BUHASDER 2021 KONGRESİ

24-28 Kasım 2021
Mirage Park Resort Hotel
Kemer - Antalya

Diyabetik Ayak İnfeksiyonlarında Bakteriyofaj Tedavisi

Dr. Oral ÖNCÜL

İstanbul Tıp Fakültesi

İnfeksiyon Hst ve Klin Mik Srv

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Sunum Planı

- Diyabetik Ayak İnfeksiyonlarına Bakış
- Yeni tedavi arayışları ve Bakteriyofaj
- Bakteriyofajların etki mekanizmaları
- Klinik kullanımları
- Başarılı tedavi örnekleri
- Sonuç

Diabetes Mellitus ve Ayak Ülserleri

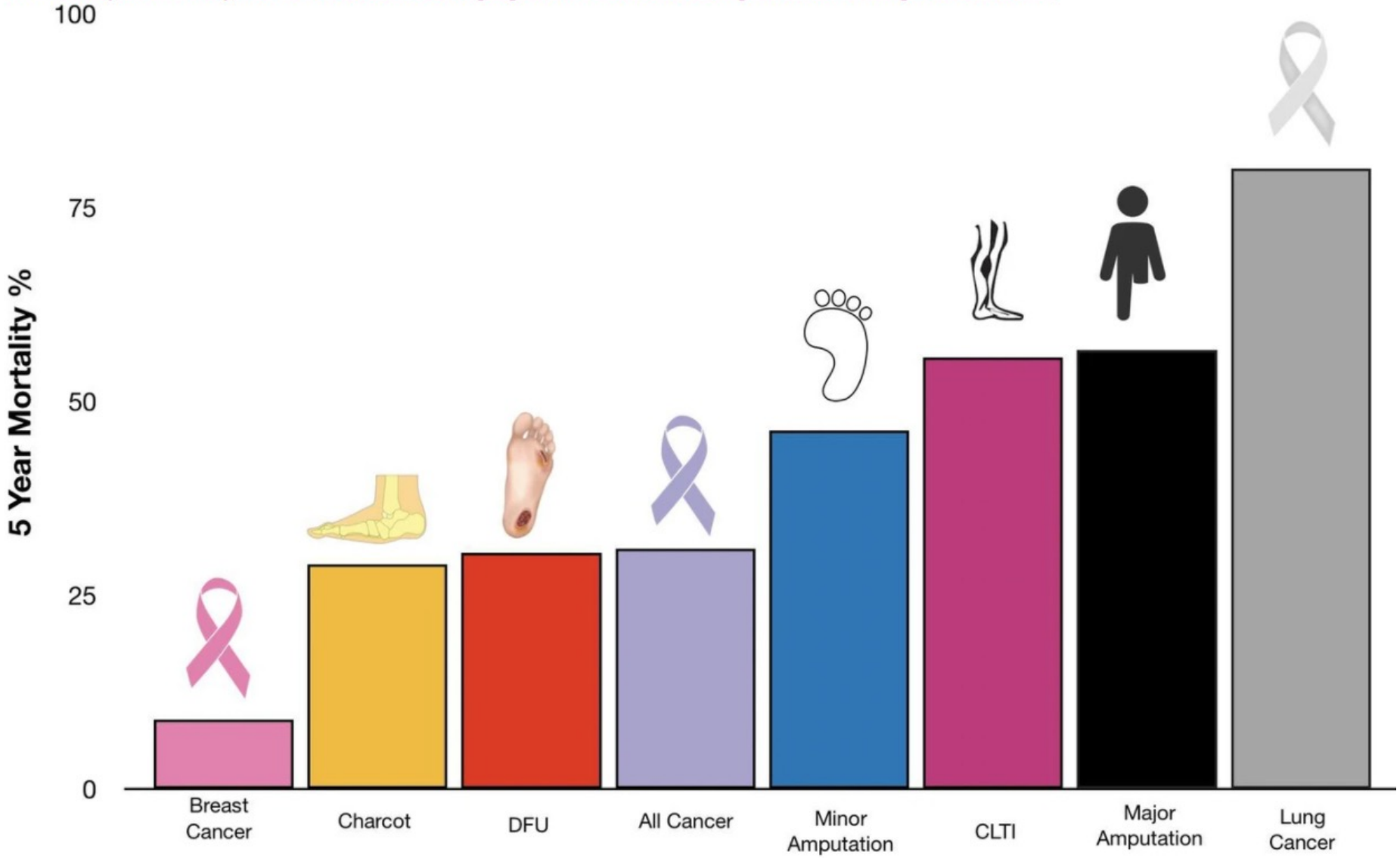
- DM'lu hastaların **%25-30'unda** ayak ülserleri gelişir
- Ayak ülserli DM hastalarının beş yıllık mortalite oranı **%40**
- Ayak ülserli hastaların **%17**'sinde ampütasyon gerekiyor

- Amputasyonlu hastalarda mortalite daha yüksek
  - **%49** / 1 yıl
  - **%69** / 5 yıl
  - **%90** / 10 yıl
- Ekonomik yükü:
  - DM 217 Milyar USD / 2017 (ABD) (2012'den sonra %26 artış)
  - DAI 74 Milyar USD /2017 (ABD)
  - Kanser 80 Milyar USD / 2015 (ABD)

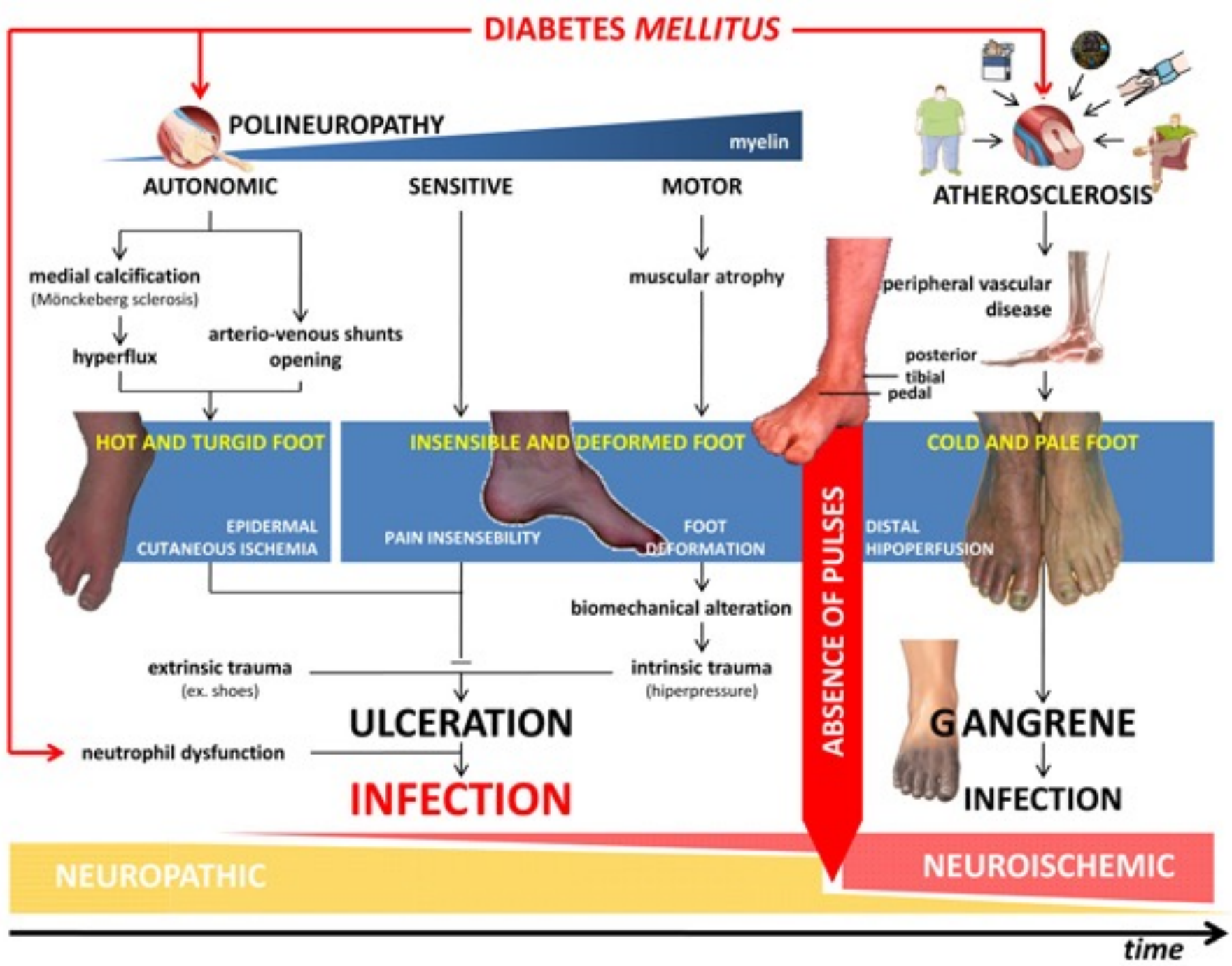
Jeyeraman K, et al. BMC Endocr Disord, 2019; 19: 1

Armstrong DG, et al. J Foot and Ankle Res, 2020; 13

From: Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer



Five Year Mortality of Diabetic Foot Complications and Cancer. Diabetic foot complications compared to cancer. DFU = diabetic foot ulcers [11] = 30.5%. Charcot = Charcot neuroarthropathy of the foot [14]. All Cancer = pooled 5 year survival of all cancers [11]. CLTI = chronic limb threatening ischemia [28, 29]. Major Amputation = above foot amputation [20,21,22, 26, 27]. Minor Amputation = foot level amputation [17, 27]


DIABETES MELLITUS
POLINEUROPATHY
myelin
AUTONOMIC
SENSITIVE
MOTOR
ATHEROSCLEROSIS
medial calcification
(Mönckeberg sclerosis)
hyperflux
arterio-venous shunts
opening
muscular atrophy
peripheral vascular
disease
posterior
tibial
pedal
HOT AND TURGID FOOT
EPIDERMAL
CUTANEOUS ISCHEMIA
INSENSIBLE AND DEFORMED FOOT
PAIN INSENSEBILITY
FOOT
DEFORMATION
COLD AND PALE FOOT
DISTAL
HIPOPERFUSION
ABSENCE OF PULSES
biomechanical alteration
extrinsic trauma
(ex. shoes)
intrinsic trauma
(hiperpressure)
ULCERATION
neutrophil dysfunction
INFECTION
GANGRENE
INFECTION
NEUROPATHIC
NEUROISCHEMIC
time

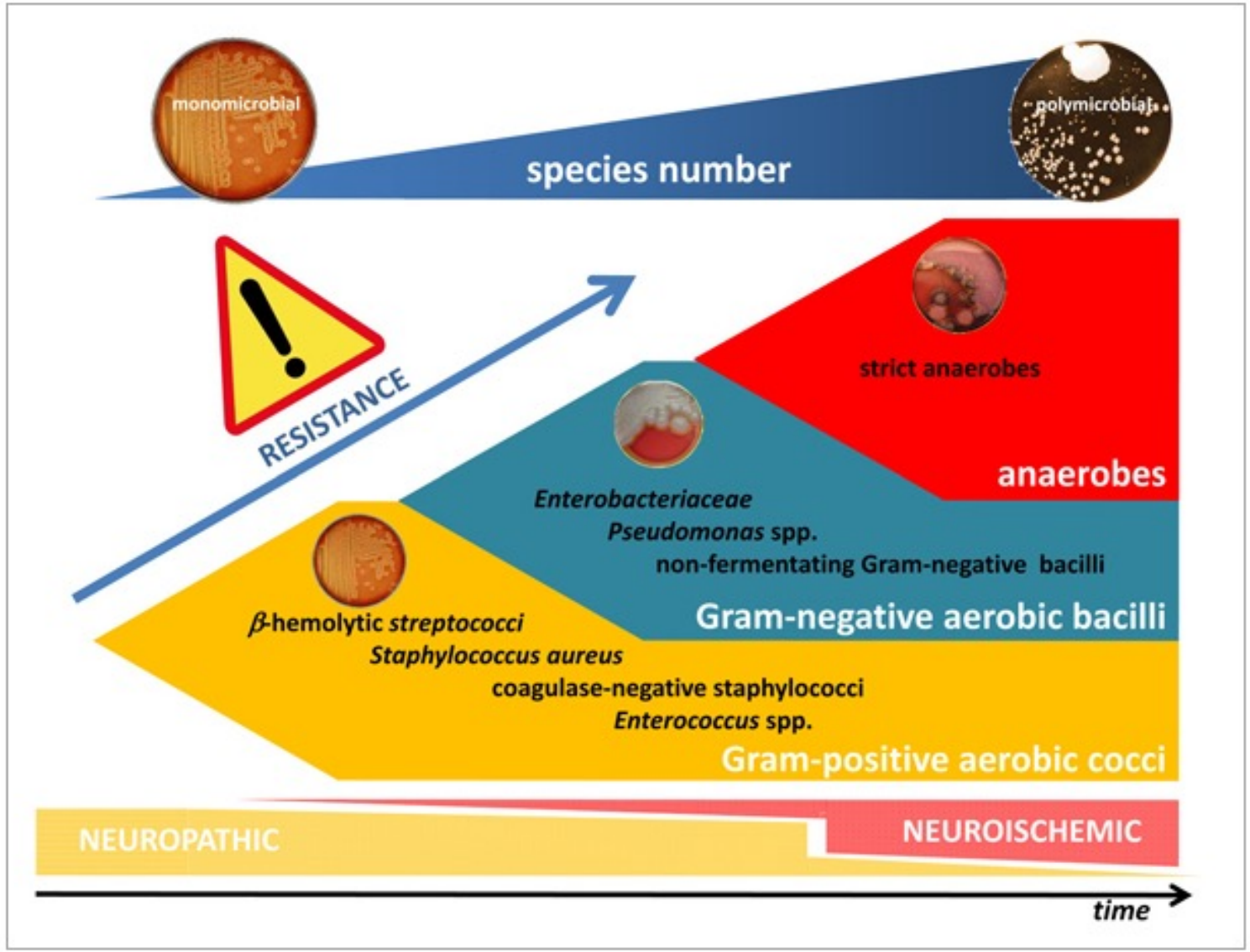


Figure 2: Qualitative and quantitative aspects of diabetic foot infections (DFIs). Staphylococcus aureus and ß-hemolytic streptococci are the first microorganisms to colonize and acutely infect breaks in the skin. Chronic wounds develop a more complex polymicrobial microbiota, including aerobic Gram-negative rods and anaerobes.

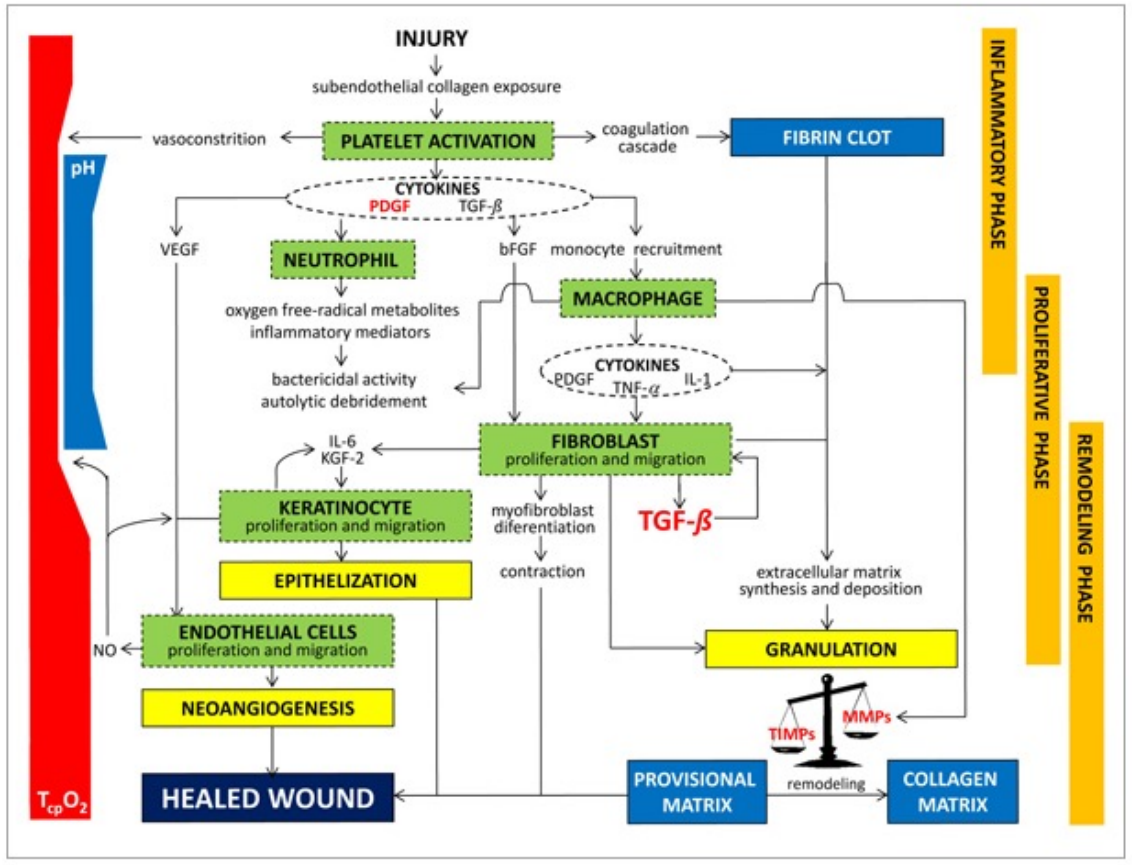
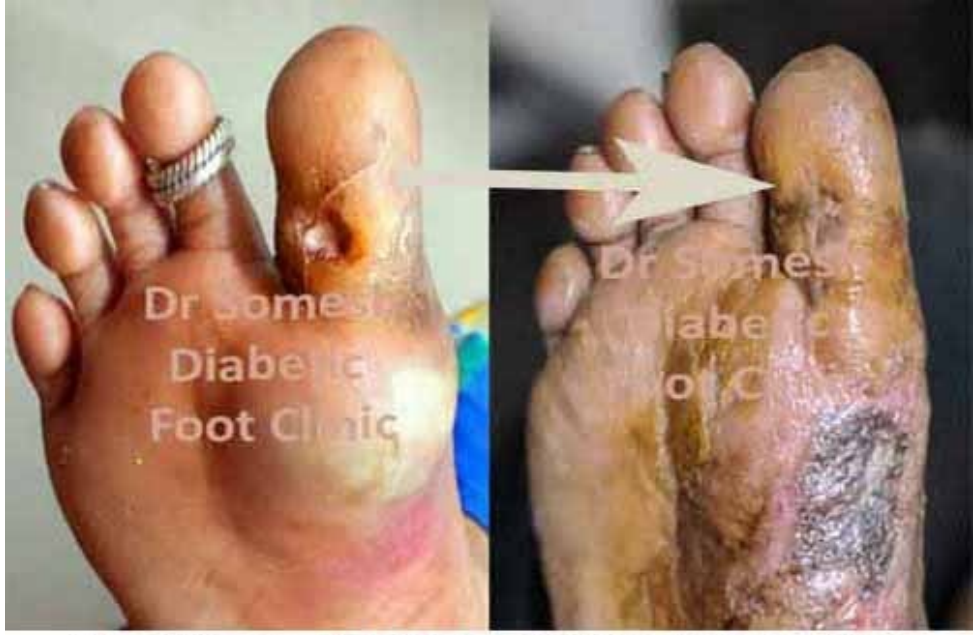
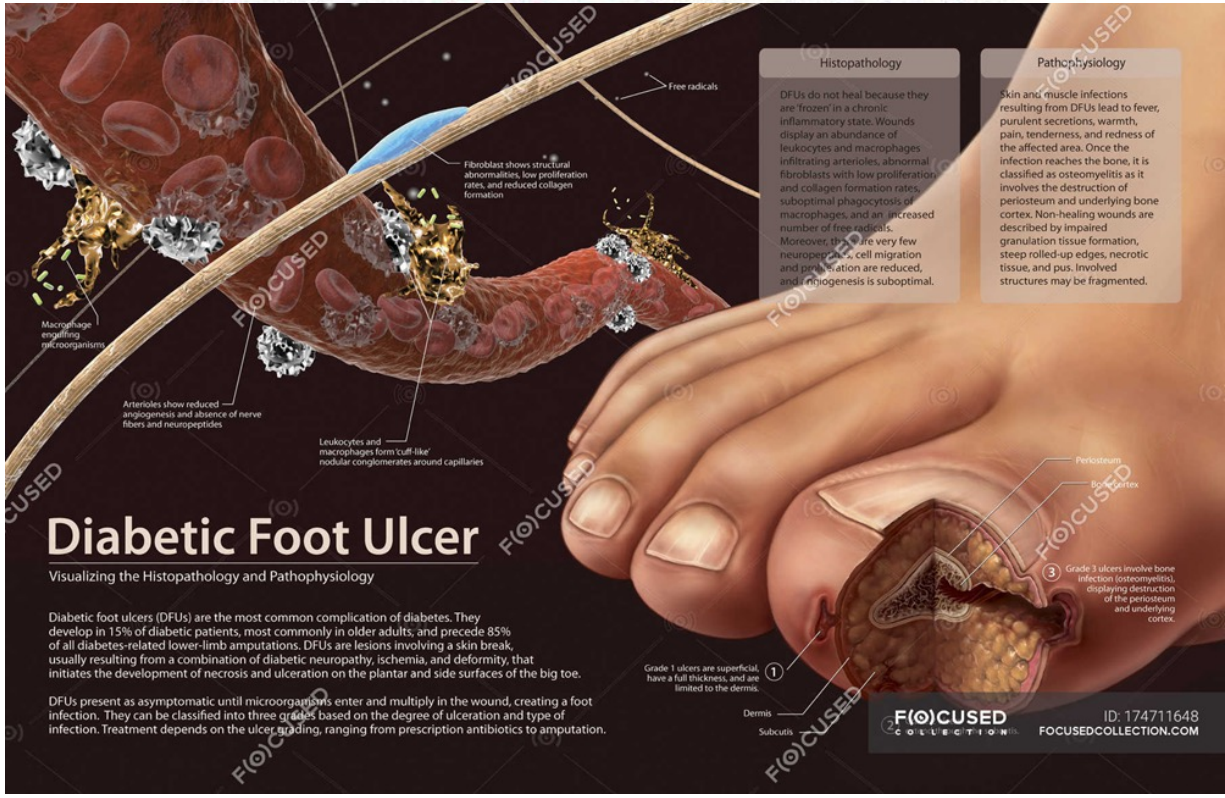


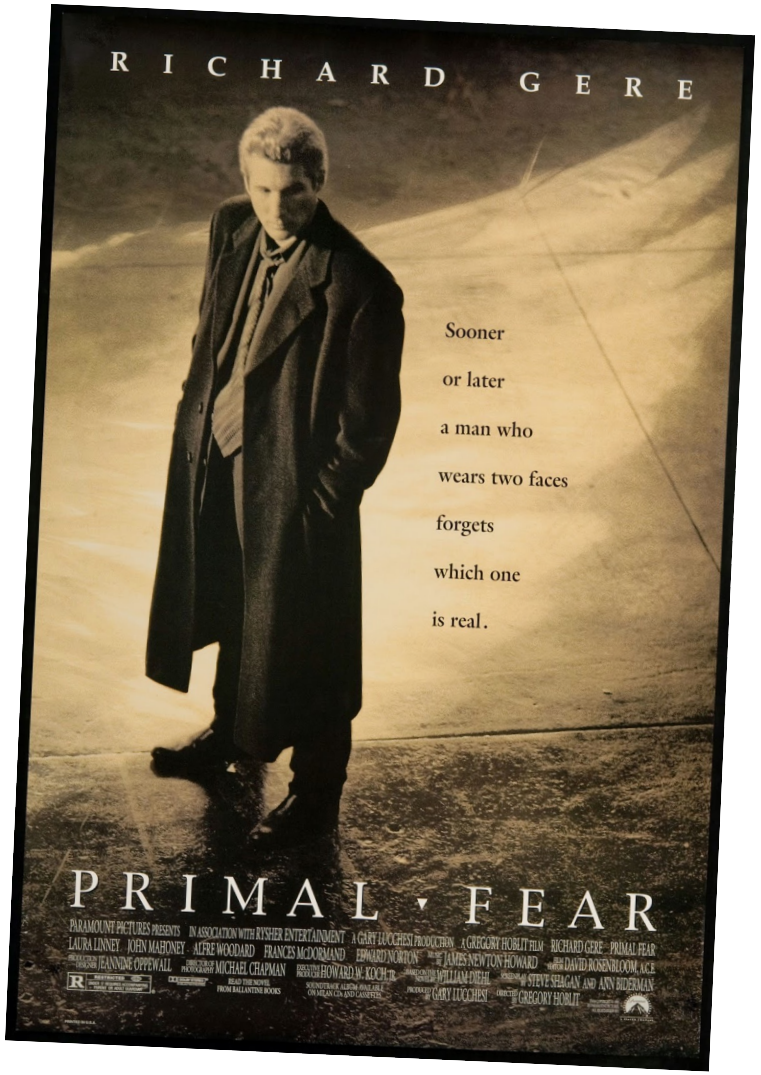
Figure 3: Model of acute wound healing. Wound healing is divided into three sequential but overlapping phases: (1) the inflammatory phase, (2) the proliferative phase (re-epithelialization, granulation, and neoangiogenesis), and (3) the remodeling phase (extracellular matrix remodeling). This complexly orchestrated biochemical cascade is characterized by signature events and cells, and their molecular regulators.





Diyabetik Ayak İnfeksiyonlarında Antibiyotik Başarısızlığı

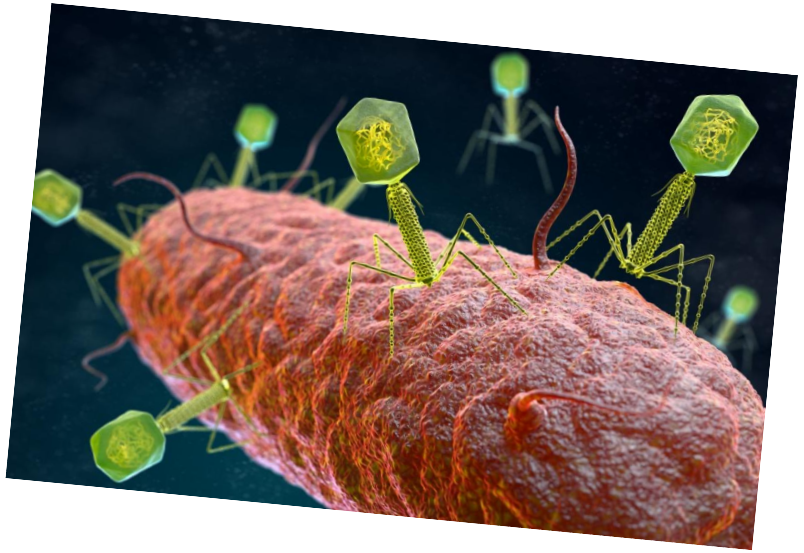
- Antibiyotiklerin hedef dokuda subteröpatik düzeyde kalması
- Polimikrobiyal etken
- Biyofilm oluşumu
- Uzun süreli antibiyotik kullanımı
- Antibakteriyel direnç artışı
- MDR bakterilerle yeni kolonizasyon riskleri
- Antibiyotik tedavisine katkı sağlayacak immün yanıt eksikliği



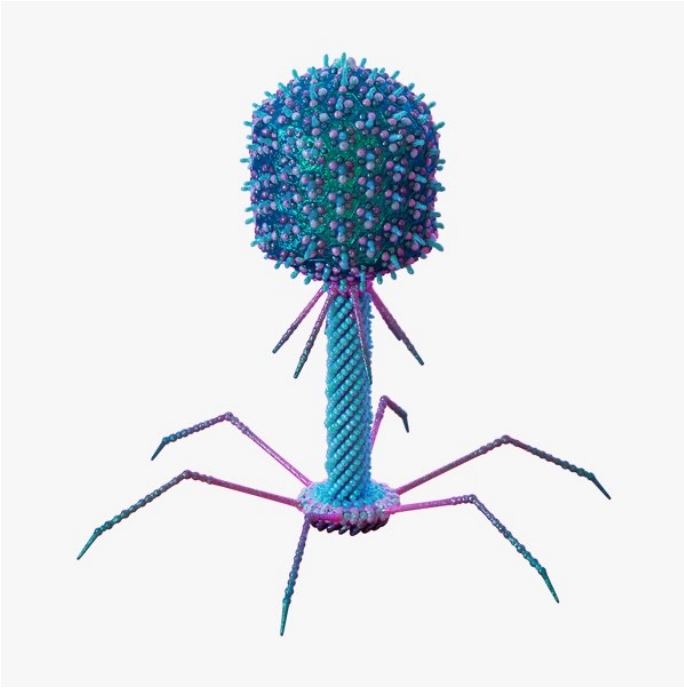
Yeni Arayışlar

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

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

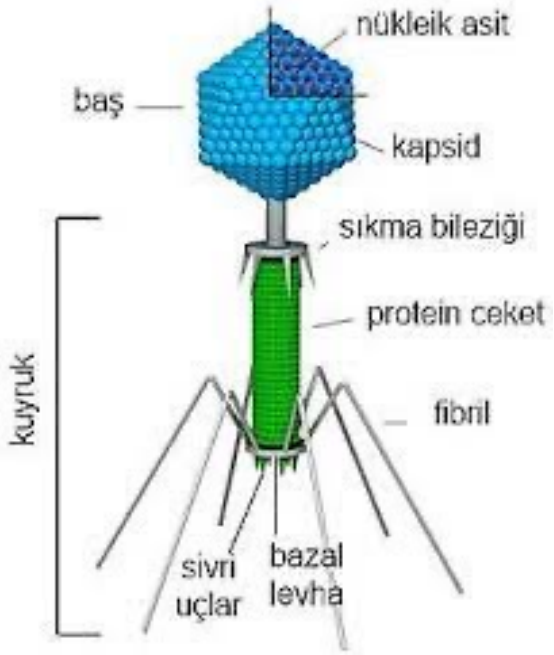


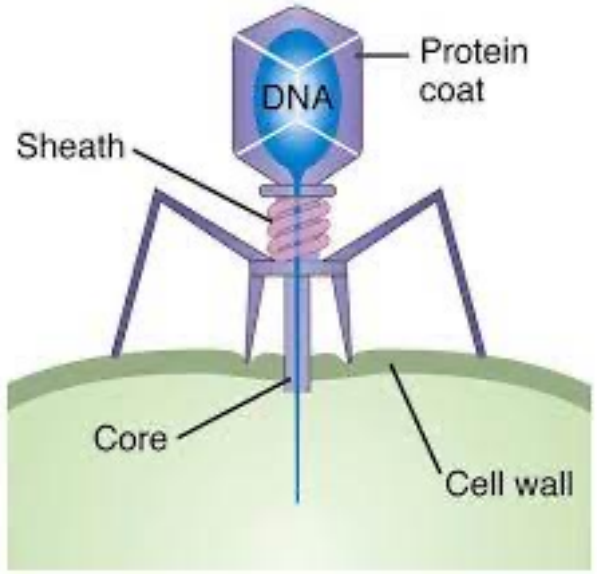
Bulunduğu Yerler

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

Bakteriyofaj Yapısı

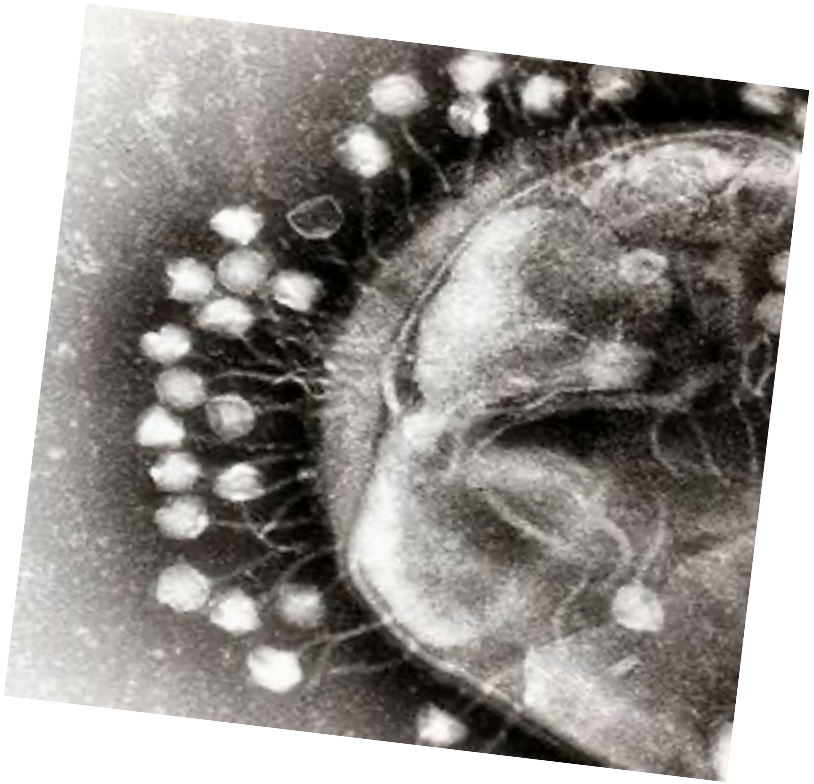
- Baş ve Kuyruk yapısından meydana gelir
- Nükleik asid baş kısmında bulunur
- Fibril uçlarla spesifik reseptörlere tutunur
- Bazal levha ile bakteri hücre zarına oturur
- Bakteri hücre yapısında porlar açılır
- Kuyruk içeriğinden gen aktarımı sağlanır

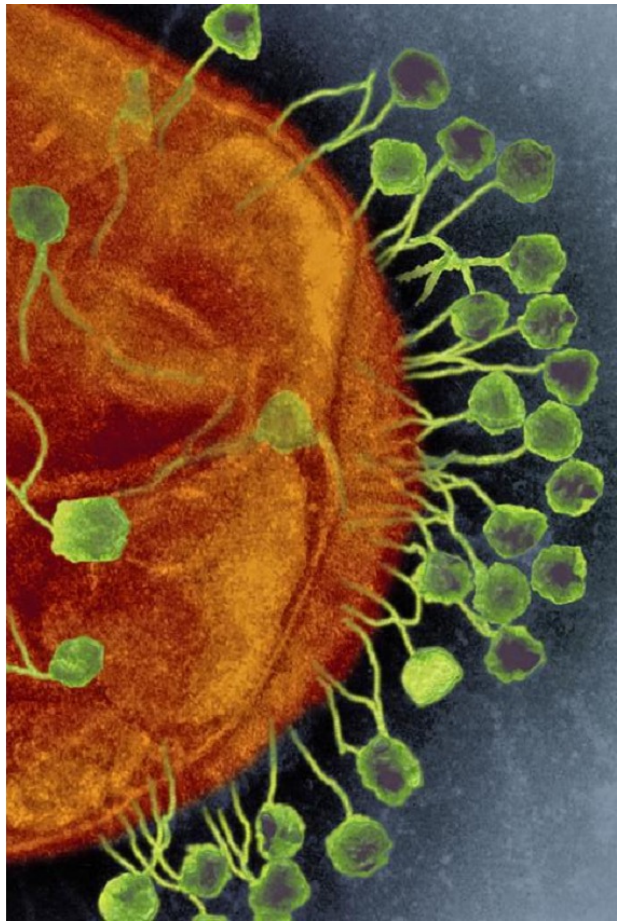


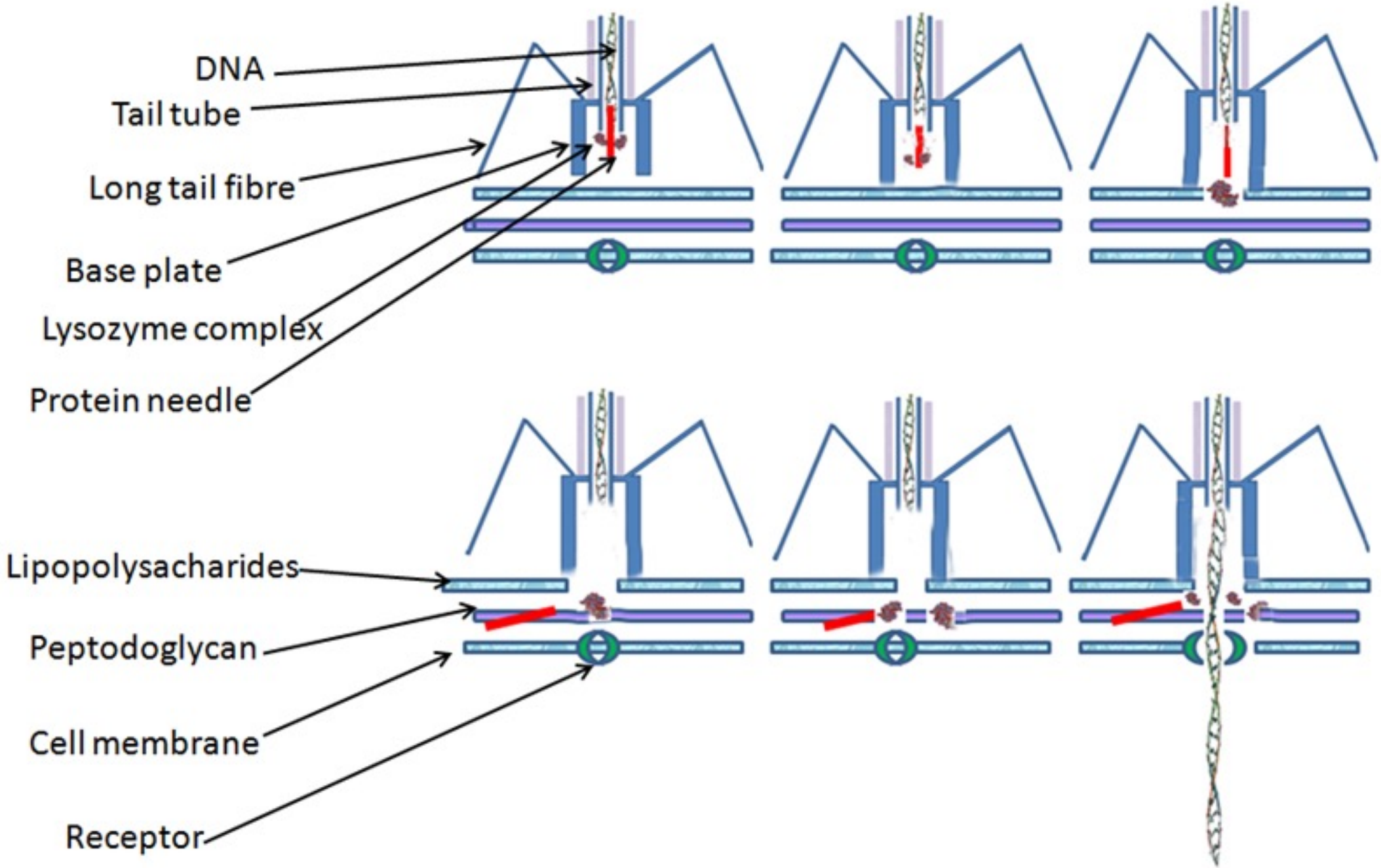


Tutunma ve Penetrasyon

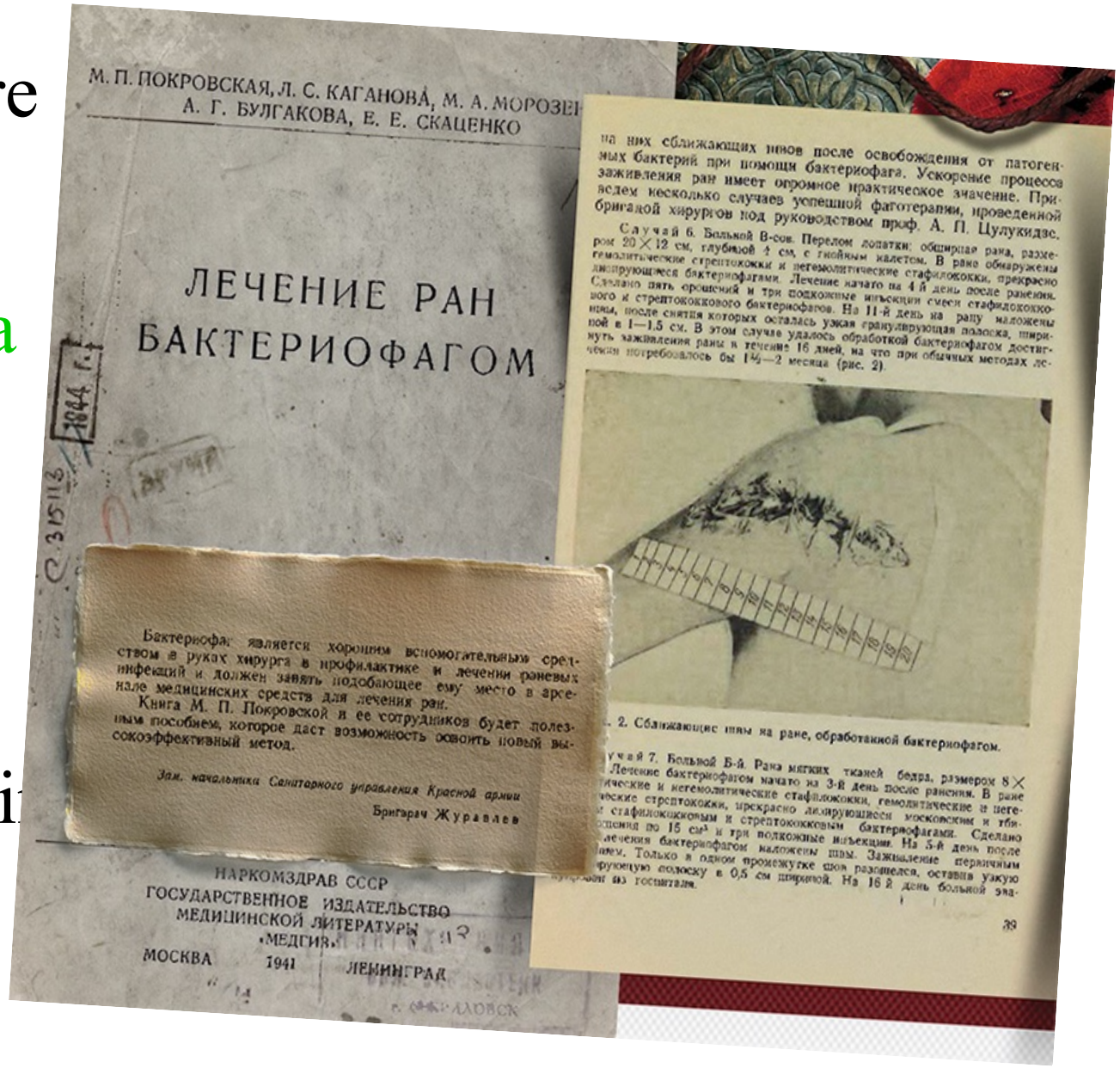
- Bakteri yüzeyinde spesifik reseptörlere bağlanır:
  - Liyopopolisakkaridler,
  - Teikokik asit,
  - Proteinler,
  - Flagella.



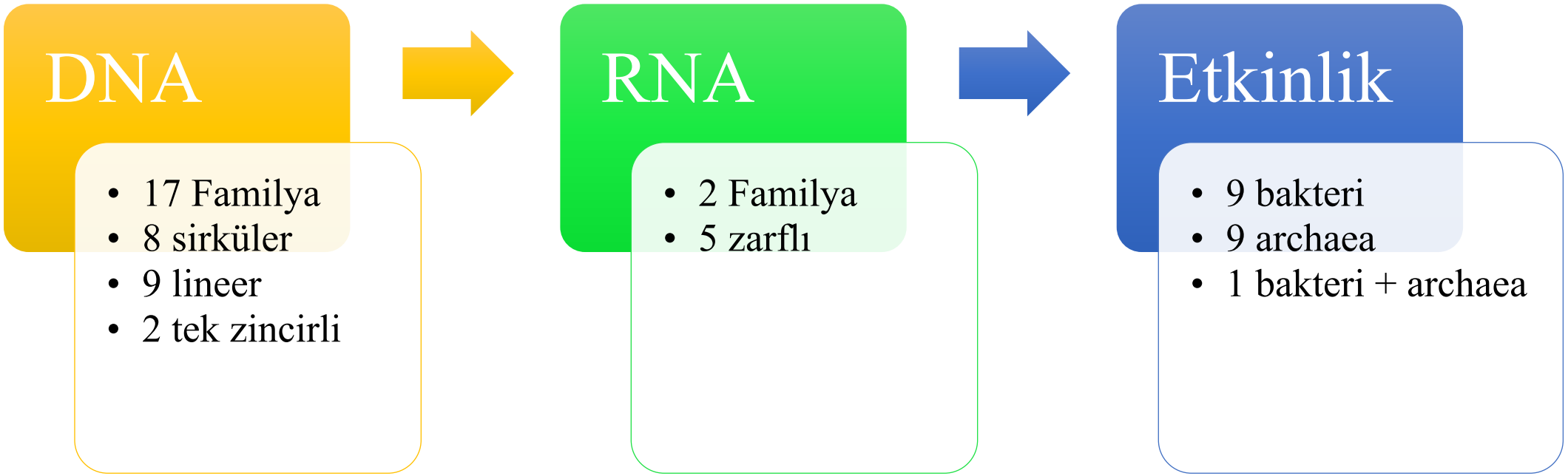



DNA
Tail tube
Long tail fibre
Base plate
Lysozyme complex
Protein needle
Lipopolysacharides
Peptodoglycan
Cell membrane
Receptor

- Yaklaşık 100 yıldır antibiyotiklere alternatif olarak kullanılmakta
- Eski SCCB (Rusya), Orta Avrupa ve Fransa'da..
- Deri ve yumuşak doku infeksiyonlarının tedavisi
- Son dönemlerde MDR bakterileri tedavisinde

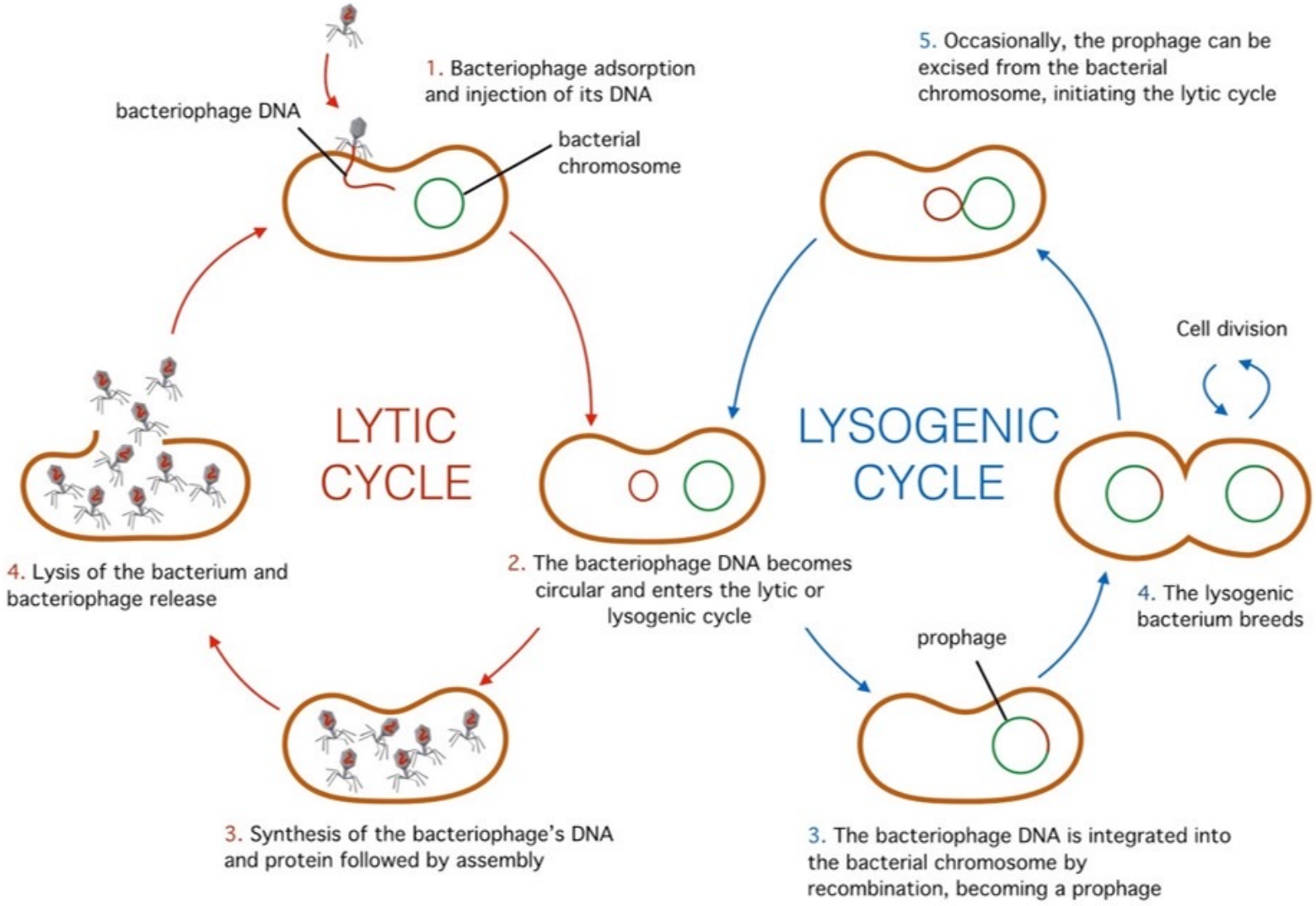


Sınıflama


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

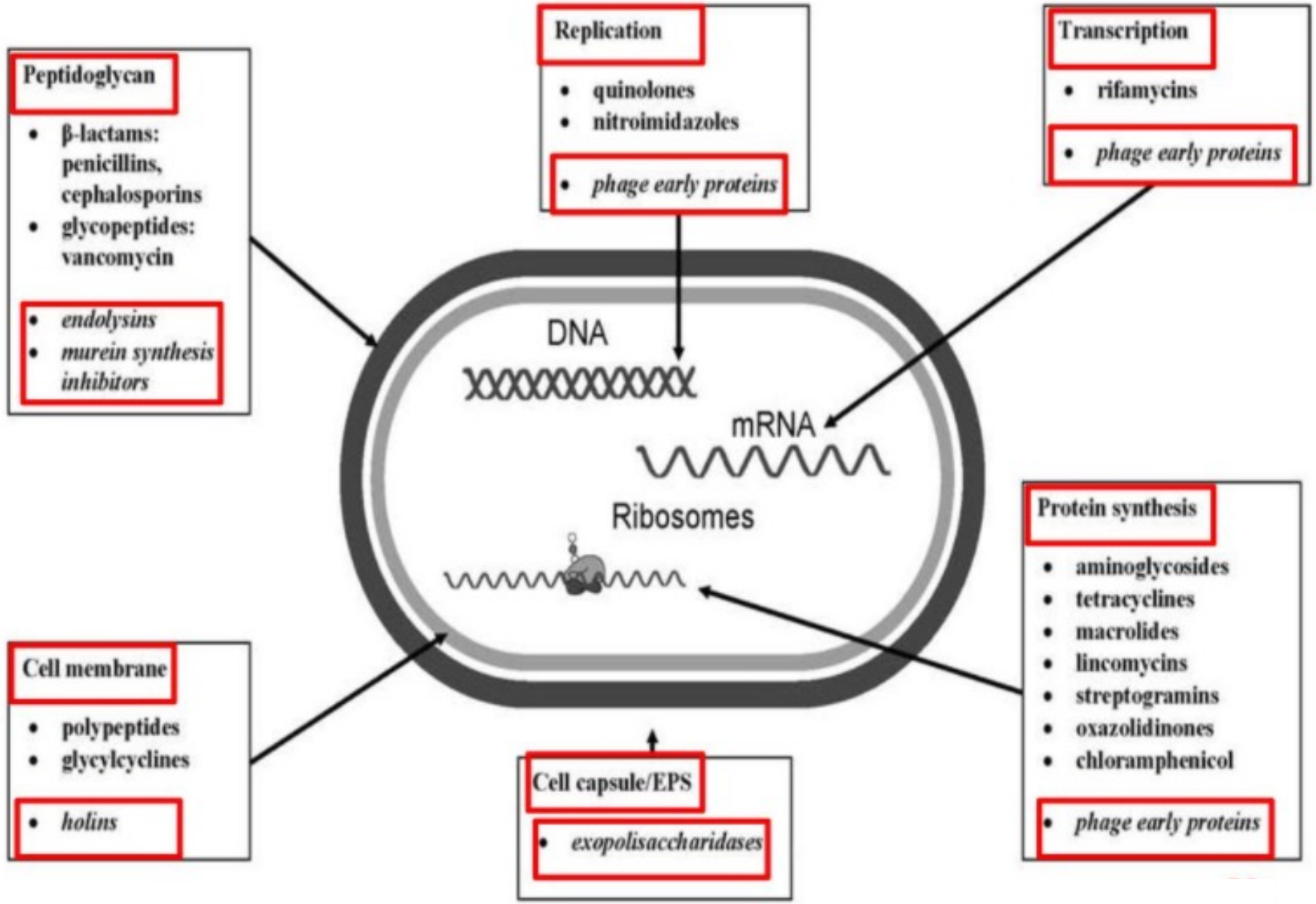
ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]				
Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (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

Bakteriyofajların Hayat Döngüsü



- Bakteriyofajların litik veya lizogenik hayat döngüleri olabilir,
- Lizogenik olabilen fajlara **ılımlı fajlar** (*temperate phage*) denir.
- Konak hücrenin sağlığı yerinde olduğu sürece Virüs sessiz bir şekilde varlığını sürdürür.
- Konağın şartları bozulursa, örneğin besin kaynaklarının tükenmesi durumunda, endojen fajlar (**profaj** olarak adlandırılırlar) etkinleşirler.
- Bir çoğalma süreci başlar, sonucunda konak hücre parçalanır.
- İlginç bir şekilde lizogenik döngü konak hücrenin çoğalmasına izin verdiği için hücrenin yavrularında da virüs varlığını devam ettirir.

Sanz-Gaitero, et al, 2014; Callewaert, et al, 2011



doi: 10.3389/fmicb.2016.00825

Bacteriophages as Weapons Against Bacterial Biofilms in the Food Industry

*Diana Gutiérrez[1], Lorena Rodríguez-Rubio[1,2], Beatriz Martínez[1], Ana Rodríguez[1] and Pilar García[1]**

[1] *Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas, Villaviciosa, Spain,*
[2] *Laboratory of Gene Technology, Katholieke Universiteit Leuven, Leuven, Belgium*

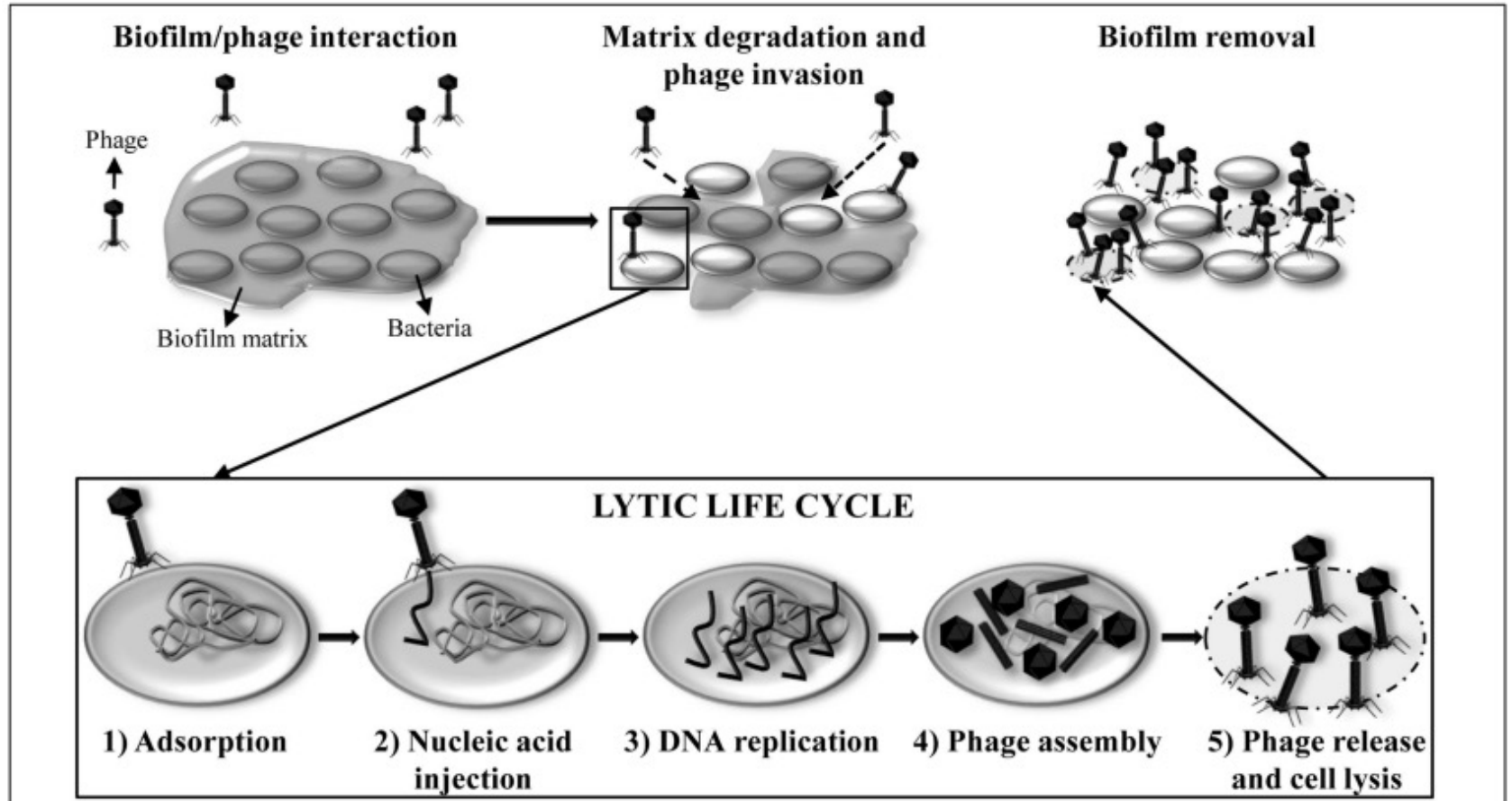


FIGURE 3 | Lytic life cycle of phages inside a biofilm. (1) Adsorption of the phage particle onto the host bacterial cell surface. Tail fibers bind to specific receptors on the cell surface. **(2)** Injection of the nucleic acid into the cytoplasm of the host bacterium. **(3)** Replication of the phage genome in multiple copies. Phage early genes are expressed to regulate the host metabolic machinery to be subjected to phage propagation. **(4)** Formation of new phage particles by expression of the phage late genes and assembly of the phage heads and tails, packaging of the nucleic acid inside the heads and maturation of the virions. **(5)** Lysis of the host bacterium and release of the new phage progeny ready to infect other cells in the biofilm and start a new cycle.

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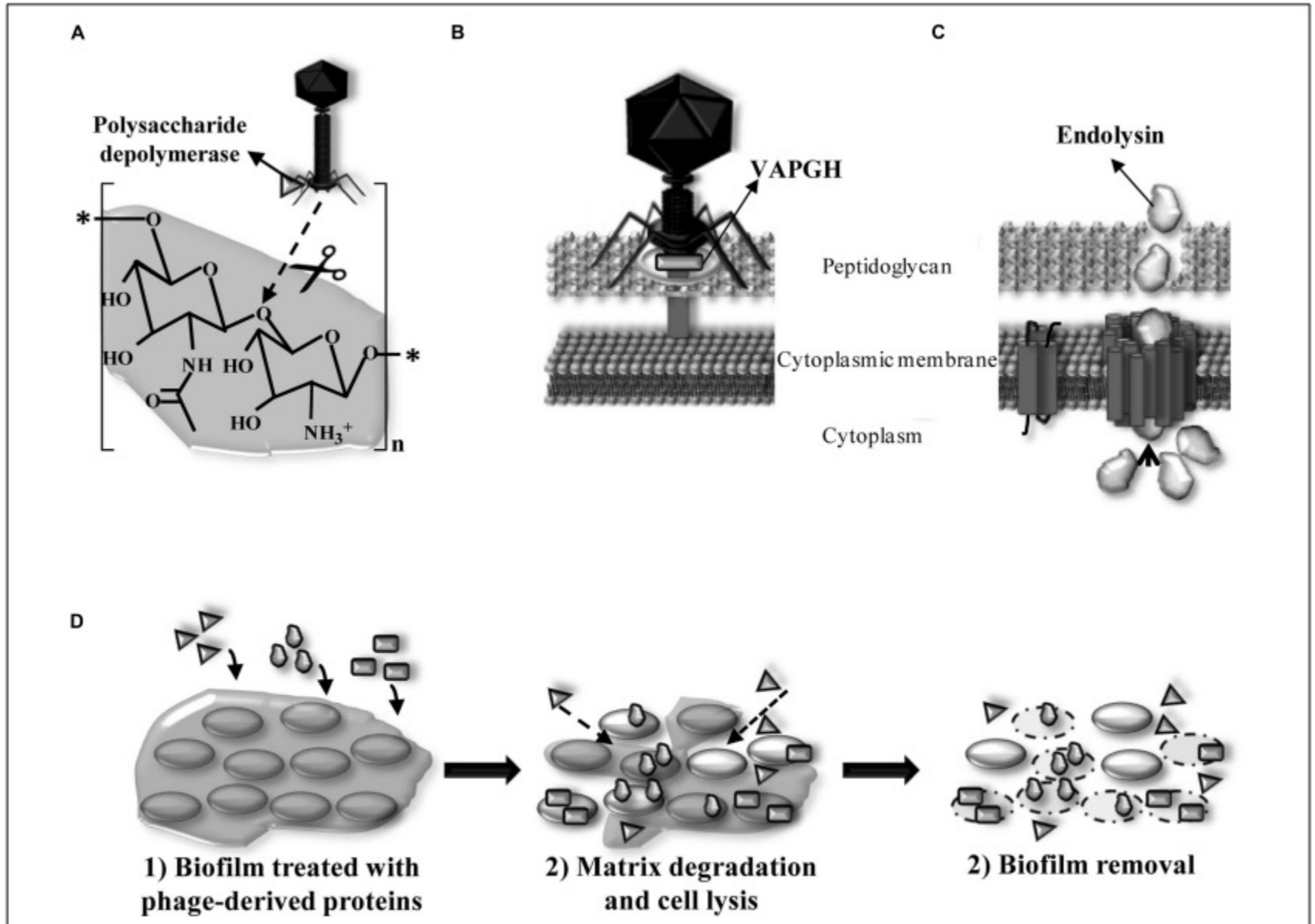

FIGURE 4 | (A) Location of exopolysaccharide depolymerase degrading β-(1,6) bonds of the biofilm extracellular matrix (PIA/PNAG) of staphylococcal species in the phage particle and mode of action. **(B)** Location of virion-associated peptidoglycan hydrolase (VAPGH) at the phage particle and its role in the infection process. **(C)** Structure of Gram-positive bacteria cell wall and role of the endolysin during the bacterial lysis. **(D)** Activity of phage derived proteins when added exogenously and their application as anti-biofilm agents degrading polysaccharidic matrices (polysaccharide depolymerases) and lysing bacteria (VAPGHs and endolysins).

antibiotics

MDPI

Review

Phages for Biofilm Removal

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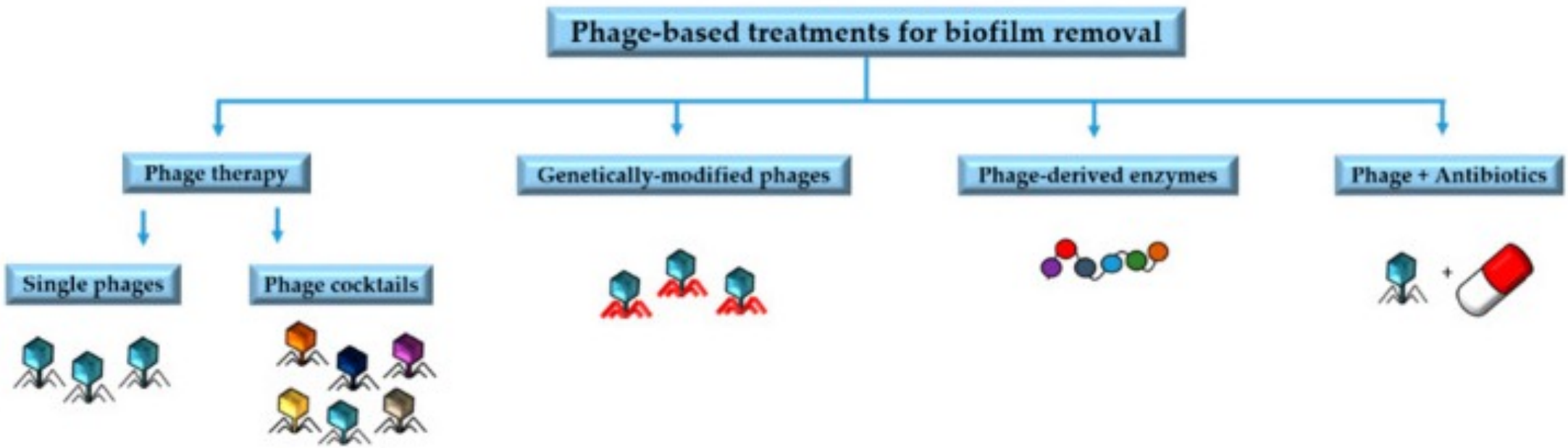
Abstract: Biofilms are clusters of bacteria that live in association with surfaces. Their main characteristic is that the bacteria inside the biofilms are attached to other bacterial cells and to



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

Thaysa Leite Tagliaferri [1,2]***, Mathias Jansen*** [1] ***and Hans-Peter Horz*** [1]*

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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*

OPEN ACCESS Freely available online

PLOS ONE

Co-Therapy Using Lytic Bacteriophage and Linezolid: Effective Treatment in Eliminating Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetic Foot Infections

Sanjay Chhibber*, Tarsem Kaur, Sandeep Kaur

Department of Microbiology, Panjab University, Chandigarh, India

Abstract

Background: *Staphylococcus aureus* remains the predominant pathogen in diabetic foot infections and prevalence of methicillin resistant *S.aureus* (MRSA) strains further complicates the situation. The incidence of MRSA in infected foot ulcers is 15–30% and there is an alarming trend for its increase in many countries. Diabetes acts as an immunosuppressive state decreasing the overall immune functioning of body and to worsen the situation, wounds inflicted with drug resistant strains represent a morbid combination in diabetic patients. Foot infections caused by MRSA are associated with an increased risk of amputations, increased hospital stay, increased expenses and higher infection-related mortality. Hence, newer, safer and effective treatment strategies are required for treating MRSA mediated diabetic foot infections. The present study focuses on the use of lytic bacteriophage in combination with linezolid as an effective treatment strategy against foot infection in diabetic population.

Methodology: Acute hindpaw infection with *S.aureus* ATCC 43300 was established in alloxan induced diabetic BALB/c mice. Therapeutic efficacy of a well characterized broad host range lytic bacteriophage, MR-10 was evaluated alone as well as in combination with linezolid in resolving the course of hindpaw foot infection in diabetic mice. The process of wound healing was also investigated.

Results and Conclusions: A single administration of phage exhibited efficacy similar to linezolid in resolving the course of hindpaw infection in diabetic animals. However, combination therapy using both the agents was much more effective in arresting the entire infection process (bacterial load, lesion score, foot myeloperoxidase activity and histopathological analysis). The entire process of tissue healing was also hastened. Use of combined agents has been known to decrease the frequency of emergence of resistant mutants, hence this approach can serve as an effective strategy in treating MRSA mediated foot infections in diabetic individuals who do not respond to conventional antibiotic therapy.

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Co-Therapy Using Lytic Bacteriophage and Linezolid: Effective Treatment in Eliminating Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetic Foot Infections

Sanjay Chhibber*, Tarsem Kaur, Sandeep Kaur

Department of Microbiology, Panjab University, Chandigarh, India

- Group 1: Diabetic mice were infected with *S.aureus* 43300(10^6 CFU/ml).
- Group 2: Diabetic mice were infected with *S.aureus* 43300 followed by administration of phage at a multiplicity of infection (MOI) – 100 [30 minutes post-infection].
- Group 3: Diabetic mice were infected with *S.aureus* 43300 followed by administration of linezolid (25 mg/kg/per oral).
- Group 4: Diabetic mice were infected with *S.aureus* 43300 followed by administration of phage at a MOI of 100 as well as simultaneous administration of linezolid (25 mg/kg/per oral).

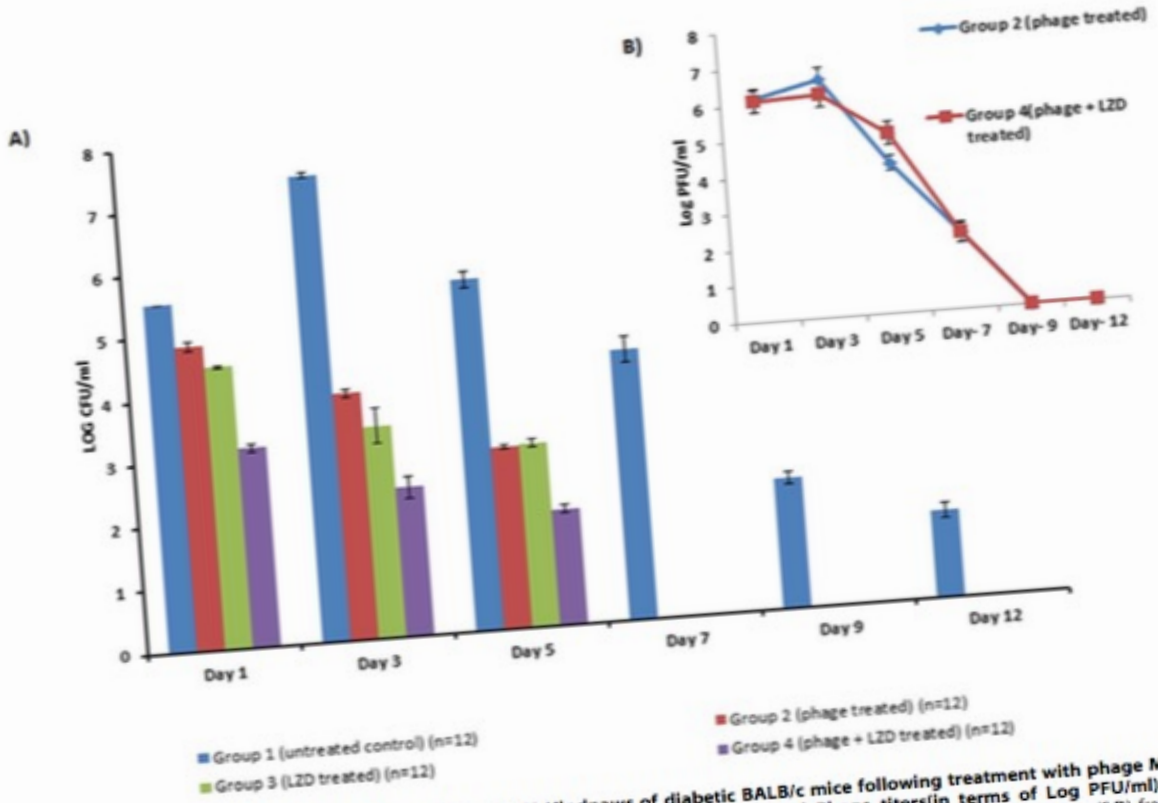


Figure 4. Bacterial load (in terms of Log CFU/ml) in A) Hindpaws of diabetic BALB/c mice following treatment with phage MR-10, linezolid and combination of phageMR-10 and linezolid (25 mg/kg/per oral) and Phage titers(in terms of Log PFU/ml) in B) Hindpaws of phage treated (group 2) and phage + LZD treated (group 4). [Error bars represent the standard deviation (S.D) from four independent values].
doi:10.1371/journal.pone.0056022.g004

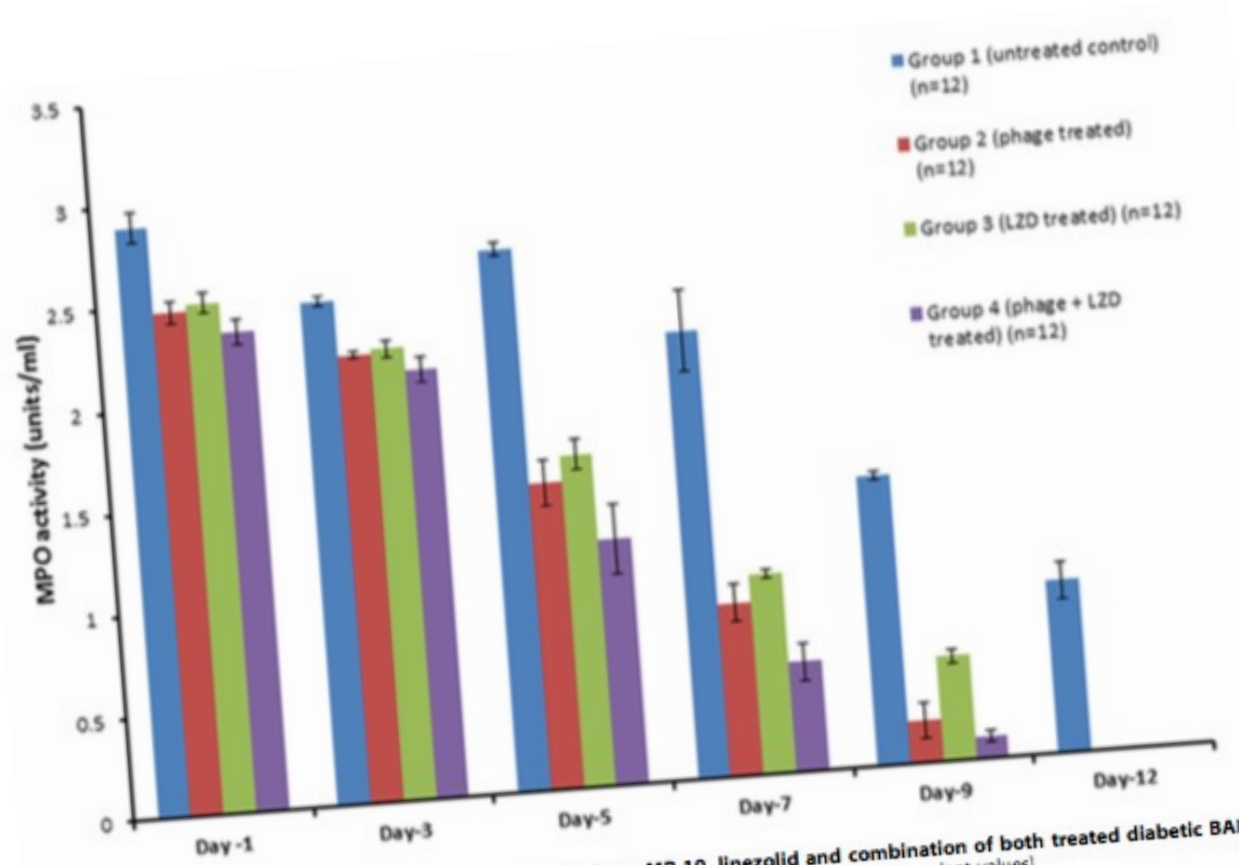


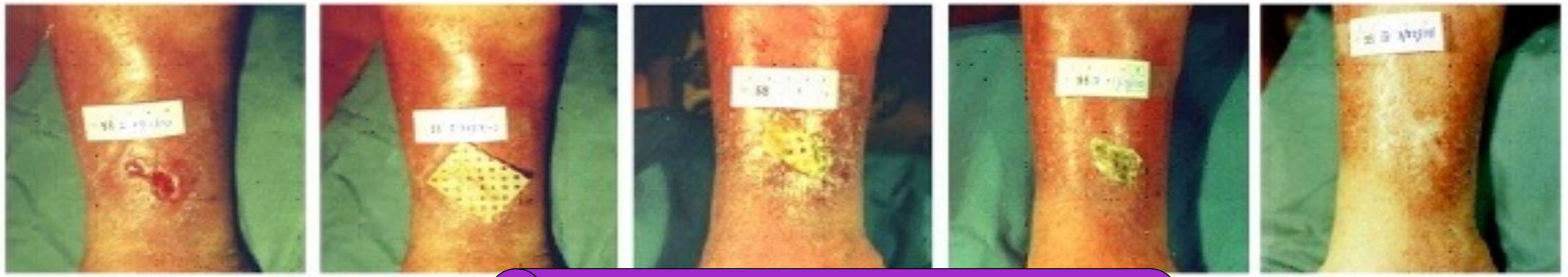
Figure 5. Comparison of MPO levels in the hindpaw of phage MR-10, linezolid and combination of both treated diabetic BALB/c mice at different time intervals. [Error bars represent the standard deviation (S.D) from four independent values].
doi:10.1371/journal.pone.0056022.g005

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

*Thaysa Leite Tagliaferri [1,2], Mathias Jansen [1] and Hans-Peter Horz [1]**

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

PhagoBioDerm: un producto para tratar infecciones bacterianas multirresistentes en quemaduras producido por el Instituto Eliava



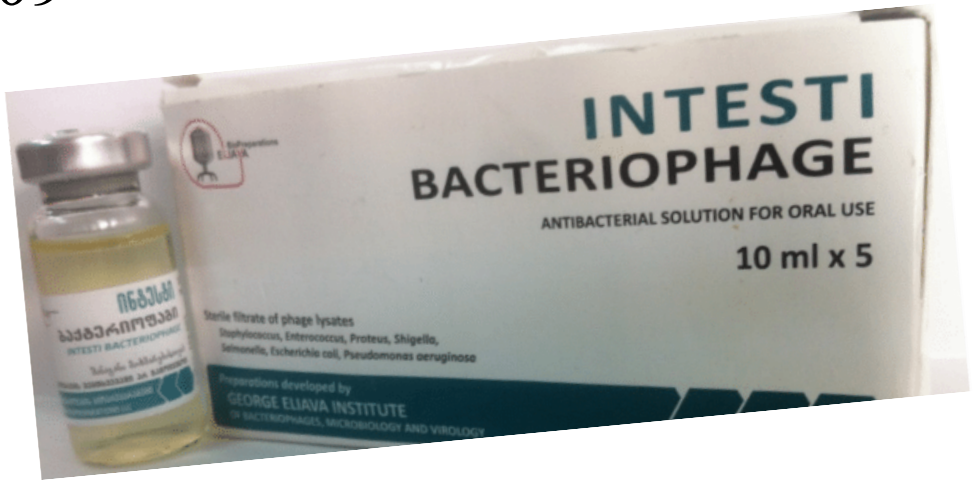
Bacteriofagos en Phago… …herida de *S. aureus*
Multirresistente a antibió… …logy, 2002, 41: 453-458

…es este producto es:
…n antibiótico),
…nestésico), los fagos,
…(proteasa que
… necrótico) y por
…o biodegradable.

Bakteriyofaj tedavisi MRSA kaynaklı kronik yaraların infeksiyonlarında başarılı

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.

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]

antimicrobial agents is available elsewhere.[5-7]

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R.D. Wolcott,[1] MD, Director;
M.A. Kuskowski, PhD, Associate Professor, Department of Psychiatry, University of Minnesota, Minneapolis, US;
B.M. Wolcott,[1] Research Technician;
L.S. Ward, PhD, Director

Journal of
Antivirals & Antiretrovirals

Golkar et al., J Antivir Antiretrovir 2013, S10
DOI: 10.4172/jaa.S10-005

Research Article Open Access

Experimental Phage Therapy on Multiple Drug Resistant *Pseudomonas aeruginosa* Infection in Mice

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[1]South Carolina Center for Biotechnology, Department of Biology, Claflin University, USA
[2]Department of Microbiology, University of Karachi, Pakistan

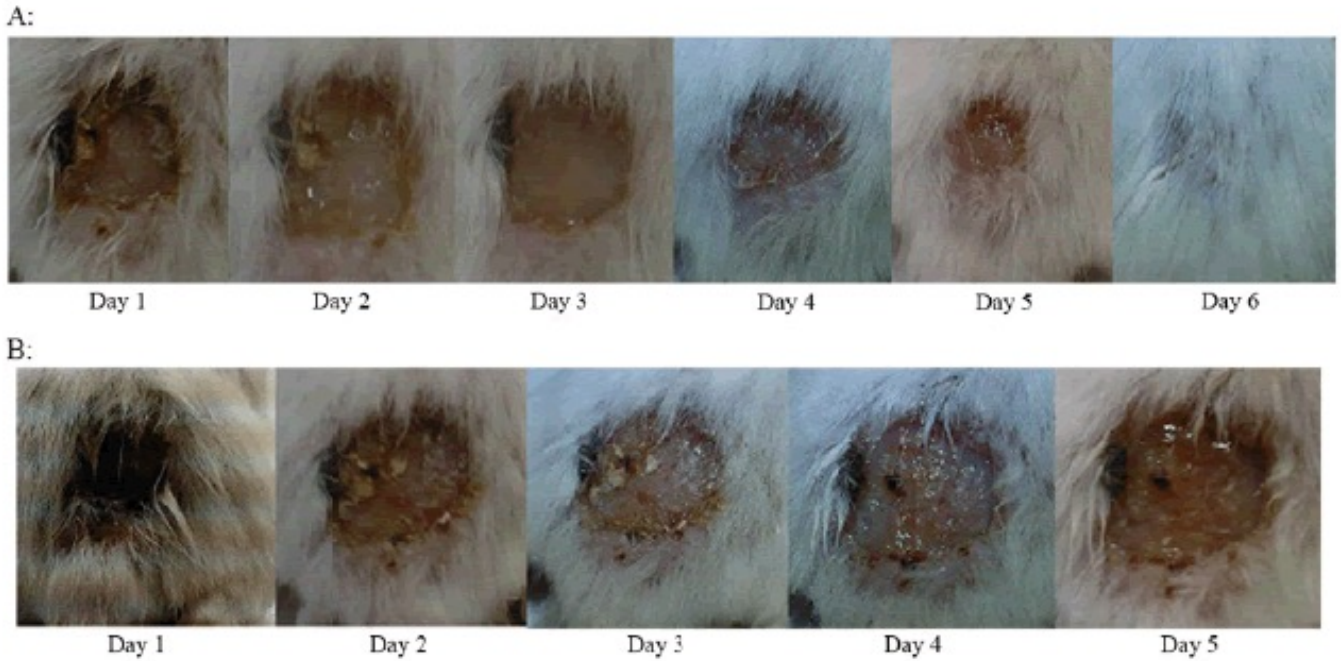
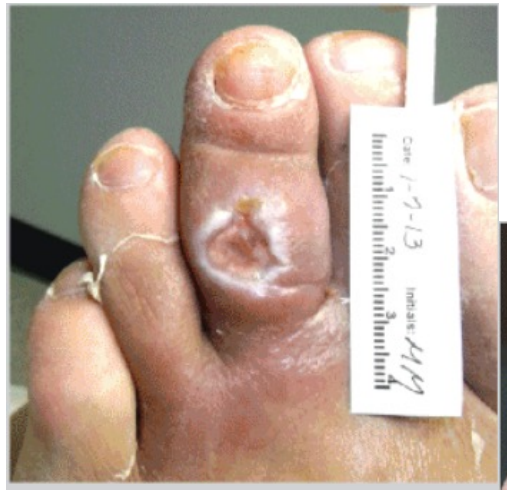


Figure 7: Phage therapy of MDR *P. aeruginosa* chronic Infection.

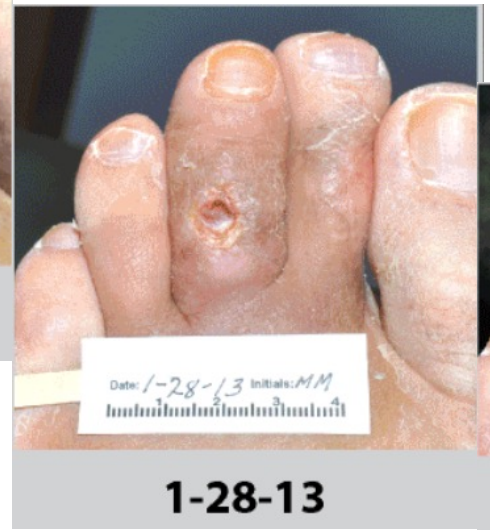
(A) *Pseudomonas* Phage PS5 (3×10^8), application after 30 minutes on infected lesion of mice infected with MDR *Pseudomonas*.
(B) Control: Lesion on mice skin infected with MDR *Pseudomonas* without Phage therapy.

Olgularla Bakteriyofaj Tedavileri

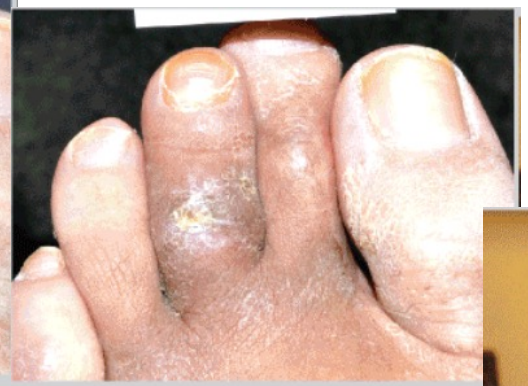
- Tiflis, Gürcistan
- 71 yaş erkek,
- DM, hiperlipidemi
- Çok sayıda ülserler
- Başarısız antibiyotik tedavisi
- Bakteriyofaj tedavisi..



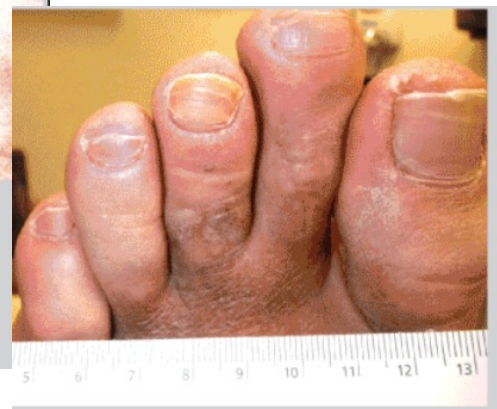
1-7-13



1-28-13



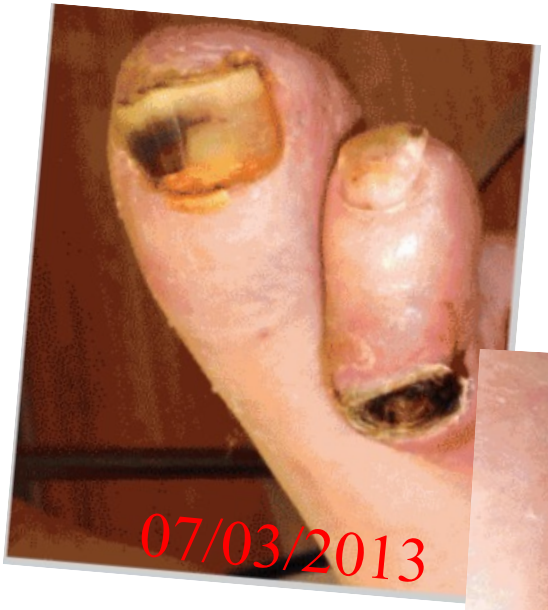
2-25-13
Wound closed

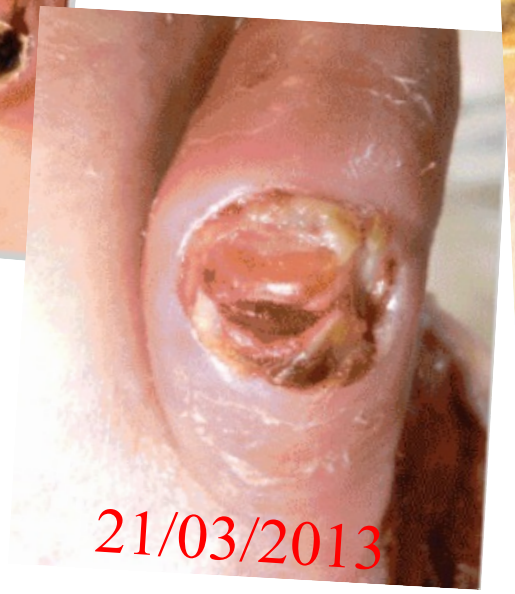


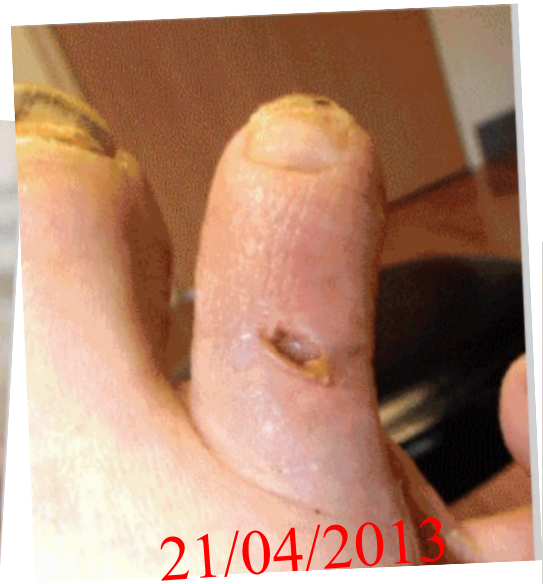
3-18-14
Wound remains closed after 1 year

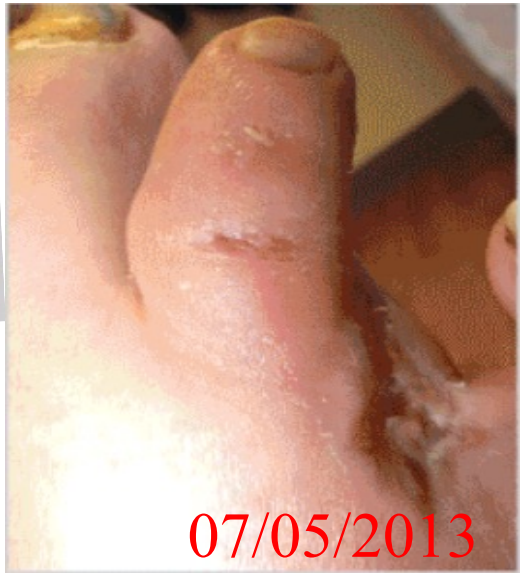
Olgularla Bakteriyofaj Tedavileri

- Tiflis, Gürcistan
- 74 yaş DM kadın,
- Hipertansiyon,
- Koroner arter bypass greft x 3
- Sağ femoral endarterektomi öyküsü
- Başarısız antibiyotik tedavisi
- Bakteriyofaj tedavisi..





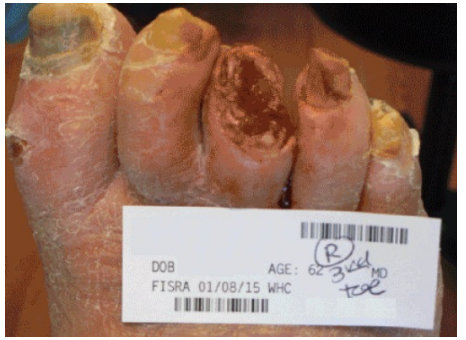




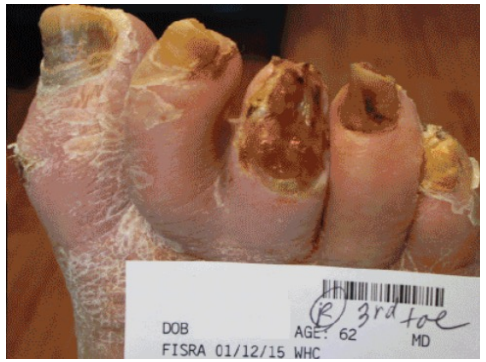
Olgularla Bakteriyofaj Tedavileri

- Tiflis, Gürcistan
- 61 yaş DM kadın,
- Hipertansiyon,
- Hiperlipidemi, kronik HCV
- Sağ femoral endarterektomi öyküsü
- Başarısız antibiyotik tedavisi
- Bakteriyofaj tedavisi..

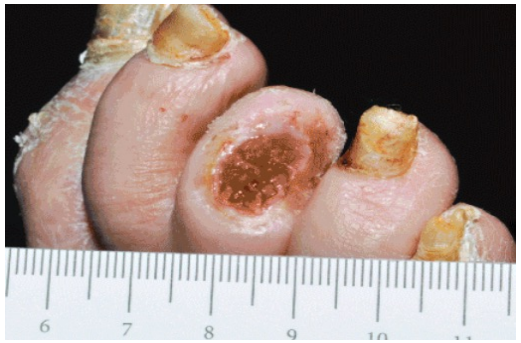
07/03/2013



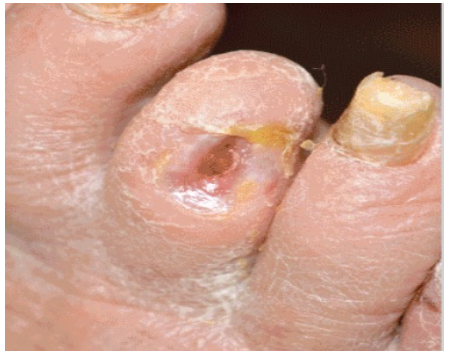
14/03/2013



21/03/2013



01/04/2013



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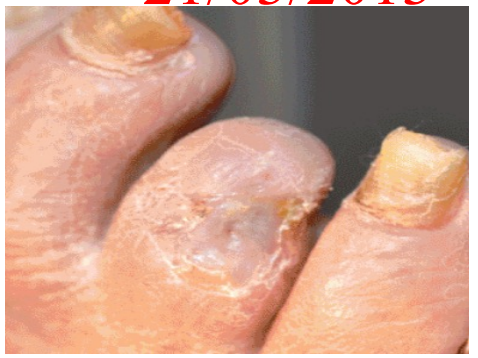

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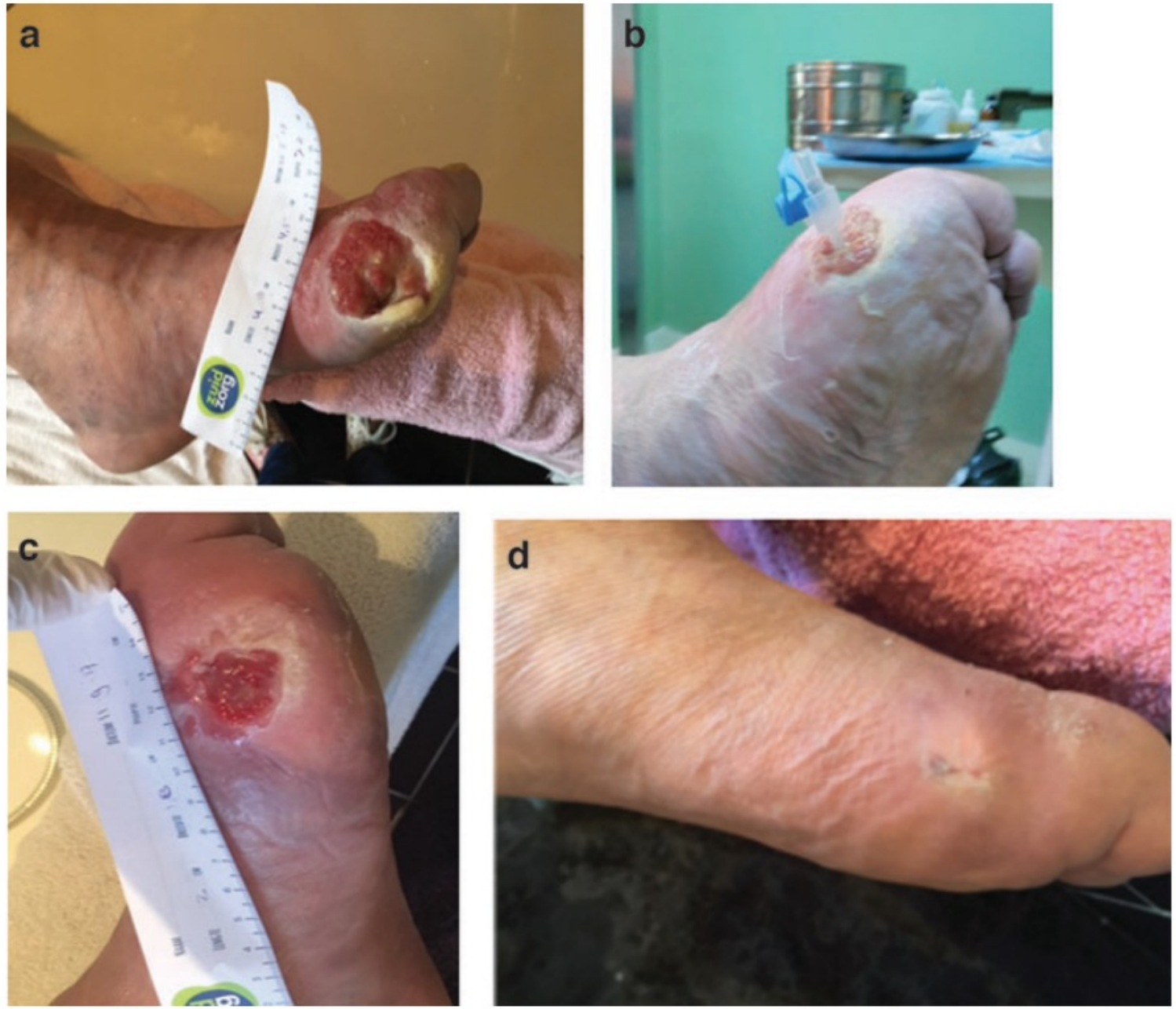


FIG. 3 **(a)** Before treatment, on the left toe, diabetic foot ulcer is seen. The wound is 5 cm in diameter. **(b)** Catheter is placed into the ulcer for direct application of phages into the tissue. **(c)** Six weeks after treatment; the size of the wound reduced to 2 cm by 1.8 cm. **(d)** Post-bacteriophage therapy, the ulcer has filled in completely and the wound has closed.

Fig 1. Patient 1. A 74-year-old male with a post-operative defect following amputation of toes 3 and 4, which closed without infection. However, the second toe wound, which originally presented without signs of infection and covered with eschar, subsequently opened to the bone with signs of infection. Only the second toe was treated with phage.

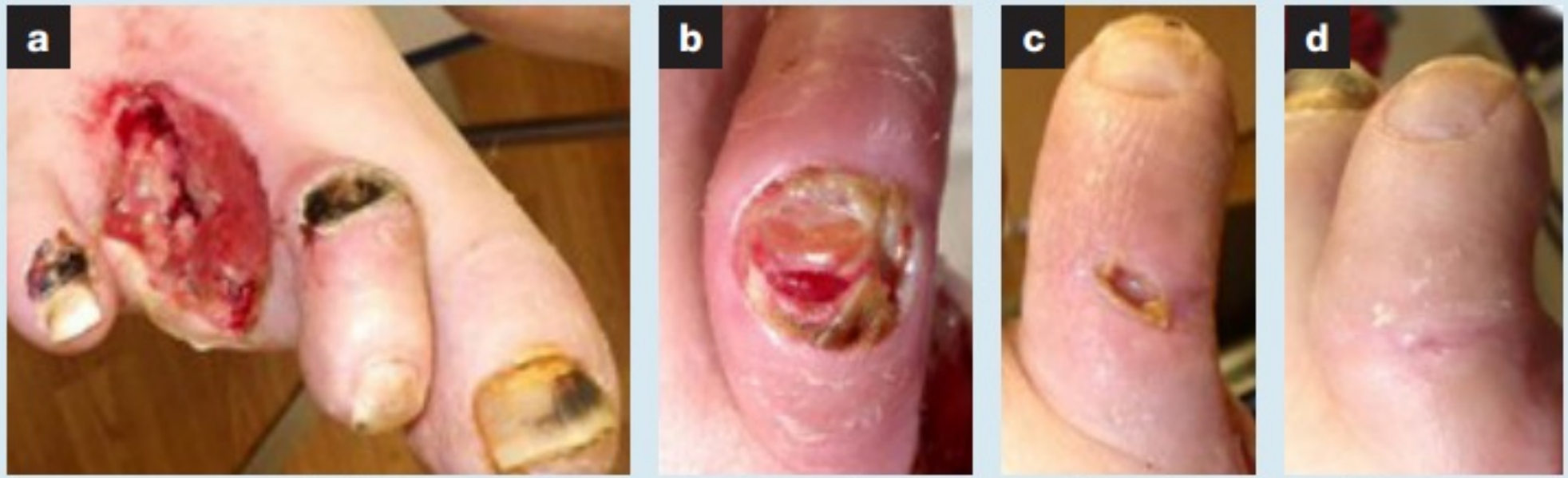


Fig 2. Patient 2. A 92-year-old male with contracted toe and shoe injury. The base of the middle phalanx was exposed in the centre of the and was excised

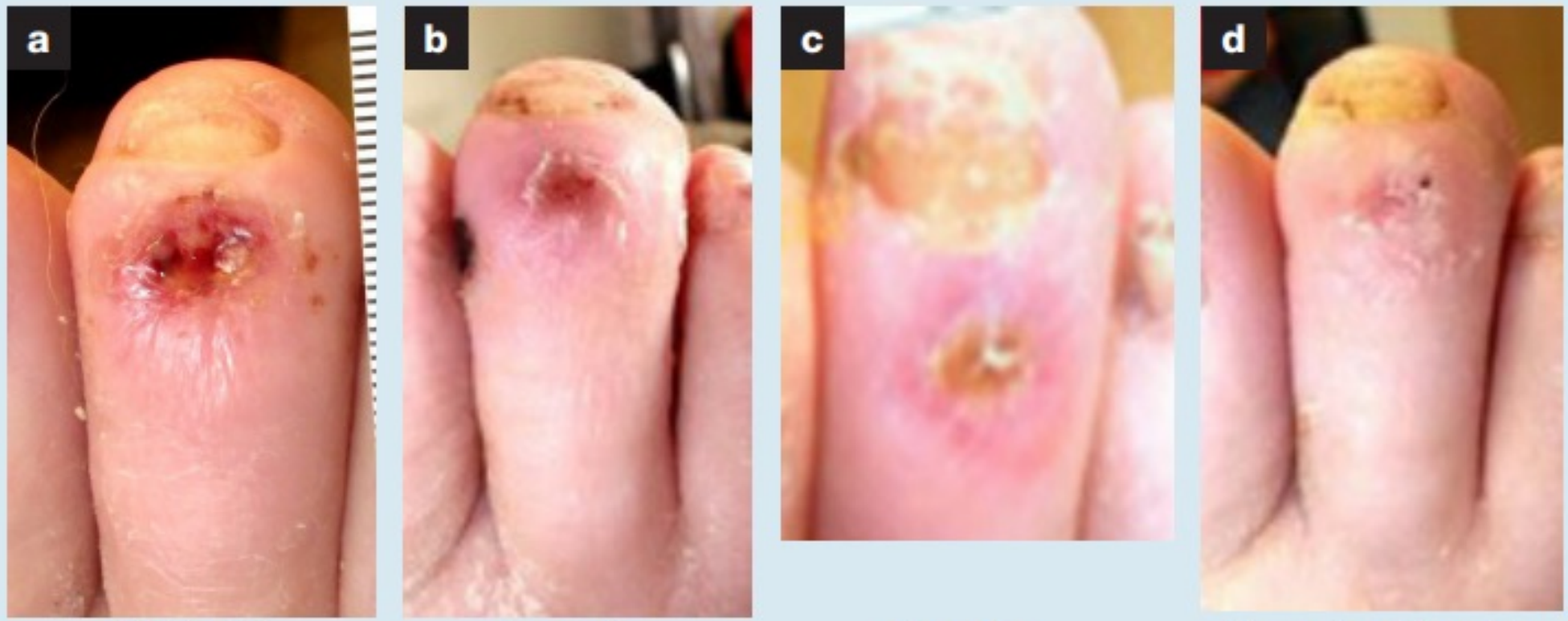
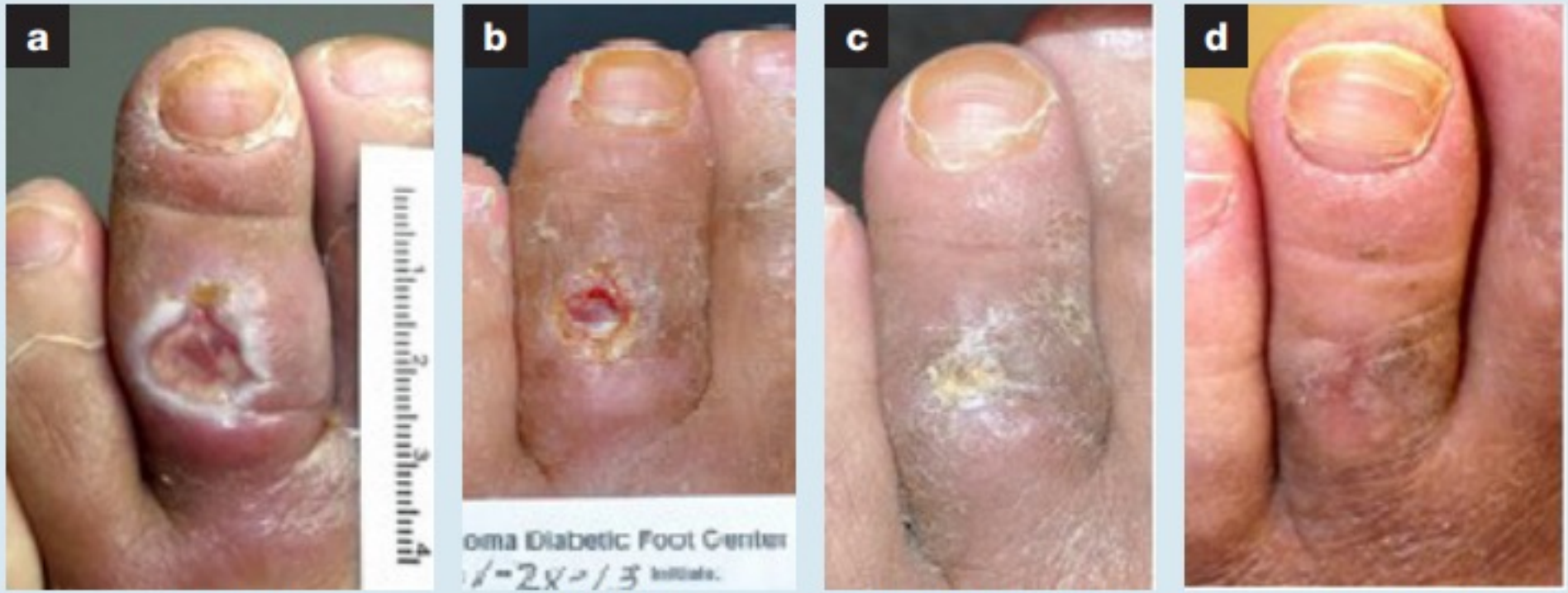


Fig 3. Patient 3. A 48-year-old male with diabetes and a resolved ulcer and cellulitis. The toe remained healed after 1 year



7-1-13 | 3 weeks | 7 weeks closed | 1 year closed

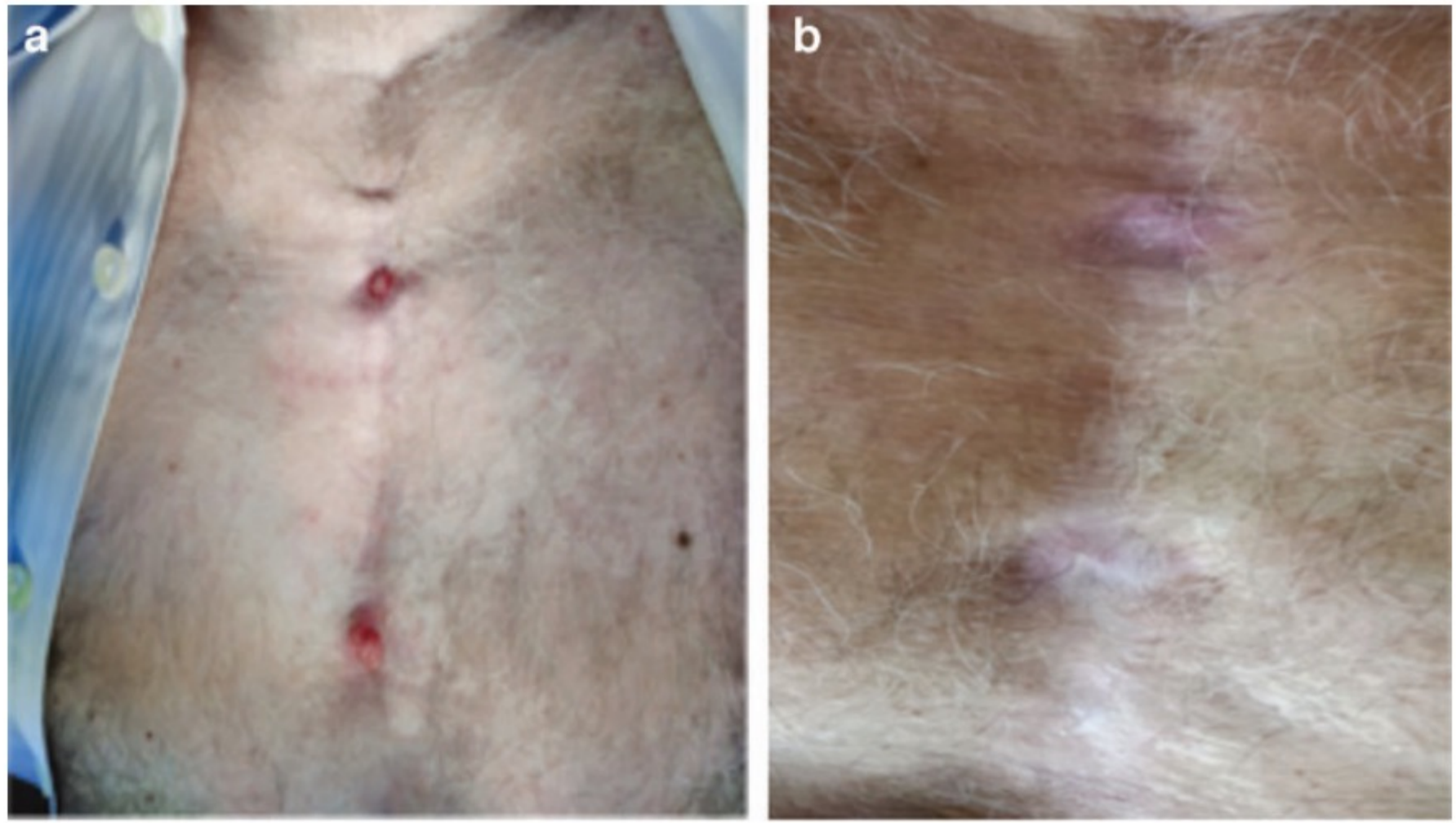


FIG. 1 **(a)** The image was taken on admission. Two purulent fistulas are seen in the sternal bone. The diameter of the upper fistula is 0.6 cm and the diameter of the lower fistula is 0.4 cm. **(b)** Postbacteriophage treatment, the fistulas have closed and there is no purulent exudate.

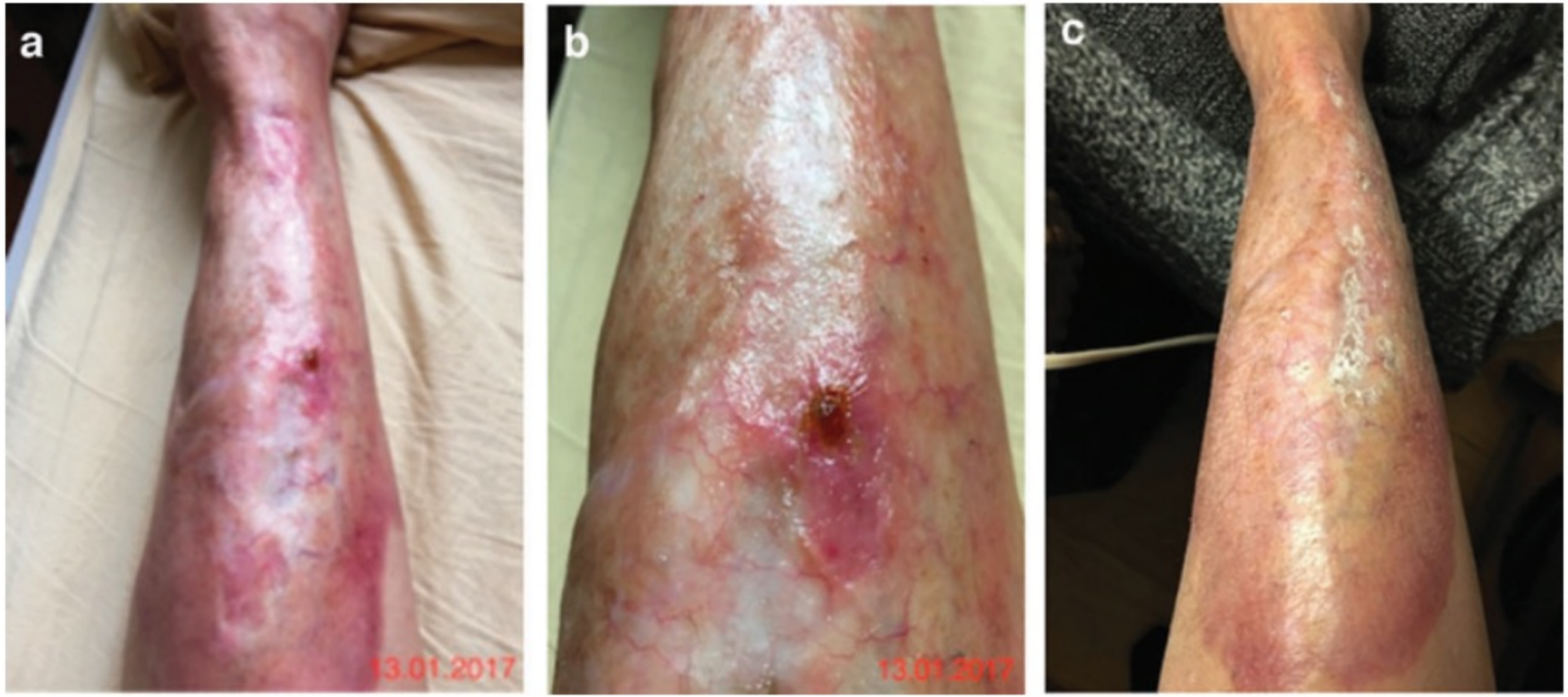
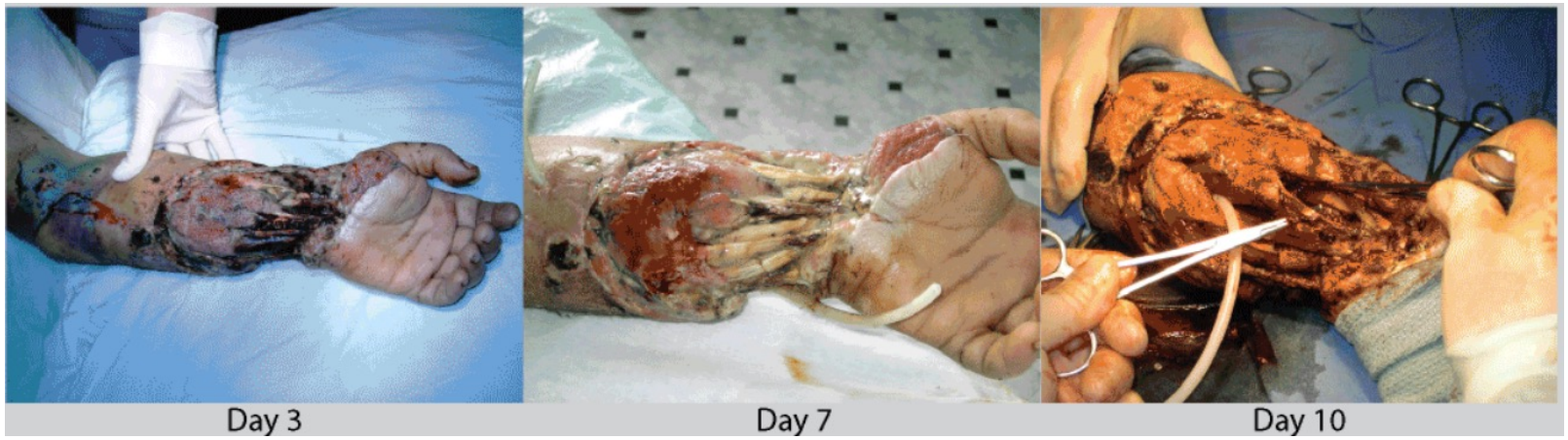


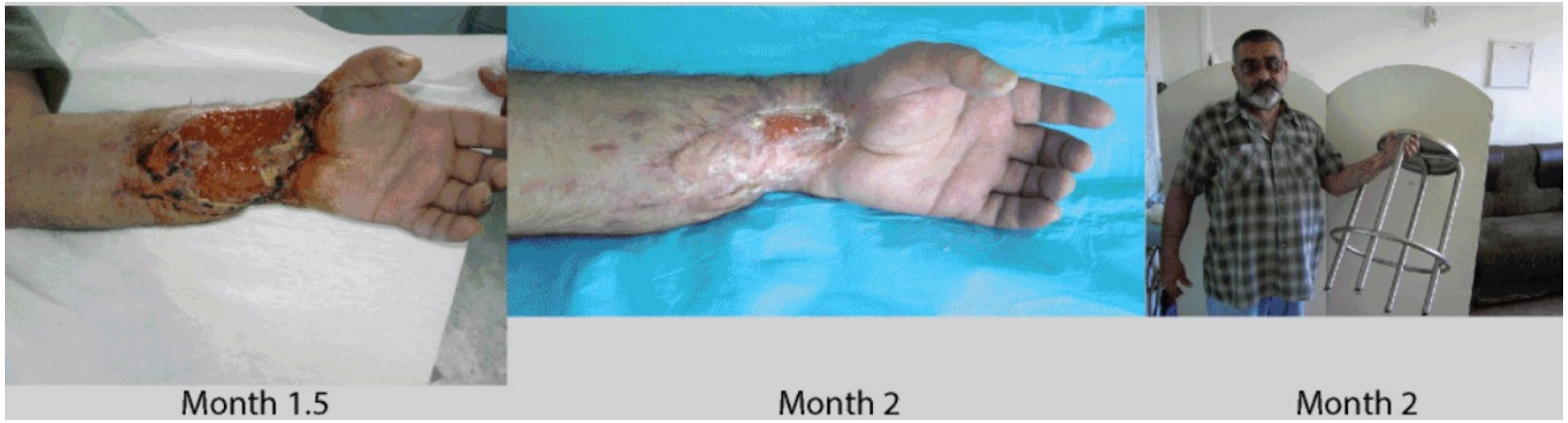
FIG. 2 **(a)** Pretreatment, the skin on the bone is markedly red, delicate, and fragile. **(b)** Purulent ulcer is visible. **(c)** Post-treatment, the color of the skin has changed from red to pink. The ulcer has filled in completely.

Olgularla Bakteriyofaj Tedavileri

- Tiflis, Gürcistan
- 55 yaş erkek,
- Maden işçisi
- Patlayıcıya maruziyet
- 12 ay IV AB tedavi
- Debritman..
- Amputasyon önerileri



- Bakteriyofaj tedavisi..



Wound Repair and Regeneration

Wound healing potential of topical bacteriophage therapy on diabetic cutaneous wounds

João J. Mendes, MD[1,2]; Clara Leandro, MSc[1]; Sof
Patrícia Cavaco-Silva, PharmD, PhD[3,4]; José Melc
Miguel Garcia, PharmD, PhD[1]

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Manuscript received: August 21, 2012
Accepted in final form: March 18, 2013

DOI:10.1111/wrr.12056

ABSTRAC

Chronic wou
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Unfortunatel
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INTRODUCTION

Diabetic foot infections (DFIs) are a frequent and
complication of diabetes mellitus (DM) and are the
leading cause of nontraumatic lower limb amputat
current clinical practice, DFI treatment includes debr
and systemic antibiotics.[2] However, because of
vascularization and insufficient local antibiotic co
tions, these treatments are often ineffective.[3] In
multidrug-resistant organisms, such as methicillin-
Staphylococcus aureus and pan-drug-resistant nonfe
Gram-negative bacilli, threaten the efficacies of the
pies in both community-dwelling and hospitalized p
Thus, it is necessary to identify new therapeutic strat
DFIs. Topical treatment is advantageous because
adverse effects from systemic treatment, increases t
concentration, and allows the use of agents that are
able for systemic therapy. Mechanical debridement
pivotal to this strategy because it significantly re
bioburden, and as elegantly demonstrated by Wolc
opens a time-dependent therapeutic window for top
microbial therapy (TAT). Nevertheless, no TAT
been proven to effectively treat DFI to date.[7]
Bacteriophages are small viral entities, existing
acids packaged within a protein capsid, that specific

NRC Research Press

865

REVIEW

Bacteriophages: the possible solution to treat infections caused by pathogenic bacteria

Ayman El-Shibiny and Salma El-Sahhar

Abstract: Since their discovery in 1915,
humans because of their unique abili
populations. The research carried out
and Poland, led to the establishmer
sulfonamide antibiotics in the West
therapy. The misuse of antibiotics ha
in the number of antibiotic-resistant
of interest with the emergence of mu
have indicated that bacteriophages
agents. Moreover, they can be used
design, and in the nanomedicine fi

Key words: bacteriophages, antibioti

Résumé : Les bactériophages ont
depuis leur découverte en 1915, à c
affecter les autres populations bac
principalement en Géorgie, dans
thérapeutiques à partir de phages
cours des années 1930 a retardé l
depuis leur découverte a résulté e
réduction de leur efficacité à c
d'intérêt comme alternative aux
menace à la santé publique. Des
ement pour détecter les bactérie
peuvent être utilisés pour dévelo
vaccins, dans la conception de
[Traduit par la Rédaction]

Mots-clés : bactériophages, antil

Introduction

Bacteriophages are small viruses that h
to infect bacteria. They have a huge infl
environment, as they play a vital role in
microbial balance. Phages are ubiquitou
found in all the natural habitats, includi
terrestrial systems, in which their bact
present. Over 6000 different phages have
and described morphologically (Ackermann
2012). They can be classified on the basis
ogy, genetic content, host, habitat, or

Received 17 January 2017. Revision received
A. El-Shibiny and S. El-Sahhar. University
12588, Giza, Egypt.
Corresponding author: Ayman El-Shibiny
Copyright remains with the author(s) or t

Formerly: Schweizerische Medizinische Wochenschrift
An open access, online journal • www.smw.ch

Review article: Biomedical Intelligence | Published 22 November 2017 | doi:10.4414/smw.2017.14553
Cite this as: Swiss Med Wkly. 2017;147:w14553

Multidrug resistant (or antimicrobia pathogens - alternatives to new an

Brunel Anne-Sophie, Guery Benoit

Infectious Diseases Service, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland

Summary

For the last few decades, multidrug resistance has become an increasing concern for both Gram-positive and Gram-negative bacteria. The number of new molecules has dramatically decreased and antibiotic resistance is now a priority in the international community. Facing this new threat, a large number of new as well as "old" solutions are now being discussed in the medical community to propose an alternative to antibiotic treatments. A first option is to potentiate the effect of existing molecules through combinations to circumvent the individual molecule resistance. The second option is to neutralise either the infectious agent itself or its by-products using specific antibodies. A third option is to use the pathogen signaling mechanism and inhibit the production of virulence factor through quorum sensing inhibition. A fourth pathway would be to interact with the patient's microbiota using either probiotics or faecal transplantation to modulate the innate immune response and improve response to the infectious challenge, but also to act directly against colonisation by resistant bacteria by replacing the flora with susceptible strains. The last option is to target the bacteria using phage therapy. Phages are natural viruses that specifically infect target bacteria independently of any antibiotic-susceptibility profile. In this review, we will discuss each of these options and provide the scientific rationale and the available clinical data. In the majority of cases, these treatments represent an interesting approach but not the ultimate solution to multiresistance. Well-performed clinical trials are still missing and the major priority remains to promote good use and appropriate stewardship of antibiotics to decrease resistance.

Key words: microbiota, phage, probiotics: quorum sensing

Introduction

With the appearance of penicillin, antibiotics became one of the most important revolutions in infectious-disease management. The medication was followed in subsequent decades by a growing number of new agents. The most obvious consequence was the rapid emergence of resistance associated with the use of each new agent. Today, the number of antiobiotic molecules is reaching a plateau, but resistance continues to grow. In 2013, the United States Centers for Disease C
top 18 drug-
carbapenem-
as urgent, an
spectrum *En*
rococcus an
nosa were
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al study on
hospitals ir
15% *E. co*
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from 5.2
to 13.5
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must b
cific t
host
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entifi

Correspondence:
Benoît Guery, MD PhD, Service des Maladies Infectieuses, Département de Médecine, BH10-549, Rue du Bugnon 46, CH-1011 Lausanne, benoit.guery[at]chuv.ch

The Journal of Infectious Diseases

BRIEF REPORT

A Prophage in Diabetic Foot Ulcer–Colonizing *Staphylococcus aureus* Impairs Invasiveness by Limiting Intracellular Growth

Jean-Philippe Rasigade,[1,2] Catherine Dunyach-Rémy,[4,5] Anaïs Sapin,[1] Nourredine Messad,[4] Sophie Trouillet-Assant,[1] Céline Dupieux,[1,2] Jean-Philippe Lavigne,[4,5] and Frédéric Laurent[1,2,3]

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The mechanisms that drive the transition from commensality to invasiveness in *Staphylococcus aureus* are poorly understood. We recently reported that >50% of *S. aureus* isolates from uninfected diabetic foot ulcers in French patients harbor a prophage, ROSA-like, that is absent from invasive isolates from diabetic foot infections, including osteomyelitis. Here we show that the ROSA-like insertion abolishes the ability of *S. aureus* to replicate within osteoblasts, the bone-forming cells, greatly reducing damage to infected cells. These results unravel an important mechanism by which particular *S. aureus* strains are maintained in a commensal state in diabetic foot ulcers.

Keywords. ROSA phage; bacterial invasion; bacterial i
cellular growth; diabetic foot infection; osteomyelitis; osteo

Staphylococcus aureus is both a frequent colonizer of h
and a versatile pathogen able to elicit invasive infection.
standing the mechanisms that drive the transition of *S*
from commensality to invasiveness is important to pr
fection risk in colonized patients and to propose tar
colonization procedures to high-risk patients. Alth
molecular epidemiology of carriage and clinical stra
markedly different [1], no genomic characteristic ab
discriminate commensal and invasive isolates has be
so far [2]. In particular, the presence of toxin-encod
the constitutive overexpression of toxins in certain
eages has been unambiguously associated with an
ience, based on epidemiological and experiment
these so-called hypervirulent *S. aureus* line

Received 28 June 2016; accepted 8 September 2016; published onli
Correspondence: J.-P. Rasigade, Faculté de Médecine Lyon-Sud Charles-
Grand Revoyet, Pierre-Bénite 69310, France (jean-philippe.rasigade@univ-lyon1.fr).
The Journal of Infectious Diseases® 2016;214:1605–8

DOI: 10.1093/infdis/jiw432

Introduction

Diabetes is one of the biggest cause of morbidity and mortality worldwide. According to a major international study, an estimated 350 million people in the world have diabetes [1]. Both type 1 and type 2 diabetes lead to hyperglycemia that further results in a number of complications, including damage to nerves (diabetic neuropathy) [2]. Peripheral neuropathy has a central role to play in the development of foot infections. Wounds leading to foot and leg amputation occur in about 30 to 50 percent of patients with diabetes [3].

One of the most common pathogen in acute, previously untreated, superficial infected foot wound in patients with diabetes is *Staphylococcus aureus*. Overuse of antibiotics and the selection of broad- rather than narrow-spectrum agents has contributed

S. aureus i
negative (Δ*rosa*) variant hav
NSA1385 was isolated from an uninfected DF

towards a high prevalence of methicillin-resistant *S.aureus* (MRSA) in diabetic foot wounds.

MRSA accounts for up to 42.86 % of the *S.aureus* isolates from diabetic foot infections [4]. The prevalence of MRSA in infected foot ulcers is as high as 30% and an increase has been noticed in its incidence in many countries [5]. A recent study from Manchester has reported MRSA isolation in 30.2% of patients, which is a 100% increase as compared to three years earlier [6]. Also MRSA bacteremia in diabetics with foot infections is associated with 43% mortality compared to 20% mortality rate reported with methicillin sensitive *S.aureus* (MSSA) bacteremia [7] The mortality rate is much higher in case of diabetic foot infections caused by MRSA undergoing amputation (43% MRSA vs 9% non-MRSA) [8].

Furthermore, there is evidence that MRSA colonization of chronic ulcers is associated with delayed healing [9,10]. Strategies

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PLOS ONE

Co-Therapy Using Lytic Bacteriophage and Linezolid: Effective Treatment in Eliminating Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetic Foot Infections

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Abstract

Background: *Staphylococcus aureus* remains the predominant pathogen in diabetic foot infections and prevalence of methicillin resistant *S.aureus* (MRSA) strains further complicates the situation. The incidence of MRSA in infected foot ulcers is 15–30% and there is an alarming trend for its increase in many countries. Diabetes acts as an immunosuppressive state decreasing the overall immune functioning of body and to worsen the situation, wounds inflicted with drug resistant strains represent a morbid combination in diabetic patients. Foot infections caused by MRSA are associated with an increased risk of amputations, increased hospital stay, increased expenses and higher infection-related mortality. Hence, newer, safer and effective treatment strategies are required for treating MRSA mediated diabetic foot infections. The present study focuses on the use of lytic bacteriophage in combination with linezolid as an effective treatment strategy against foot infection in diabetic population.

Methodology: Acute hindpaw infection with *S.aureus* ATCC 43300 was established in alloxan induced diabetic BALB/c mice. Therapeutic efficacy of a well characterized broad host range lytic bacteriophage, MR-10 was evaluated alone as well as in combination with linezolid in resolving the course of hindpaw foot infection in diabetic mice. The process of wound healing was also investigated.

Results and Conclusions: A single administration of phage exhibited efficacy similar to linezolid in resolving the course of hindpaw infection in diabetic animals. However, combination therapy using both the agents was much more effective in arresting the entire infection process (bacterial load, lesion score, foot myeloperoxidase activity and histopathological analysis). The entire process of tissue healing was also hastened. Use of combined agents has been known to decrease the frequency of emergence of resistant mutants, hence this approach can serve as an effective strategy in treating MRSA mediated foot infections in diabetic individuals who do not respond to conventional antibiotic therapy.

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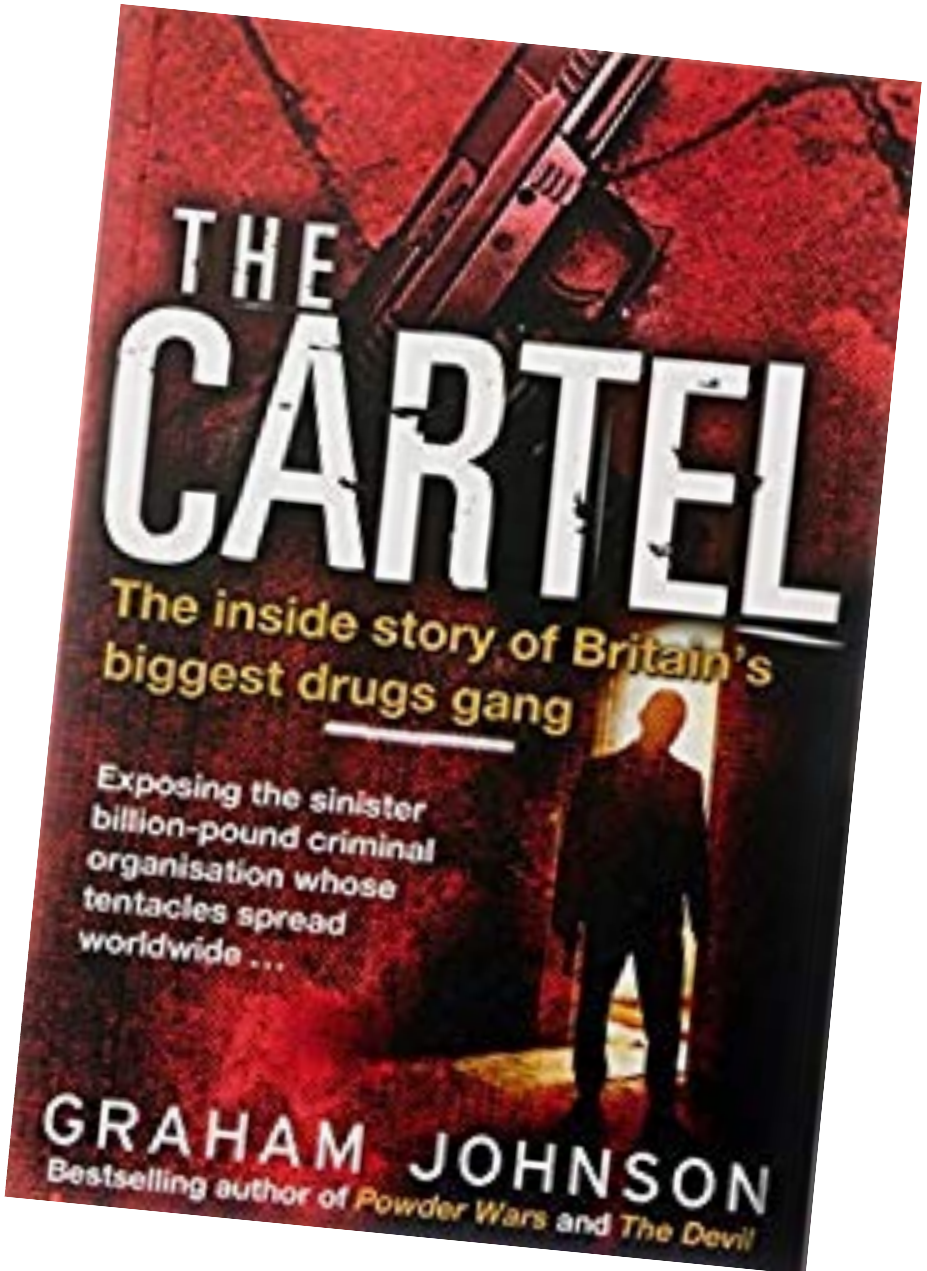
Neden Fajlarda Direnç Gelişmez

- 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

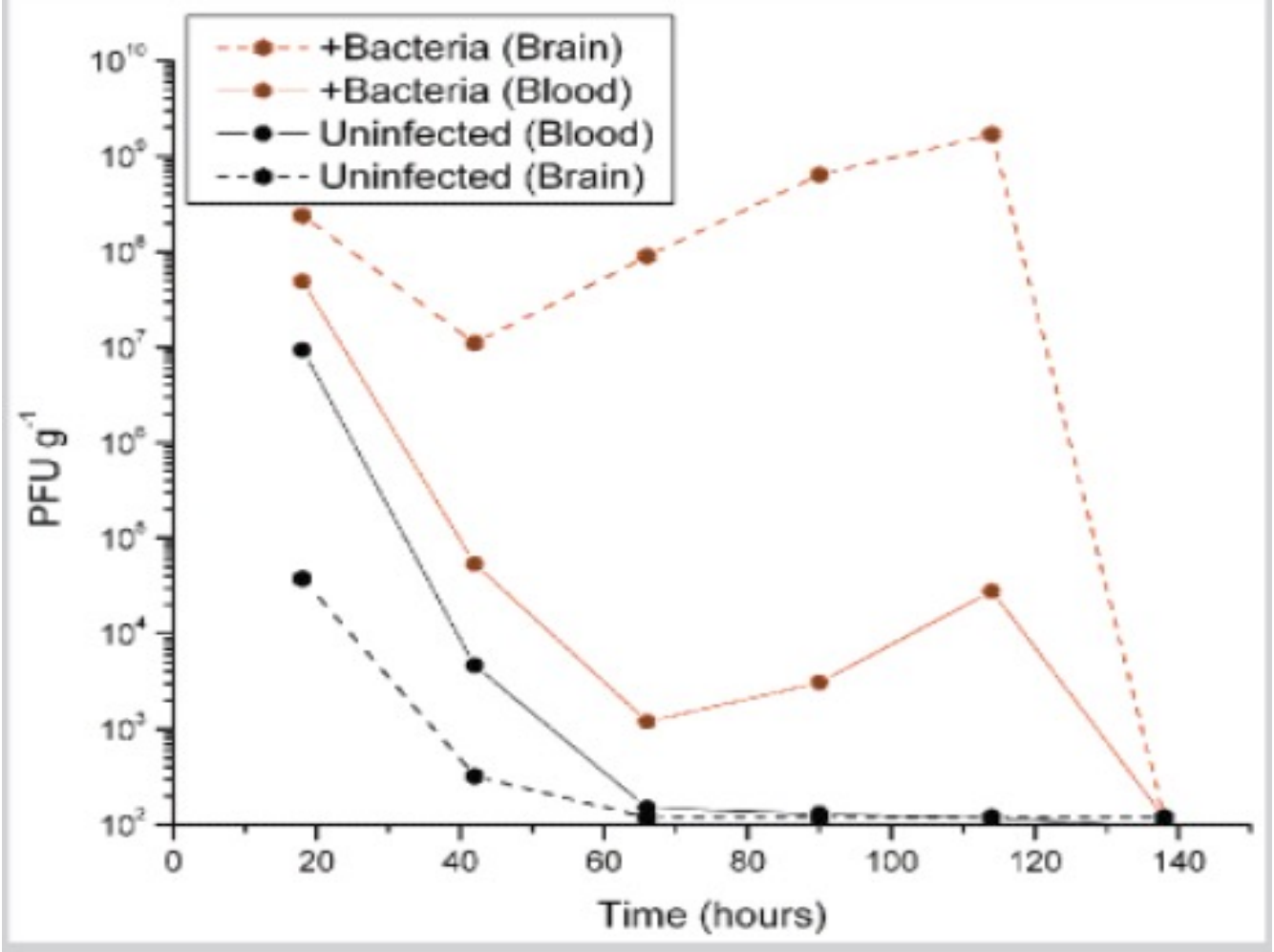
Klinik Kullanımı Neden Yaygınlaşmadı?

- Batıda kullanılmamasının birkaç nedeni var:
- Antibiyotiklerin yapımı, depolanması ve kullanımı daha pratik ve kolaydı
- Fajla ilgili çeşitli medikal girişimler yapıldı, sistemik kullanımını geliştirecek ARGE bütçeleri yeni antibiyotiklere kaydırıldı,
- Antibiyotik direnci henüz sorun olmadığından bakteriyofaj tedavisi önemini kaybetti
- İlaç firmaları için Antibiyotik geliştirmek ve buna dönük ARGE yatırımları daha karlı ve prestijli idi
- Uluslararası alanda Eski Sovyetler Birliği'nde gerçekleştirilen ve Rusça ya da Gürcü diliyle yazılan makaleler yeterince anlaşılamadı ve geniş okuyucu kitlesine ulaşmadı.



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..



antibiotics

MDPI

Review

The Safety and Efficacy of Phage Therapy for Superficial Bacterial Infections: A Systematic Review

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BURN WOUND INFECTIONS	CHRONIC WOUND INFECTIONS-ULCER	DERMATOLOGIC INFECTIONS
8 Articles, (156 Cases) Clinical Cure 77.5% (n=111)	12 Articles, (327 Cases) Clinical Cure 86.1% (n=310)	(10 Articles, 1096 Cases) Clinical Cure 94.1% (n=734)

Konular	Antibiyotiklerin Dezavantajları	Bakteriyofajların Avantajları
İlaç Molekülünün Kaderi	Antibiyotikler bulunduğu ortamda metabolik yıkıma uğrar	Eksponensiyel artışla infeksiyon bölgesinde daha güçlü etkinlik sergiler
Spektrumdaki belirli bir bakteriyi öldürmek için gereken ilacın konsantrasyonu	Antibiyotikler bulundukları ortamda diğer ilaçlarla etkileşerek teröpatik güçlerini kaybedebilirler	Bakteriyofajların bulundukları ortamda diğer ilaç ya da moleküllerle etkileşmeleri söz konusu değildir
Bakteri Mutasyonuna karşı yanıt	Antibiyotikler bakterinin geliştirdiği mutasyonlar karşısında etkisiz kalmaktadır. Bu da antibiyotik direnci ile sonuçlanır.	Fajlar, bakteriyel mutasyonların üstesinden gelebilen ve mutasyona uğrayan canlılardır. Örneğin fajlar mutasyona uğrayan bakteriye de bağlanabilir ya da bakterinin mutasyon etkisinden kendisini koruyabilir.
Bakteri Direnci	Bakterilerin antibiyotiklere karşı gelişen direnç mekanizmaları nesiller ve türler arasında yayılmaktadır.	Fajlar açısından durum daha farklıdır. Bakteri faja karşı spesifik reseptör içermediğinde faj bağlanamaz, ancak bir başka faj o reseptörlere bağlanır, direnç gelişmesi görülmez.
Etkinlik	Hedef bakteri grubu dışında non-patojen türlere ve flora bakterilerine de zarar verebilir. Spektrumundaki bütün bakterileri hedef alır	Sadece bakteriyofajın hedefinde bulunan bakteri türüne karşı etki eder. Diğer bakteriler bundan etkilenmez
Üretimi ve Hazırlanması	Antibiyotiklerin geliştirilmesi son derece maliyetli ve uzun süreli işlemleri gerektirir. Bazen aylar ve yıllarca süren çalışmalar daha sonra başarısızlıkla sonuçlanabilir. Yüksek maliyetlidir.	Kısa süre içerisinde düşük maliyetle hazırlanabilir.
Yan Etki ve Diğer İlaçlarla Etkileşim	Lokal ve sistemik yan etkileri mevcuttur. İlaç etkileşimleri yaygındır. Tedavide bu durum dikkate alınmalıdır.	Bilinen yan etkileri yoktur. İlaç etkileşimine girmez

TEŞEKKÜRLER..