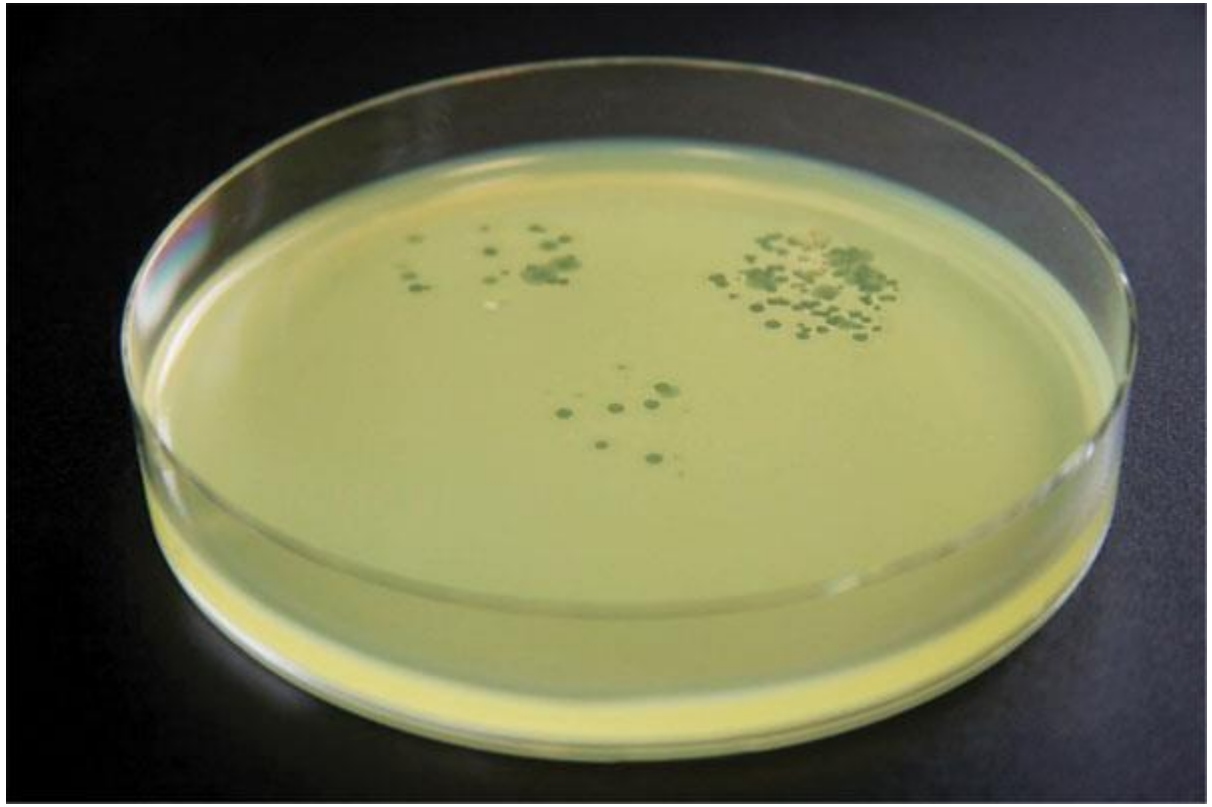


1917



İlk gözlemler-bulgular

- Basilli dizanteri kültür plaklarında «litik» alanlar
- Bakteri filtresinden geçebiliyor.
- Hastalık şiddetlendiğinde titrasyonu artıyor
- *bacteriophage*



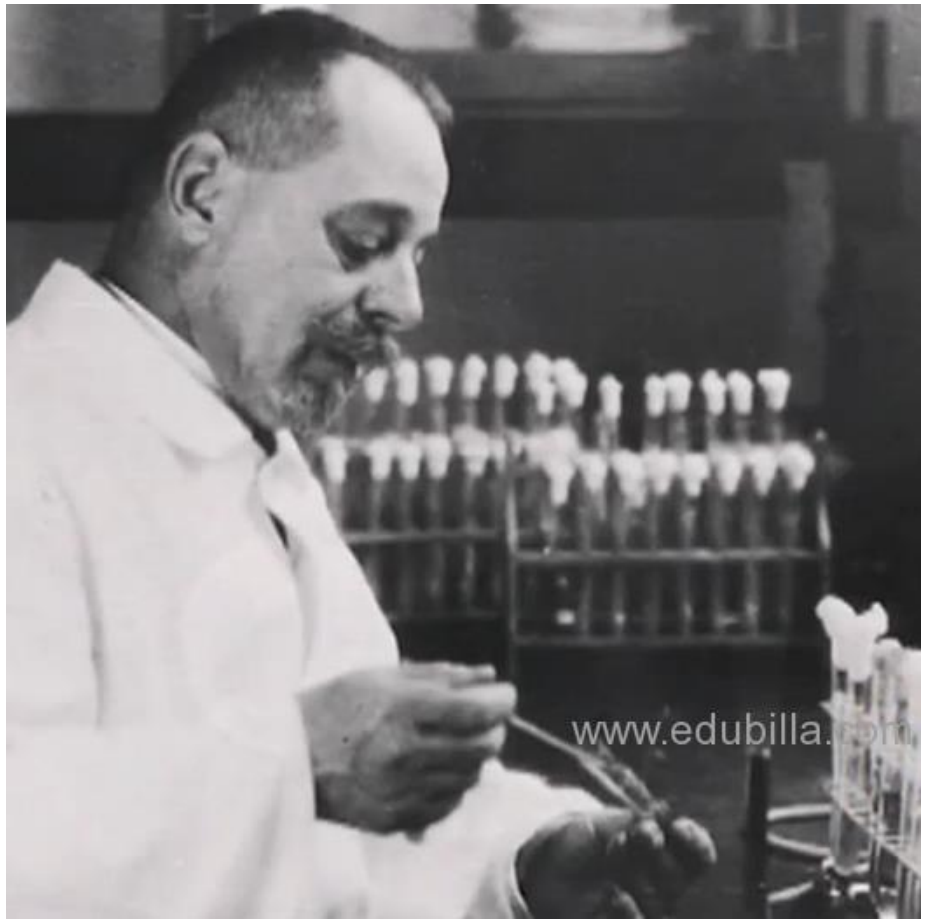
İlk tedavi denemeleri

- Felix D'Herelle faj süspansiyonunu kendisinde, ailesinde, çalışma arkadaşlarında ve sonrasında hastalarda tedavi amaçlı kullandı.
  - Basilli dizanteri ve kolera (1919)

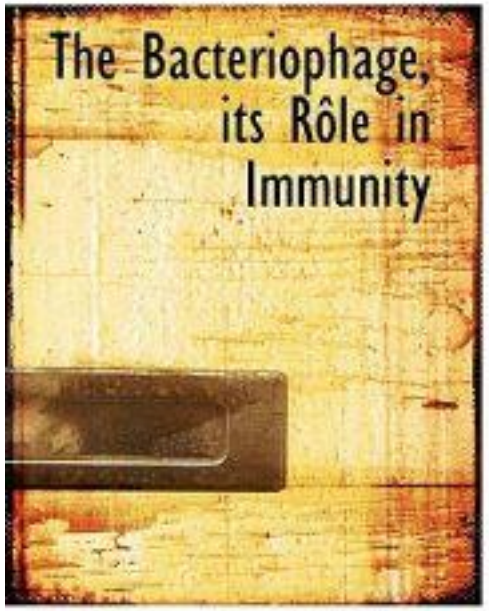
- Stafilokokal cilt enfeksiyonu tedavisinde denendi.

- Kümes hayvanlarında *Salmonella gallinorum* enfeksiyonunda etkinliği için çalışmalar yapıldı (1926)

- *Pasteurella multocida* enfeksiyonunda etkinliği gösterildi.



1922

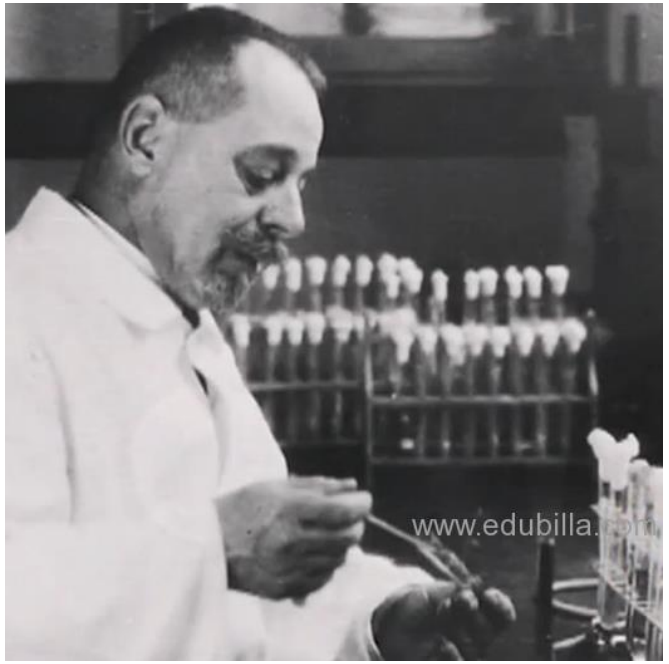


Felix D'Herelle, George Hathorn Smith Ph.D.

Bilimsel itirazlar - eleştiriler

- Tedavi başarısı olmakla birlikte başarısız sonuçlar da mevcut
- Başarılı çalışmalar birtakım hatalar içeriyor
- Fajların biyolojik yapısı bilinmiyor, tedavide kullanmak riskli!
- Faj hazırlama yöntemleri standart değil
- Araştırmacıların sonuçları kıyaslanabilir değil

Curr Microbiol (2017) 74:277–283



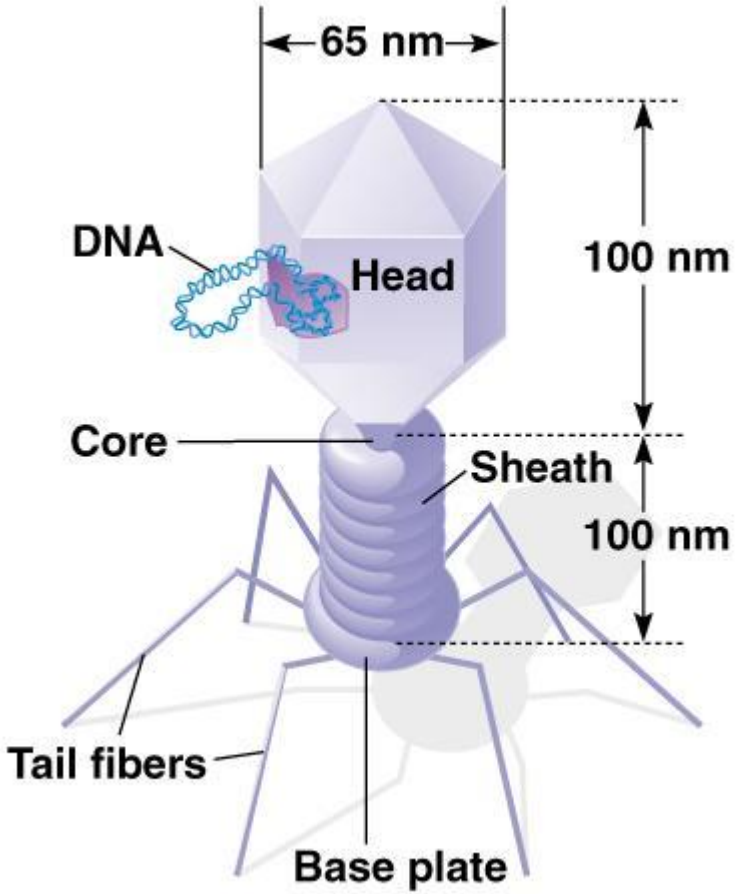
Bakterofaj
Laboratuvarı
PARİS

Yale Üniversitesi
ABD
1928-1933

Eliava Enstitüsü
TİFLİS

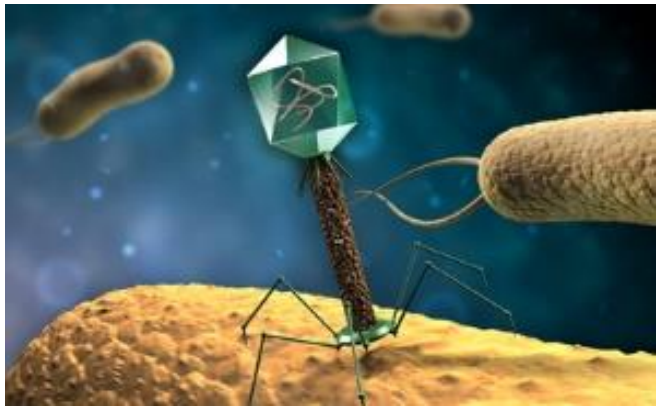
George Eliava Bakteriofaj Enstitüsü
Tiflis/ GÜRCİSTAN

Textbook of Natural Medicine


65 nm
DNA
Head
100 nm
Core
Sheath
100 nm
Tail fibers
Base plate

Bakteriofaj - Mikrobiyoloji

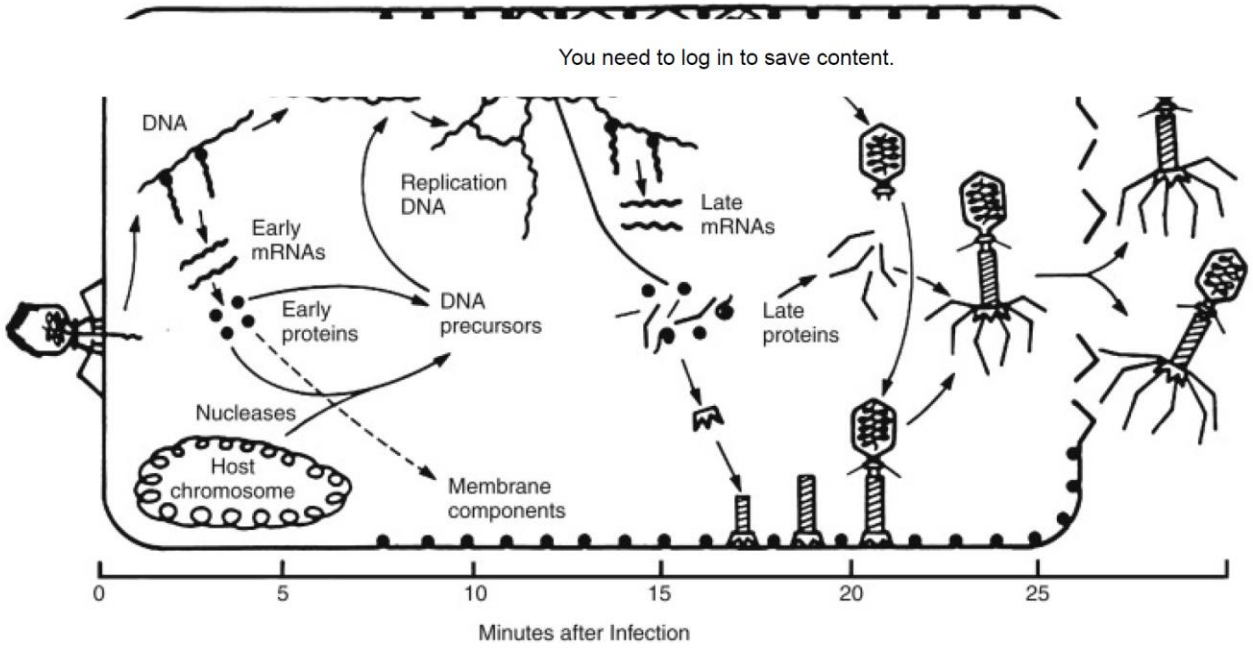
- Hedef bakteri yüzeyindeki spesifik moleküle *(lipopolisakkarit, teikoik asit, protein)* tutunur.
  - *Yalnızca duyarlı bakteriyi etkiler*
  - *Diğer bakteri veya memeli hücrelerini etkilemez*.
- Her bakteriye spesifik fajlar genellikle doğada bu bakterilerin üredikleri çevreden izole edilir.
  - Lağım suları
  - Dışkı
  - Toprak
  - Deniz suyu
  - Kaplıcalar



Faj tipleri

- **Litik Faj**
  - Bakteriyi enfekte eder,
  - Bakteri içinde kısa sürede çoğalır
  - Bakteri duvarı parçalanır ve dış ortama salınır.
  - Bakteriyel enfeksiyonların tedavisi için uygun

- **Lizojenik (Temperate - Ilımlı) Faj**:
  - Bakteri DNA'sına entegre olur



Litik fajlarda mekanizma

- Holin – Lizin sistem

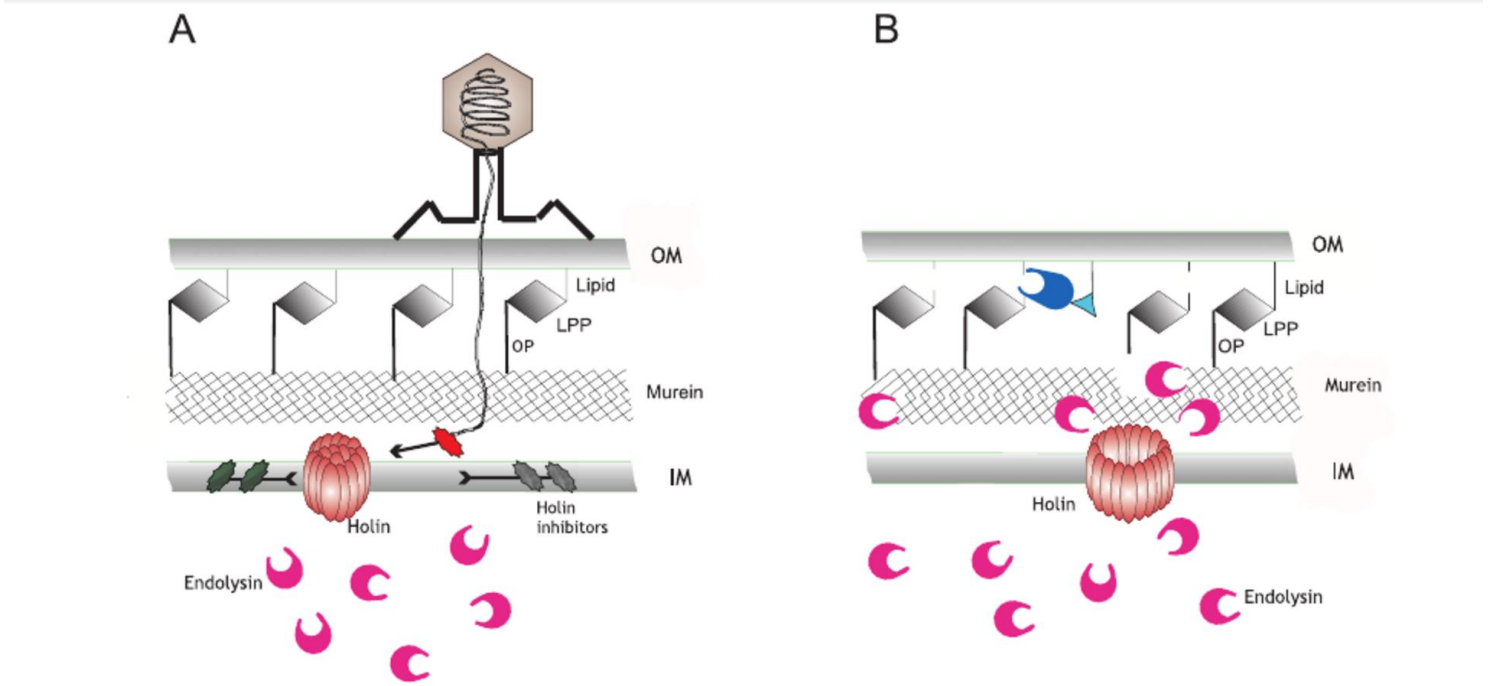


Fig. 1. (A) The pre-hole configuration of a Gram-negative cell wall during holin-endolysin lysis. The envelope consists of inner (IM) and outer (OM) membranes, OP- oligopeptide, LPP-lipoprotein. Holins accumulate in membrane aggregates and endolysin accumulates in the cytosol. Triggering of holin action can be inhibited by inhibitors, which can be membrane proteins or a periplasmic protein. (B) Hole configuration. An oligomeric membrane lesion is formed by the holin, allowing escape of endolysin, which attacks the murein. Also shown is a model for a complex of λ Rz and Rz1 endolysins attaching the oligonucleotide links to the OM, with Rz1 bind to the OM by its lipoid moiety (Young et al., 2000).

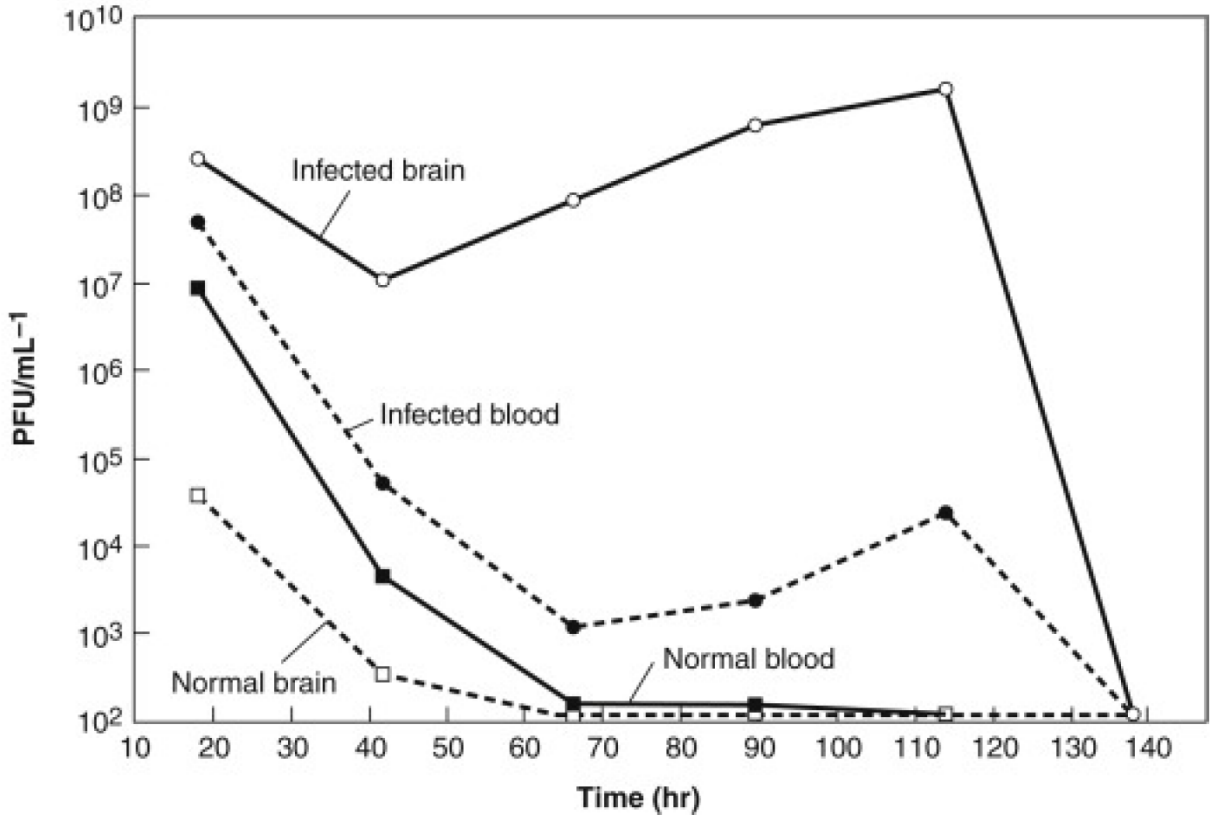
Memeli vücudu – Faj etkileşimi

- Fajlar doğada yaygın olarak bulunur
- Gıdalar ile alınır, insan/hayvan bağırsaklarına saptanır
  - Hayvan/insan serumlarında anti-faj antikorlar saptanır
    - Doğal immünite
  - Bakteriyel enfeksiyonların faj tedavisi sonrasında anti-faj antikor geliştiği saptanmıştır.
    - Nötralizan antikorların serumdaki miktarı , yarılanma ömrü, tedavi yanıtına etkisi ???

  - Vücuda alınan faj, kendine spesifik bakteri ile karşılaşırsa bakteri içinde replikle olur
  - Spesifik bakteri ile karşılaşmaz ise fagositik hücreler tarafından vücuttan elimine edilir.

Deneysel amaçlı yüksek miktarlarda faj maruziyetine rağmen; immünolojik bir yan etki saptanmadı.

- Fare deneyi (1943, ABD)
- *Shigella dysenteriae*



Shigella	Shigella + Faj
Canlı kalan 3/84 (%3,6)	Canlı kalan 46/64 (%72)

IMAGE

Phage Therapy: Bacteriophages as Natural, Self-limiting Antibiotics

Textbook of Natural Medicine.

- **Yıllardır standart tedavi olarak verilmektedir**.
  - Yanık tedavisinde
  - Cerrahi yara tedavisinde
  - Gastroenterit tedavisi

- **Sovyetler Birliği döneminde**
  - Faj üretim fabrikasında 700 kişi çalışıyor
  - Haftada 2 ton faj solüsyonu üretiliyor.

  - *Staphylococcus, Streptococcus, Proteus, P. aeruginosa, Clostridium*

- 1999-2000 yıllarında yapılan bir çalışma,
- 107 hasta
- Antibiyotik ve diğer tedavi yöntemlerine yanıt vermeyen yaralarda faj terapisi
  - % 70 tam iyileşme

Hirszfeld Institute of Immunology and Experimental Therapy Wrocław/ POLONYA

- **1981-86 arasında 10 merkezde 550 hasta**
  - Persistan süpratif fistül/abse
  - Septisemi
  - Solunum yolunun süpüratif enfeksiyonalrı
  - Pnömoni
  - Peritonit
- **Ekenler**
  - *Staphylococcus, Klebsiella, Proteus, Escherichia, Shigella, Pseudomonas, Salmonella*
- **Tedavi şekli**
  - 3*10 ml faj solüsyonu PO
  - 3*1 faj solüsyonu ile yara pansumanı
  - İV TEDAVİ VERİLMEDİ, Fajlar kanda, idrarda teröpatik düzeylerde saptandı
  - SÜRE: 1,5-14 hafta (5,3 hafta)

- Tedavi başarısı % 92
- Lokal yaralarda %82 tam iyileşme
- Yaşlı hastalarda başarı daha düşük
- Önemli bir yan etki görülmedi

- 1970 li yıllarda DSÖ öncülüğünde Pakistan’da **kolera** bakteriofajlar ile tedavi ediliyordu.
  - Faj tedavi Tetrasiklin kadar etkili değildi
  - Vücutta vibrio dışındaki mikroorganizmalar etkilenmiyordu.
  - Faj tedavisinde yan etki yoktu.

Curr Microbiol (2017) 74:277–283

Faj Tedavisi - Staphylococcus aureus Enfeksiyonu

- Yara tedavisinde
- Hastanelerde cerrahi alet ve çevre sanitasyonunda

- Faj solüsyonu ile el yıkama
  - *Faj içeremeyen yıkamaya göre; Stafilokok sayısında 100 kat daha fazla azalma*

- Nazal stafilokok eliminasyon tedavisinde
- MRSA taşıyan sağlık personeline oral faj solüsyonu tedavisi

- 550 Stafilokok enfeksiyonu epizodunda % 90 üzerinde başarı

Faj Tedavisi - Ürogenital Sistem Enfeksiyonları

- 42 ürogenital enfeksiyonda % 93 başarı
- Akut sistite tedavi yanıtı 4-5 saatte görülür, 1-3 günde kür.
- Kronik sistitte başarı daha düşük

Faj Tedavisi - Solunum Sistemi Enfeksiyonları

- 180 enfeksiyon atağı % 87 başarı

- Kistik fibrozis gibi akciğer hatalıklarında faj solüsyonu nebülüzatör ile uygulanmış

Faj Tedavisi - Kulak ve Göz enfeksiyonları

- Otitis eksterna tedavisinde kullanıldı
- 16 Konjonktivit vakasında % 94 başarı

Faj Tedavisi - Yan etki

- Temiz su içerisinde 10^8 faj/ml bulunur
- Normal koşullarda vücutta; cilt, idrar, ağız, bağırsak içeriklerinde fajlar mevcuttur.
- Hasta serumlarında, ticari aşı içeriklerinde saptanmıştır.
- Deney hayvanlarına, insana verilen dozun 3500 katı verilmiş ve toksik etki saptanmamıştır.

Genel Kabul: Bakteriofajlar insanlar için güvenlidir.

İntavenöz uygulama

Lokal veya oral uygulanan faj sistemik dolaşıma geçiyor – güvenli

Faj solüsyonu yeterince pürifiye edilemeyebilir, Bakteremi riski ???

Faj Tedavisi - İlaç Etkileşimi

- Bilinen bir etkileşim yok.
- Bu konuda geniş araştırmalar yok
- «Biyo-kimyasal» olarak faj ile ilaçların etkileşimi öngörülmüyor.

Faj Tedavisi – Doz

- Düşük doz stratejisi
  - Faj enfeksiyon bölgesinde replikle olur,
  - Teröpatik konsantrasyonlara ulaşır.
  - Yüksek doza gerek yok ?

- Standart doz yöntemi
  - Replikasyon dikkate alınmaz
  - Standart doz verilir.

Solüsyon 10^{6} - 10^{7} PFU/mL içerir ve
Genellikle 5 - 10 mL uygulanır

	Antibiyotik	Bakteriofaj
Dokulara penetrasyon	Dokuya göre değişken	İyi penetrasyon
Hedef mikroorganizma seçicilik	Yok – sınırlı	Mükemmel
Sekonder bakteriyel enfeksiyon riski	Olası	Yok
Bakteriyel direnç gelişimi	Olası-Kaçınılmaz!	Yok (?)
Ciddi Yan etki	Görülebilir	Yok (?)
Antibiyotiklere alerji varlığında	Kullanılmaz	Engel yok
Hastane sanitasyonunda kullanım	Kullanılmaz	Mümkün
Ekosisteme etki	Olumsuz	Milyonlarca yıldır mevcut
Kontrolü klinik çalışma deneyimi	Mevcut	Sınırlı
Endüstriyel standartlar (üretim, endikasyon, doz vb)	Mevcut	Henüz tanımlanmadı
Lizojenik fajların direnç geni transferi	-	Bilinmiyor
Hızlı bakteri yıkımı, sitokin fırtınası	Olası	Olası

Günümüzde faj tedavisi

- Gürcistan
- Rusya
- Polonya

100 yıldır

- Fransa
- Belçika
- İsviçre
- ABD

Eliava Est. GÜRCİSTAN

Enterococcus faecalis
E. coli
Proteus vulgaris
P. mirabilis
Pseudomonas aeruginosa
Salmonella
Shigella
Staphylococcus aureus
S. epidermidis
S. saprophyticus
Streptococcus pyogenes
S. sanguis
S. salivarius

Wroclaw Est. POLONYA

Acinetobacter
Burkholderia
Citrobacter
Enterobacter
Enterococcus
Escherichia
Klebsiella
Morganella
Proteus
Pseudomonas
Shigella,
Salmonella
Serratia
Staphylococcus
Stenotrophomonas.

Bacteriophage. 2016 Oct 21;6(4):e1251379. doi: 10.1080/21597081.2016.1251379.

Phagebiotics in treatment and prophylaxis of healthcare-associated infections.

Aleshkin AV[1], Ershova ON[2], Volozhantsev NV[3], Svetoch EA[3], Popova AV[3], Rubalskii EO[4], Borzilov AI[3], Aleshkin VA[5], Afanas'ev SS[5], Karaulov AV[6], Galimzyanov KM[7], Rubalsky OV[7], Bochkareva SS[5]

- **Abstract**
- We have developed a phagebiotic composition using 8 virulent bacteriophages (2 strains of each species) which are able to lyse *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
- Our small-scale clinical trial was aimed to evaluate therapeutic effectiveness of the phagebiotic composition in an epidemiological emergency situation in an intensive care unit, caused by multi-resistant strains of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Seventy nine per cent of the initial samples from 14 patients' endotracheal aspirate, blood and urine were contaminated. Twenty-four hours after the 3-day phage therapy (20 ml of cocktail at a titer for each phage 10^8 pfu/ml were introduced intragastrically through a tube once a day) contamination level dropped to 21%. Hence the obtained results enabled us to create a new phagebiotic composition that may be used as an alternative to antibiotics to treat these healthcare-associated infections.

Özgün Çalışma/Original Article *Mikrobiyol Bul 2013; 47(1): 27-34*

Klinik MRSA İzolatlarından Elde Edilen Yeni Bir Litik Bakteriyofajın Tanımlanması ve Antibakteriyel Etkisinin Değerlendirilmesi

Identification of a Novel Lytic Bacteriophage Obtained from Clinical MRSA Isolates and Evaluation of Its Antibacterial Activity

Fikret ŞAHİN, Djursun KARASARTOVA, T. Murat ÖZSAN, Devran GERÇEKER, Mehmet KIYAN

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Ankara University Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey.

antibiyotiklerin, özellikle metisiline dirençli *Staphylococcus aureus* (MRSA) suş-
dirençli enfeksiyon etkenleri üzerindeki etkinliklerinin giderek azaldığını bildir-
ni antibiyotik geliştirme çalışmalarının yetersizliği nedeniyle, patojen bakterile-
(faj) kullanılabileceği alternatif tedavi yöntemleri gündeme gelmiş; faj tedavisi
sından da önem kazanmıştır. Bu çalışmada, 20 klinik MRSA izolatından elde edi-
ı MRSA suşlarını enfekte edebilme özelliklerinin araştırılması sırasında tanımla-
bakteriyel ve sitotoksik etkilerinin değerlendirilmesi amaçlanmıştır. ϕ LizAnk ola-
n, farklı MRSA izolatları üzerindeki antibakteriyel etkisi, BHI (Brain Heart Infusi-
sıvı besiyerlerinde in vitro koşullarda belirlenmiş; ayrıca fajın MRSA'lara in vivo
antibakteriyel etkisi ve memeli hücresine muhtemel sitotoksik etkisi fibroblast hücre kültürleri (3T3) kul-
lanılarak araştırılmıştır. Çalışmada, hastanede yatan hastalardan izole edilen 20 MRSA suşu kullanılmış;
izolatlar konvansiyonel yöntemlerle tanımlanmış; metisilin direnci ise oksasilin disk difüzyon testi ve PCR
ile *mec*A geni saptanarak belirlenmiştir. MRSA'lardan faj indüksiyonu ve izolasyonu, Kaneko ve arkadaşla-
rı [*Biosci Biotechnol Biochem 1997; 61(11): 1960-2*] tarafından tanımlanan yöntemde bazı modifikasyon-
lar yapılarak gerçekleştirilmiştir. Yaptığımız in vitro deneylerde, ϕ LizAnk fajının, 10^7 cfu/ml miktarındaki
altı farklı MRSA izolatını sekiz saat içerisinde tamamen ortadan kaldırdığı saptanmış; bu güçlü litik etki-
nin her iki sıvı besiyerinde de aynı olduğu belirlenmiştir. İn vivo çalışmalarda ise, mikroplaklarda hazırla-
nan hücre kültürlerini içeren kuyucuklara; sadece faj, faj + MRSA karışımı ve sadece MRSA eklendikten
sonra, 2 ve 24. saatte hem mikroskopik hem de canlı hücreleri kolorimetrik olarak saptayabilen MTT yön-

frontiers in Microbiology

ORIGINAL RESEARCH
published: 03 November 2016
doi: 10.3389/fmicb.2016.01761

In vitro Effectiveness of Commercial Bacteriophage Cocktails on Diverse Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* Strains

Aycan Gundogdu[1,2], Darajen Bolkvadze[3] and Huseyin Kilic[1]*

[1] Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey, [2] Genome and Stem Cell Center (GENKOK), Erciyes University, Kayseri, Turkey, [3] Laboratory of Molecular Biology, G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

The objective of this study is to determine the *in vitro* susceptibility of Georgian bacteriophage cocktails on multidrug resistant (MDR) extended-spectrum beta-lactamase producing *Escherichia coli* (ESBL-EC) isolated from patients' blood and urine cultures. A total of 615 *E. coli* isolates were included in this study. Phene Plate (PhP)-typing and phylogenetic grouping were used for the typing. Antimicrobial resistance profiles and ESBL production of all isolates were confirmed according to Clinical and Laboratory Standards Institute (CLSI) criteria. The activities of four bacteriophage cocktails (Enko-phage, SES-bacteriophage, Pyo-bacteriophage, and Intesti-bacteriophage) were determined against 142 ESBL-EC using *in vitro* spot tests. According to this, Enko-phage were active against 87.3% of the tested strains while that ratio was 81.7% for Intesti-bacteriophage, 81.7% for Pyo-bacteriophage, and 59.2% for SES-bacteriophage cocktails. Based on the contingency tests, the phage cocktails were observed to be statistically significantly ($p < 0.001$) more effective on ESBL-EC strains belonging to phylogenetic groups D and B2. The employed phage cocktails were found to be affective against all tested resistant types. These results are promising especially for the infections that are caused by MDR pathogens that are difficult to treat. As this is a preliminary step to the potential clinical trials to be designed for the country, *in vitro* confirmation of their success on a MDR ESBL-EC collection should be accepted as an initial action, which is encouraging to consider clinical trials of phage therapy especially in countries which are not introduce phage therapy.

Keywords: ESBL-*E. coli*, bacteriophage, antibiotic resistance, phage therapy, phylogenetic grouping

- Mikrobiyol Bul. 2016 Apr;50(2):215-23.
- **[Susceptibilities of multidrug-resistant pathogens responsible for complicated skin and soft tissue infections to standard bacteriophage cocktails].**
- [Article in Turkish]
- Gündoğdu A[1], Kılıç H, Ulu Kılıç A, Kutateladze M.
- **Author information**

- **Abstract**
- The aim of this study was to investigate the in vitro susceptibilities of multidrug-resistant (MDR) pathogens isolated from patients with complicated SSTIs, against standard bacteriophage (phage) cocktails.
- Six different ready-made phage preparations used in this study have been **provided by G. Eliava Institute**, Georgia.

- Accordingly, a total of **33 isolates**, nine of them were E.coli, nine were MDR P.aeruginosa; nine were MDR A.baumannii; three were MRSA and three were K.pneumoniae

- In the study, **29 (87.9%) out of 33 MDR pathogens were found to be susceptible** to at least one of the tested phage/phage preparations. All MRSA (3/3) strains were susceptible to ENKO, SES, Fersisi and Sb phage cocktails, while all A.baumannii isolates (9/9) were susceptible to Φ5 and Φ7 phages.

- However, **two E.coli, one K. pneumoniae and one P.aeruginosa strains were resistant** to the all phage preparations tested.

Clin Invest Med. 2016 Dec 1;39(6):27504.

Lytic Activity of Various Phage Cocktails on Multidrug-Resistant Bacteria.

Ozkan I[1], Akturk E, Yeshenkulov N, Atmaca S, Rahmanov N, Atabay HI.

Author information

•[1]. ismail.ozkan@sifa.edu.tr.

S. aureus		*P. aeruginosa*	
Duyarlı:	**8**	**Duyarlı:**	**9**
Dirençli:	**2**	**Dirençli:**	**1**

Abstract

PURPOSE:

A bacteriophage is a virus that infects and replicates within a
bacterial cytoplasm. They are seen as a possible therapy for
study is to evaluate the lytic activity of the Pyo, Intesti and Fe

METHODS:

Ten different S. aureus and P. aeruginosa strains, which were isolated from hospitalized patients in Turkey, were used in the study. The identification and antibiotic susceptibility of the isolates were performed using Vitec 2 system. The identities of the isolates were confirmed by a species-specific Polymerase Chain Reaction (PCR) assay. Lytic activity of the bacteriophage cocktails on bacteria was determined by spot test and plaque assay methods.

RESULTS:

The lytic activity of the Pyo phage cocktail was evaluated on P. aeruginosa and S. aureus strains. It was found that eight isolates of MDR S. aureus were susceptible to Pyo phage cocktail and two isolates were resistant. Nine isolates of antibiotic-resistant P. aeruginosa were found to be susceptible to this phage cocktail and one isolate was resistant. Thus, the Pyo, Intesti and Fersisi cocktails are very effective in treating clinical strains of multidrug-resistant P. aeruginosa and S. aureus isolated in Turkey.

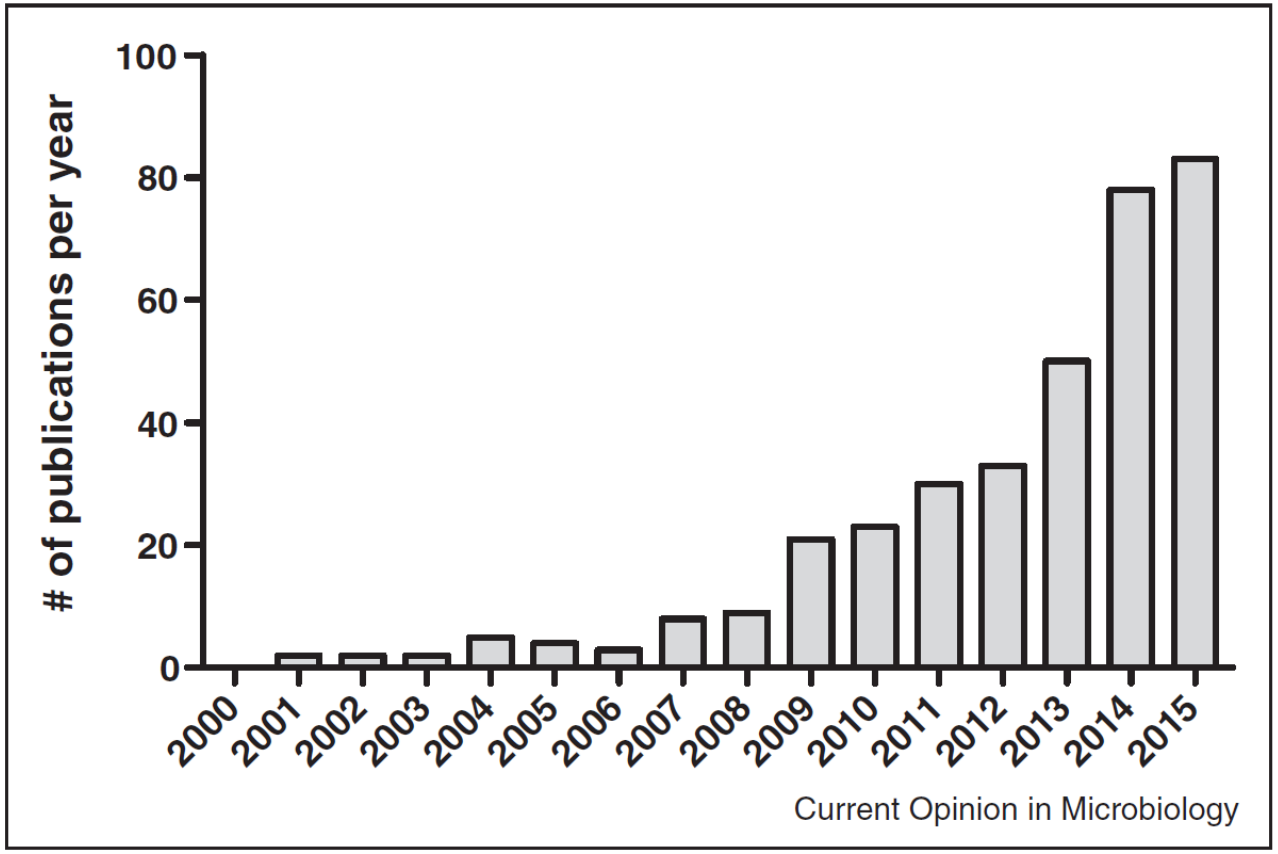
CONCLUSION:

The Pyo, Intesti and Fersisi cocktails may prove useful in the treatment of various infections caused by those bacteria.

Anti-Virülan Tedavi

- Bakterinin viabilitesini, üremesini etkilemeden;
  - hastalık oluşturmasında rol oynayan virülans faktörlerini *(toksin, enzim, yüzey proteini, kapsül vb)* veya bu faktörlerin sentezini yöneten süreçleri inhibe etmeyi amaçlayan tedavi yöntemlerini içerir
- Bu yöntem ile;
  - Antibiyotik kullanımı azaltılabilir
  - Antibiyotiklere direnç gelişimi azaltılabilir
  - Vücut florasına antibiyotik etkisi azaltılabilir

Toxins **2016**, 8, 72 5



Growth in publications with the keywords 'anti-virulence' or 'antivirulence' in PubMed. Some relevant publications without these keywords were likely not included.

Anti-virülan tedavide hedeflenen temel virülans faktörleri

- **Spesifik virülans faktörleri**
  - Tip3 sekresyon sistemi (T3SS)
  - Enterotoksinler
- **Virülans regülatörleri**
  - quorum sensing (QS)
- **Konak savunma sistemine ve antibiyotiklere direnç**
  - Kapsül
  - Staphyloxanthin
  - Biofilm

Table 1

Selected examples of anti-virulence targets and inhibitors for ESKAPE pathogens

Target	Pathogen	Example inhibitor
Hla	*S. aureus*	Morin hydrate
Staphlyoxanthin	*S. aureus*	Phosphonoacetamide derivative
Enterotoxins	*S. aureus*	Menthol
Sortase A	*S. aureus*	Chlorogenic acid
Biofilm	*S. aureus*	Black pepper oil
	A. baumannii	TAGE-triazole conjugates
	K. pneumoniae	GarO (garlic ointment)
	P. aeruginosa	Mix of sugars
QS	*S. aureus*	C14-TOA (3-acyltetronic acid)
	P. aeruginosa	M64
C-di-GMP	*P. aeruginosa*	Ebselen
Protein secretion	*P. aeruginosa*	Anti-PcrV antibody
Capsule	*S. aureus*	Fascioquinol E
	K. pneumoniae	Triazines

Current Opinion in Microbiology 2016, 33:41–46

Table 2
Examples of inhibitors of virulence that have shown efficacy in animal models.

Target	Inhibitor	Mode of action
Adhesion	Pilicides/curlicides (Cegelski et al., 2009)	Inhibit *E. coli* pilus and/or curli assembly
Biofilm production/Quorum-sensing	RNAIII-inhibiting peptide (Balaban et al., 1998)	Inhibits *S. aureus* quorum sensing
	Furanone derivates (Hentzer et al., 2003)	Inhibit *P. aeruginosa* quorum sensing
Toxins	Hydroxamate lethal factor inhibitor (Shoop et al., 2005)	Inhibits *B. anthracis* lethal factor protease activity
	Cisplatin (Moayeri et al., 2006)	Inhibits *B. anthracis* protective antigen
	Polyvalent inhibitors of anthrax toxin (Mourez et al., 2001)	Inhibit *B. anthracis* toxin assembly
	β-cyclodextrin derivatives (Karginov et al., 2005)	Block the *B. anthracis* protective antigen pore
	BPH-652 (Liu et al., 2008)	Inhibits *S. aureus* production of staphyloxanthin
	Ac-PPP-tet (Watanabe-Takahashi et al., 2010)	Alters the intracellular transport of *E. coli* Stx2
Type III secretion	INP0007 and INP0403 (Hudson et al., 2007)	Block type III secretion in *Y. pestis* and *Salmonella enterica* serovar Typhimurium
Virulence gene regulation	LED209 (Rasko et al., 2008)	Inhibits QseC signaling in several Gram-negative pathogens
	Virstatin (Hung et al., 2005)	Inhibits *V. cholera* production of cholera toxin and the toxin coregulated pilus

Anti-virülan ürün adayları

- **Anti-virülan ürünler;**
  - Doğal maddelerden elde edilen ürünler
    - Sarımsak, mentol, karanfil, karabiber enterotoksinlere karşı etkili (?)
  - Doğal maddelerin modifikasyonu
  - Kimyasal laboratuvarda üretilen sentetik ürünler

Anti-virulan tedavi - problemler

- **Ürüne karşı rezistans gelişmesi**
  - In vitro çalışmalarda direnç gelişimi saptandı.
  - İnvivo çalışmalar henüz yapılmadı

- **Vücut moleküllerine etki**
  - Anti-biofilm inhibitörleri bakterinin karbonhidrat bağlayan **lektin** molekülünü hedefler.
  - İnvivo ve invitro çalışmalarda ürünün etkinliği gösterildi.
  - Ancak lektin insan vücudunda da var!

Current Opinion in Microbiology 2016, 33:41–46

Teşekkürler

- https://youtu.be/vmZBRhVxatk

- https://youtu.be/RIht7UDqGS0
- https://youtu.be/Mw8FiwSRMok