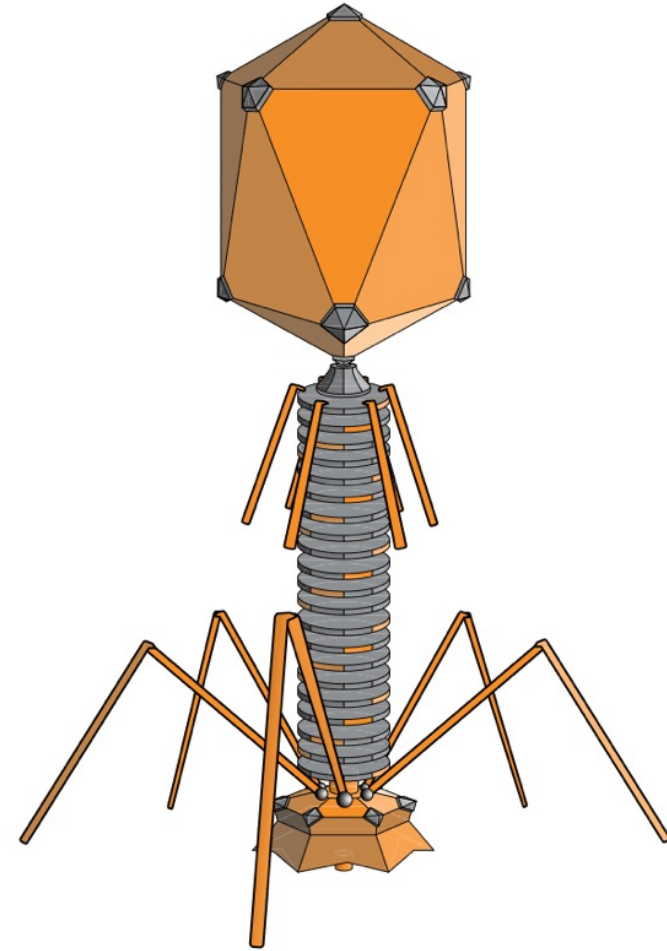


MESTO BAKTERIOFAGOV PRI ZDRAVLJENJU SEPSE

Doc. Dr. Nina Grasselli Kmet, dr. med.

KIBVS-RC

11.9.2025



O virusu podobnem povzročitelju z antibakterijskimi lastnostmi je prvi poročal M. E. Hankin leta 1896.



Značilnosti:

- temperaturno občutljiv
- sposoben prehajanja skozi porcelanasti filter
- zmanjšal je titer bakterije *Vibrio cholerae* v laboratorijski kulturi.

➤ Hankinovi zaključki:

"To bi lahko pomagalo zmanjšati pojavnost kolere pri ljudeh, ki uporabljajo vodo iz Gangesa."



Troubled waters? Bathers in the Ganges were thought to be protected from cholera by phage.

BAKTERIOFAG

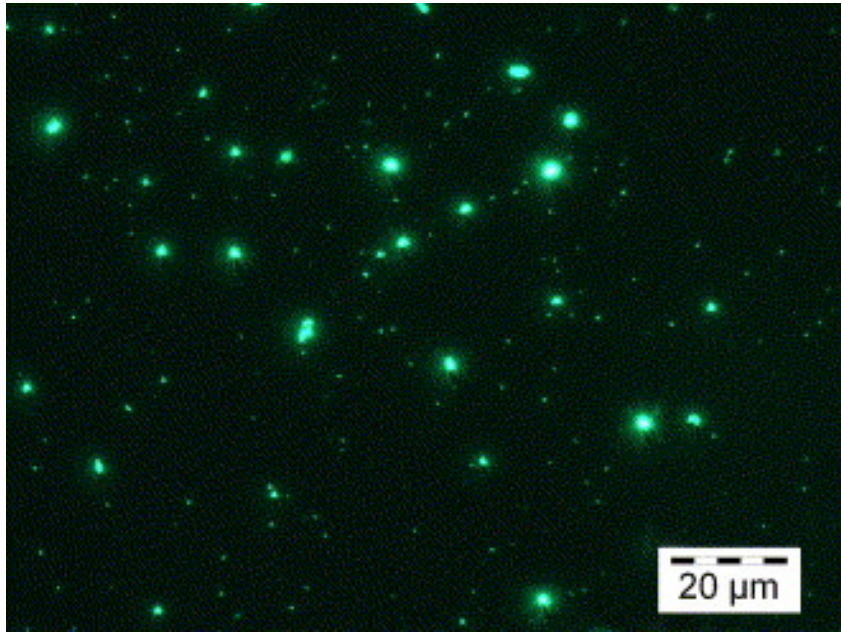
Beseda izvira iz stare grščine

bacteria

φαγεῖν (phagein) 'požreti'

Bakteriofági, ali samo fági (gr. φαγεῖν phagein = žreti), **so virusi, ki zajedajo v bakterijah, in so navadno specifični za vrsto ali sev bakterij.**

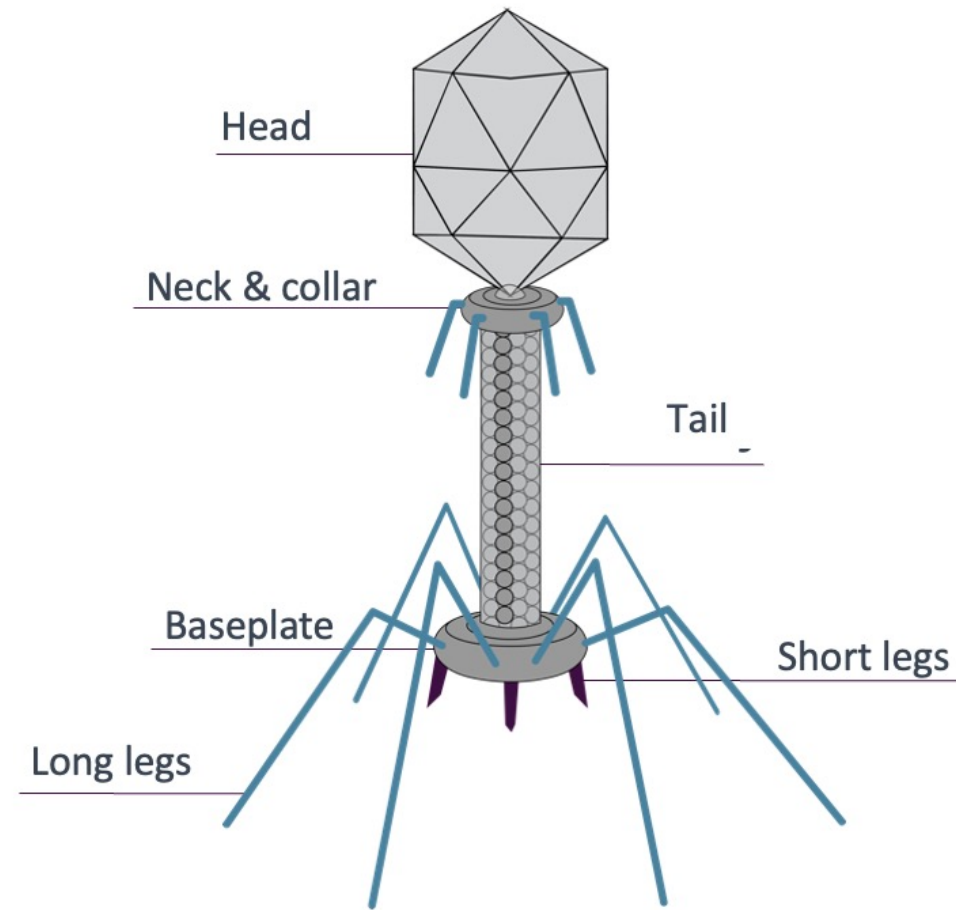
POJAVNOST



Na planetu je po ocenah 10^{31} fagov, ki so vsi potencialno sposobni zdraviti bolezni. Za primerjavo, v nam vidnem delu vesolja je »le« 10^{24} zvezd!

Do sedaj je bilo izoliranih in preučenih
le 2000–3000 fagov.

STRUKTURA



NAČIN DELOVANJA

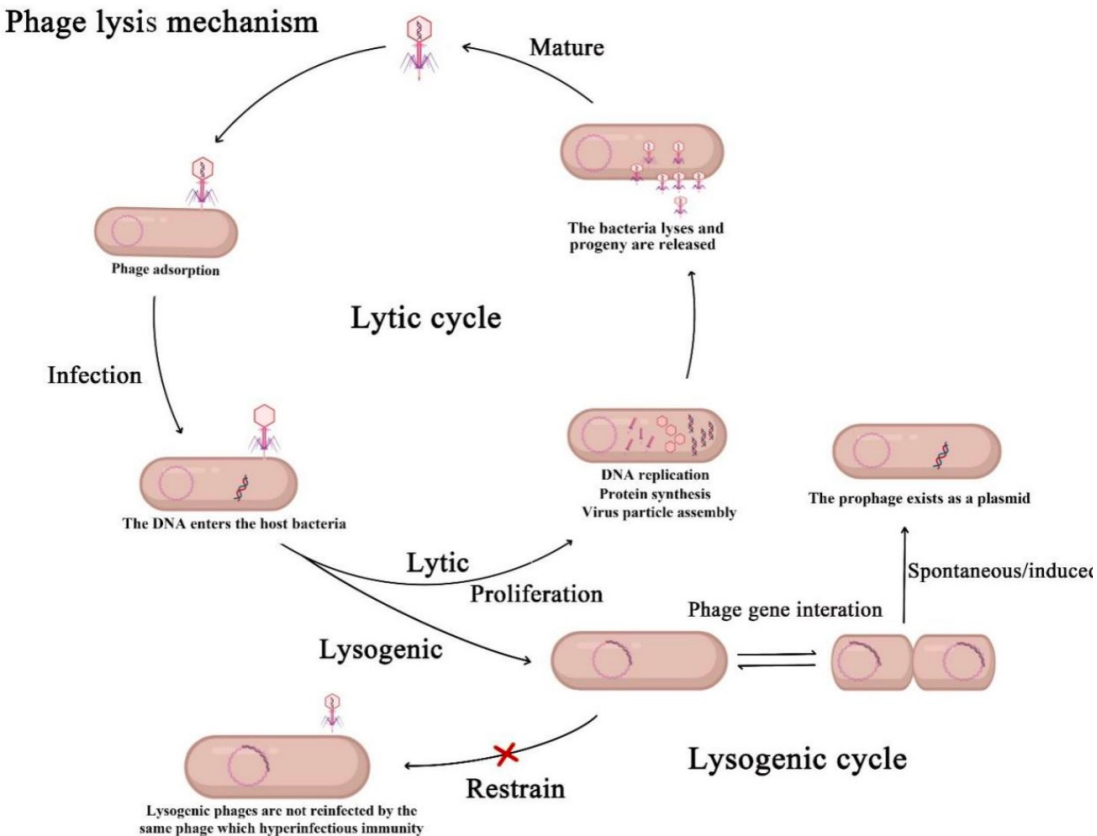


Figure 1. Mechanisms of action of lytic phage and lysogenic phage.

Litični in lizogeni cikel: primerjava

Značilnost	Litični cikel	Lizogeni cikel
Izid za bakterijo	Bakterija je uničena (lizirana)	Bakterija ostane živa
Integracija DNK faga	Ne integrira se v bakterijsko DNK	Integrira se v bakterijsko DNK (profag)
Replikacija	Hitra proizvodnja novih fagov	Fagova DNK se replicira skupaj z bakterijsko DNK
Sprožitev aktivnosti	Takoj po okužbi	Lahko ostane v mirovanju, aktivira ga stres

ODKRITJE BAKTERIOFAGOV



1898

**Nnikolai
Gamalea**

Russia

*Russ Arch
Pathol Clin Med
Bacteriol 6
(1898), pp. 607–
613.*



1915

**Frederick Twort
UK**

*An investigation
on the nature of
ultramicroscopi
c viruses (1915),
Lancet 11: 1241*



1917

**Felix D'Herelle
France**

*Sur un microbe
invisible antagoniste
des bacilles
dysentériques (Comptes
rendus de l'Académie
des Sciences, Paris,
1917. 165 :p.373-5.*



1918

**George Eliava
Georgia**

“THE WINTER WAR” (TALVISOTA)

1939-1940



Experience of phage prophylaxis based on the results of 3 mobile brigades.

I brigade applied phages for 2,500 solders, among them gangrene symptoms were revealed in 35 (1,4%) cases. In the control group of 7,918 solders gangrene was registered in 342 (4.3%) cases.

II brigade applied phage treatment for 941 solders, only 14 (1,5%) got sick. In the control group 6,8% were infected.

III brigade applied phage treatment for 2,584 soders, gangrene was developed in 18 (0,7%) cases, while in the control group incidence of infection was 2,3%



BITKA ZA STALINGRAD (1943)



Zinaida Ermolyeva



PENICILLIN, New Wonder Drug from Mold

By IRMIS JOHNSON

A GREENISH BLU mold like the one that grows on stale bread, or lends aroma and flavor to Roquefort cheese, now promises to be an important ally in helping wounded soldiers fight their way back to health.



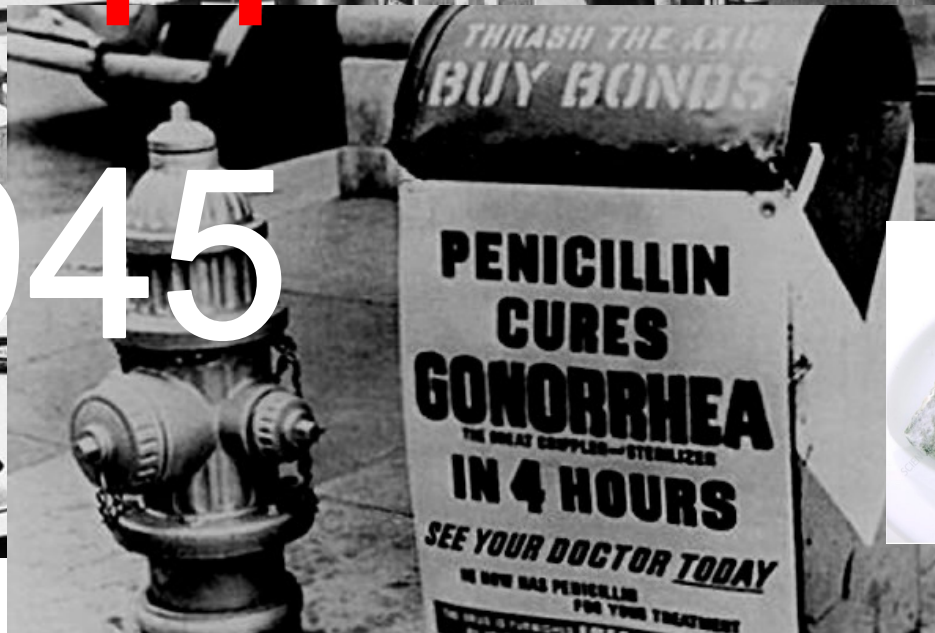
1928

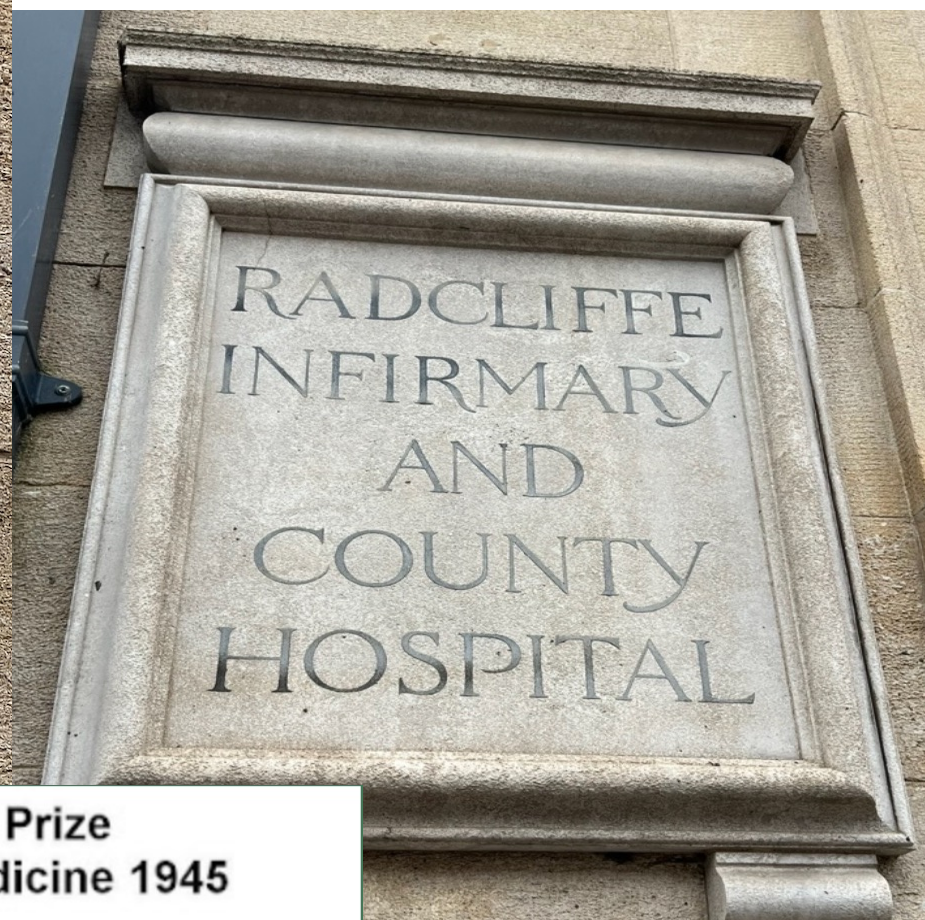


1941



1945





The Nobel Prize Physiology/Medicine 1945



Sir Alexander Fleming
1881 - 1955

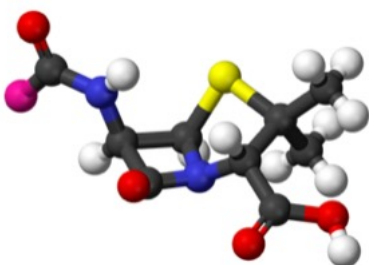


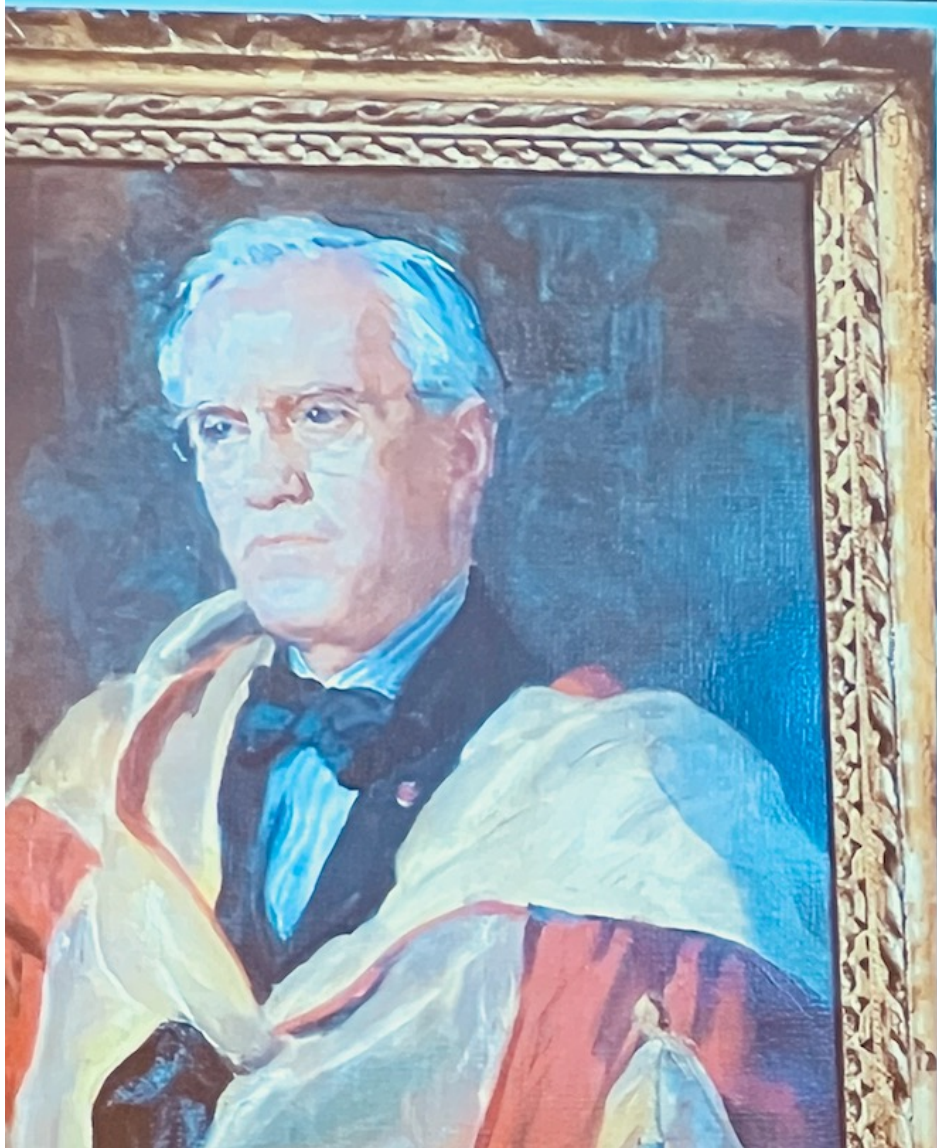
Sir Howard Walter Florey
1898 - 1968



Ernst Boris Chain
1906 - 1979

Alexander Fleming discovered the antimicrobial properties of penicillin in 1928. years later, Howard Florey and Ernst Chain developed the processes to produce penicillin





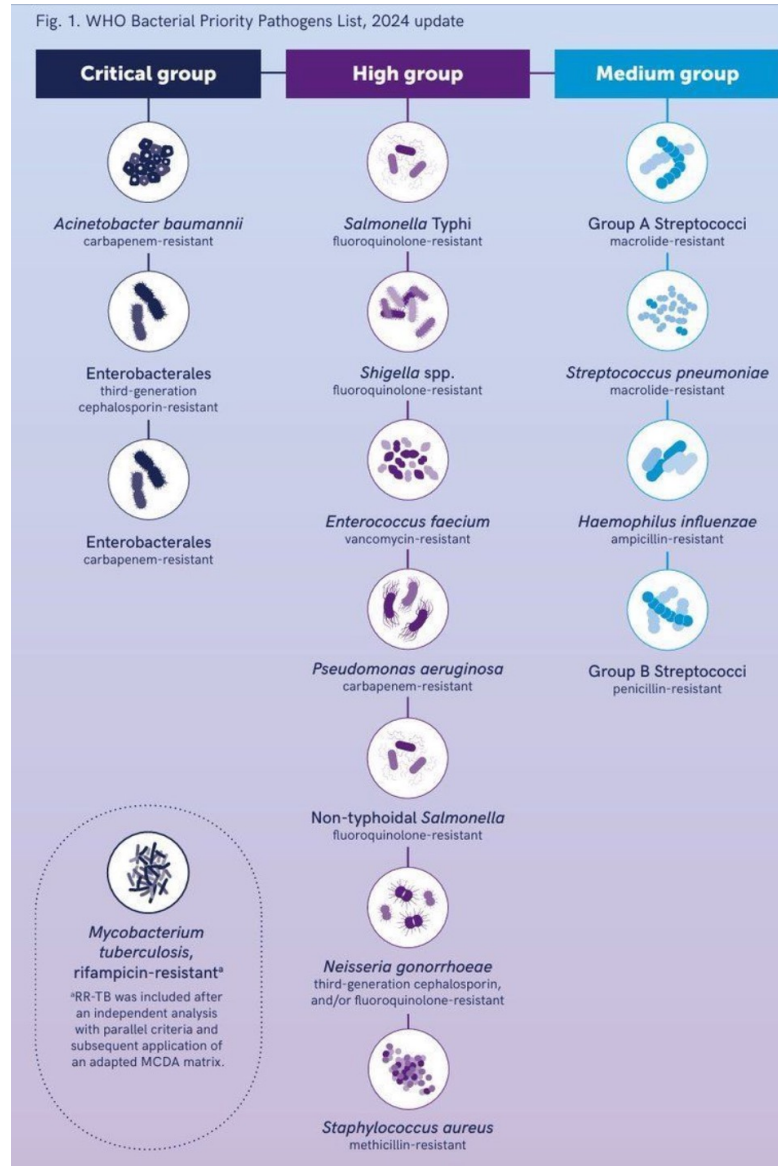
"The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism."

Fleming, 1945

KAJ JE PROTIMIKROBNA ODPORNOST?

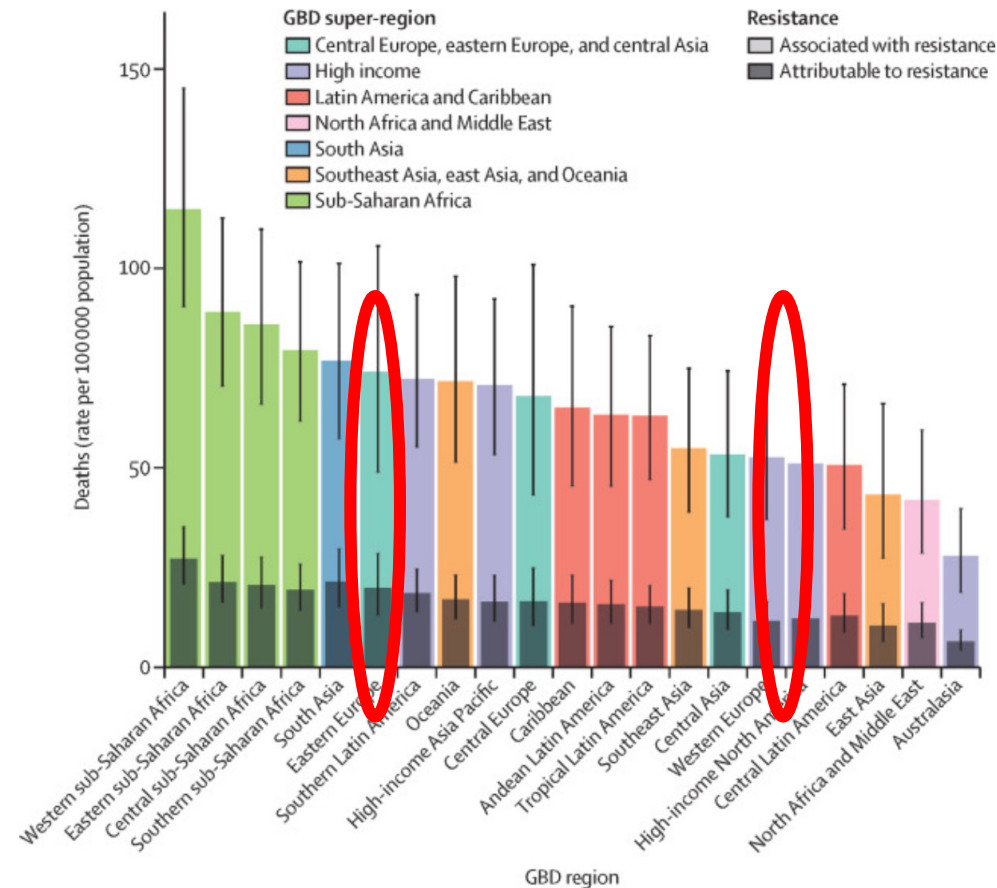
- Protimikrobna odpornost (AMR) je sposobnost mikroorganizmov, da vztrajajo ali rastejo v prisotnosti zdravil, ki so namenjena njihovem zaviranju ali ubijanju.
- VOB – vrsta mikroorganizma kaže odpornost na vsaj eno protimikrobno zdravilo v treh ali več protimikrobnih kategorijah

WHO “PRIORITY” LIST



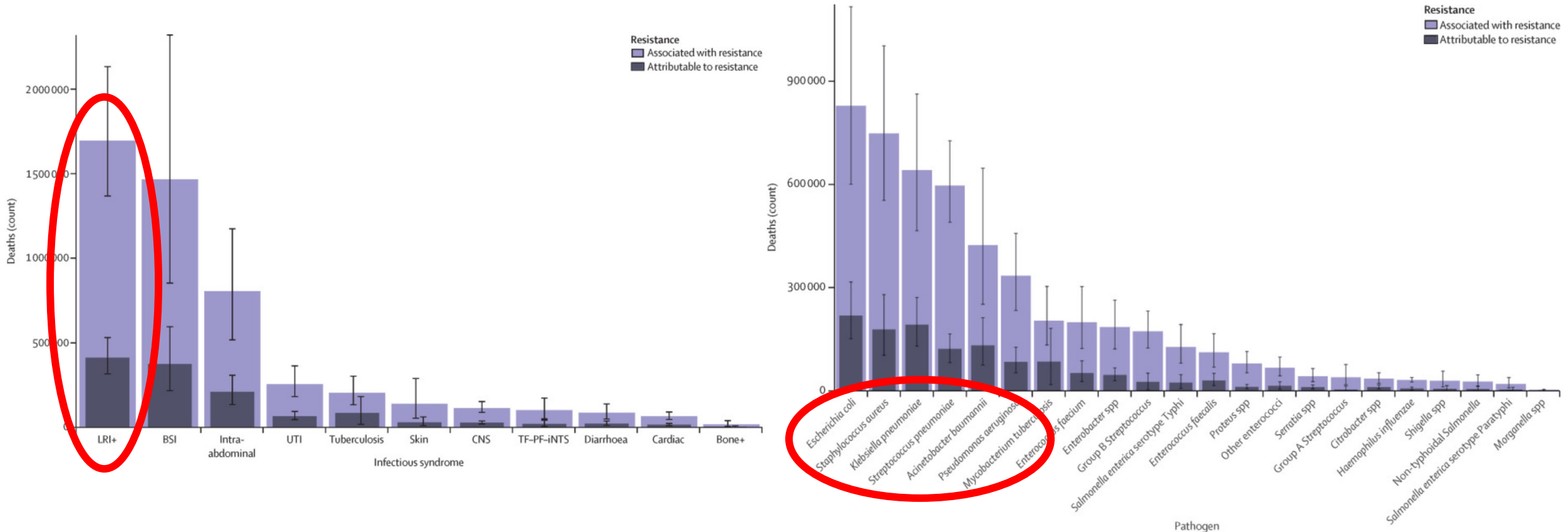
Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis

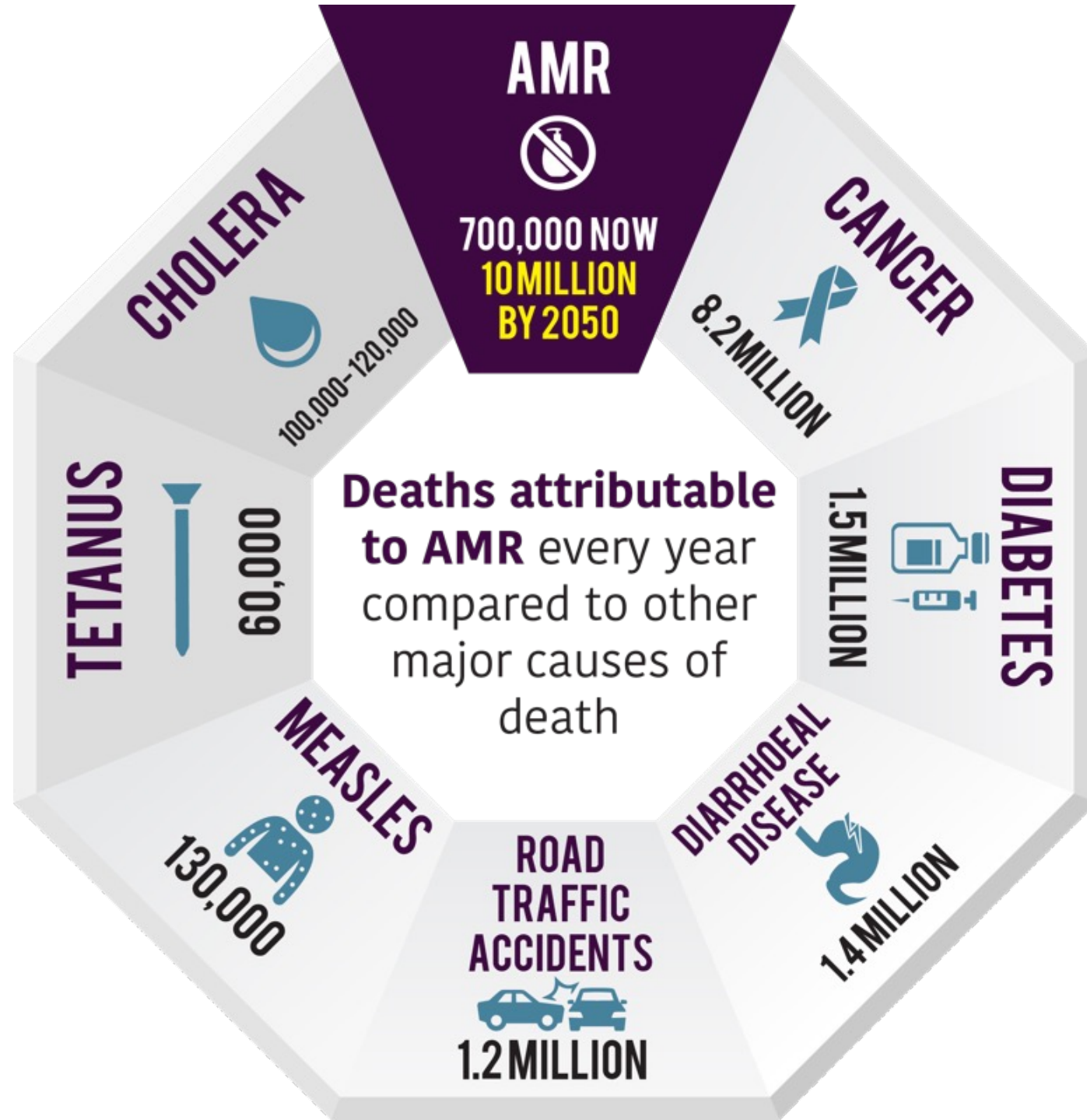
*Antimicrobial Resistance Collaborators**

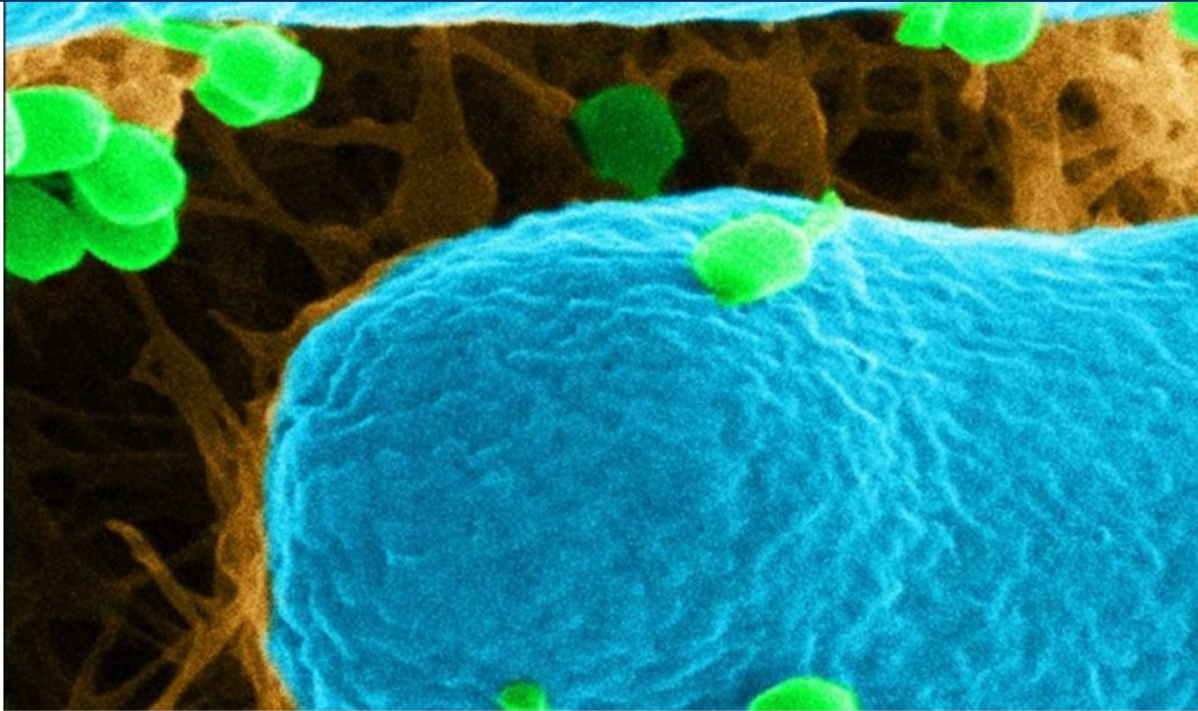


Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis

Antimicrobial Resistance Collaborators*







Bacteriophages and their use in combating antimicrobial resistance

Key facts

- Bacteriophages (phages) are viruses that selectively target and kill bacteria. They are the most abundant commonly occurring natural entities, playing crucial roles in regulating bacterial populations and influencing microbial ecosystems.
- Phages are useful as they can destroy bacteria resistant to drugs such as antibiotics. Phages infect their bacterial hosts with great specificity. They do not infect human cells.
- Antimicrobial resistance (AMR) poses a serious global threat to our ability to treat bacterial infections. New antibiotics have often proved difficult and expensive to develop. This has led to an interest in an older approach to treating microbial infections by using phages. Phage therapy can be a promising tool for controlling AMR, which is one of the top global public health and development threats.
- In the WHO European Region, AMR is directly responsible for 133 000 deaths each year and indirectly linked to 541 000 deaths. Estimates show that AMR costs the European Union and European Economic Area about €11.7 billion each year due to health expenditure and workforce productivity losses.
- Addressing AMR requires a multifaceted approach that considers the interconnectedness of human, animal, and environmental health – known as the One Health approach. Phages provide biologically innovative approaches to addressing the challenge of AMR across sectors, ranging from therapeutic use in humans and animals to potentially replacing antibiotic use in the agricultural sector.
- Currently, phages are primarily used on compassionate grounds, in life-threatening situations, when all other treatments have been exhausted.

FAGI vs. ANTIBIOTIKI

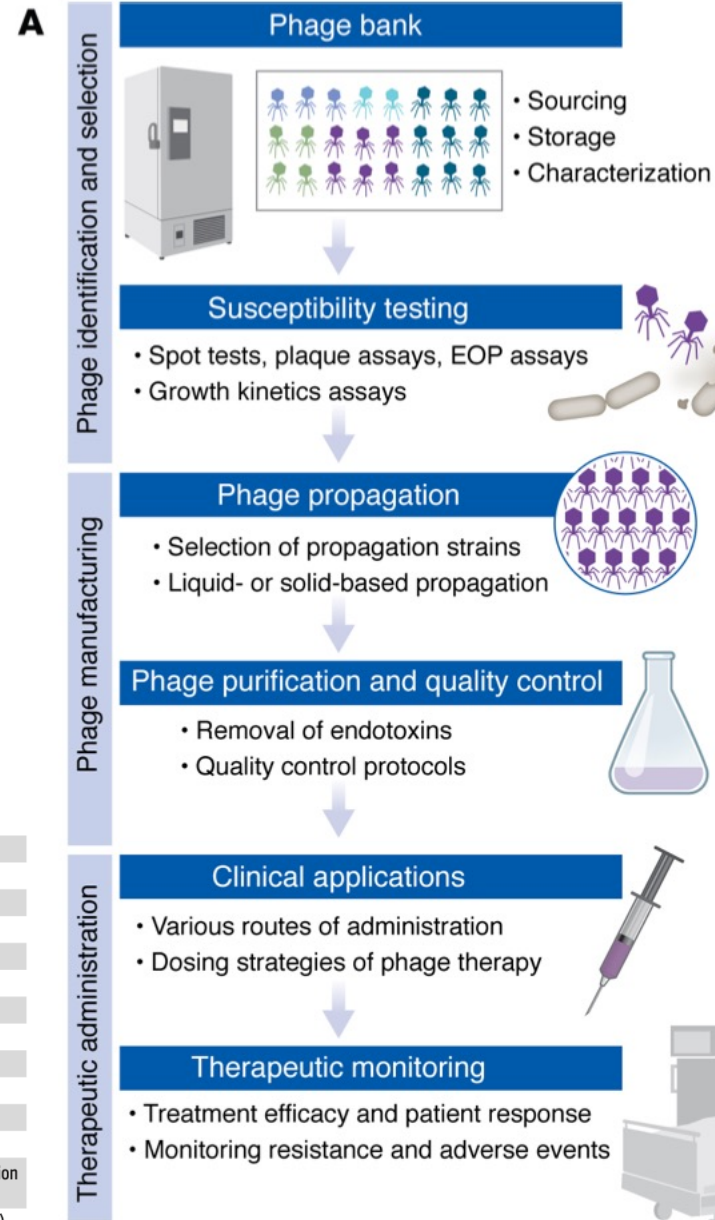
- delujejo specifično na eno klico – ni stranskih učinkov na telesno mikrobioto
- se samoreproducirajo
- s proizvodnjo so povezani nižji stroški v primerjavi z antibiotiki
- učinkoviti so tudi proti večkratno odpornim bakterijam
- redke interakcije in neželeni učinki

NAČINI UPORABE

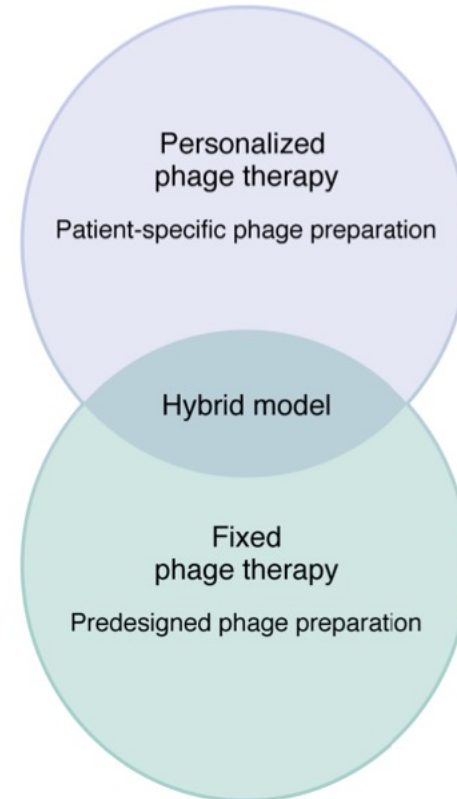
- Magistralni pripravki
- Sočutna raba/
personaliziran pristop (EU,
ZDA)

RCT raziskave

Parameter	Personalized phage therapy	Fixed phage therapy
Phages isolated in advance?	Variable	Yes
Phages characterized in advance?	Variable	Yes
Phage-phage interactions known?	Variable	Yes
Cocktail defined in advance?	No (customized per patient)	Yes
Phagogram done before treatment?	Yes	Variable
Therapeutic monitoring during therapy?	Variable	Variable
GMP production required for compassionate use?	Not currently	Not currently
GMP production required for scaled-up product?	Yes	Yes
Cost per patient for compassionate use?	Low if non-GMP	Low if non-GMP
Cost per patient at scale?	High	Low (Economies of scale)
Controlled clinical trials completed?	No	Yes (114)
Success in case reports?	Yes (9)	Yes (10, 111)
Straightforward regulatory pathway for compassionate use?	Yes, in most countries (eIND in USA, Helsinki Declaration in Europe; SAS in Australia)	Yes, in most countries (eIND in USA, Helsinki Declaration in Europe; SAS in Australia)
Defined regulatory pathway for scaled up drug?	No (allowed in Georgia; allowed through magistral phage in Belgium; unclear in other countries)	Yes (traditional biologic drug development pathway)
Potential for rapid availability for acute infections?	Unlikely	Yes



B Phage therapy approaches



MAGISTRALNI PRIPRAVKI



PHAGE BANK Seed lot

- Characterized phages
- Stored using a (tiered) banking system



CONTROLLED ENVIRONMENT

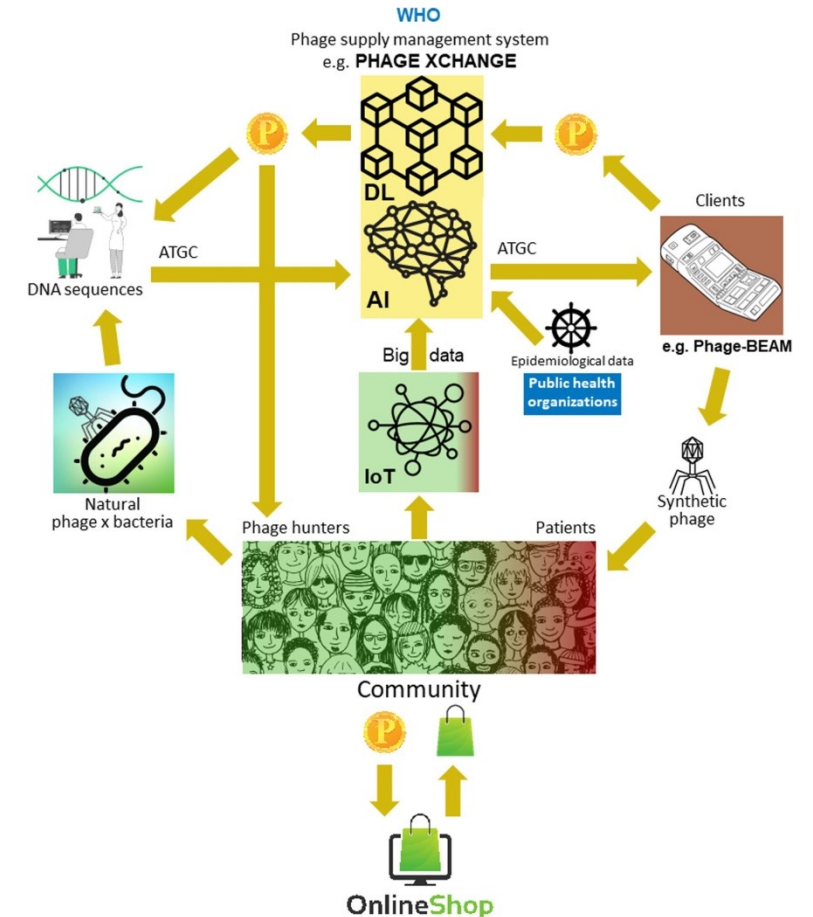
Active Pharmaceutical Ingredient (API)

- A phage API (single phage) is produced using a suitable host
- Produced according to a monograph
- External quality testing based on a monograph, performed by a "Belgian Approved Laboratory"

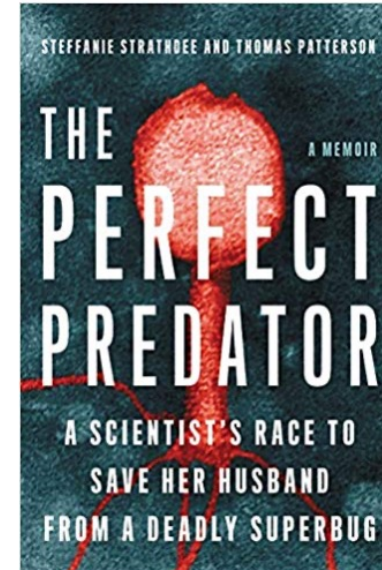
HOSPITAL PHARMACY

Magistral preparation

- Upon prescription
- One or more phage APIs are selected (phagogram)
- Phage APIs are mixed with a carrier (e.g. a hydrogel)



PERSONALIZIRANA (SOČUTNA) RABA



Antimicrobial Agents
and Chemotherapy

📄 | Research Article | 22 September 2017



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

 This article has been corrected. [VIEW CORRECTION](#)

Authors: Robert T. Schooley, Biswajit Biswas, Jason J. Gill, Adriana Hernandez-Morales, Jacob Lancaster, Lauren Lessor, Jeremy J. Barr, [SHOW ALL \(34 AUTHORS\)](#), Theron Hamilton | [AUTHORS INFO & AFFILIATIONS](#)

<https://doi.org/10.1128/aac.00954-17> •  Check for updates

PERSONALIZIRANA (SOČUTNA) RABA



ARTICLE



<https://doi.org/10.1038/s41467-021-27056-z>

OPEN

Combination of pre-adapted bacteriophage therapy and antibiotics for treatment of fracture-related infection due to pandrug-resistant *Klebsiella pneumoniae*

Analís Eskenazi^{1,9}, Cédric Lood^{2,3}, Julia Wubbolts⁴, Maya Hites¹, Nana Balarjishvili⁵, Lika Leshkasheli⁵, Lia Askilashvili⁵, Leila Kvachadze⁵, Vera van Noort^{3,6}, Jeroen Wagemans², Marc Jayankura⁷, Nina Chanishvili⁵, Mark de Boer⁴, Peter Nibbering⁴, Mzia Kutateladze⁵, Rob Lavigne², Maya Merabishvili⁸ & Jean-Paul Pirnay⁸

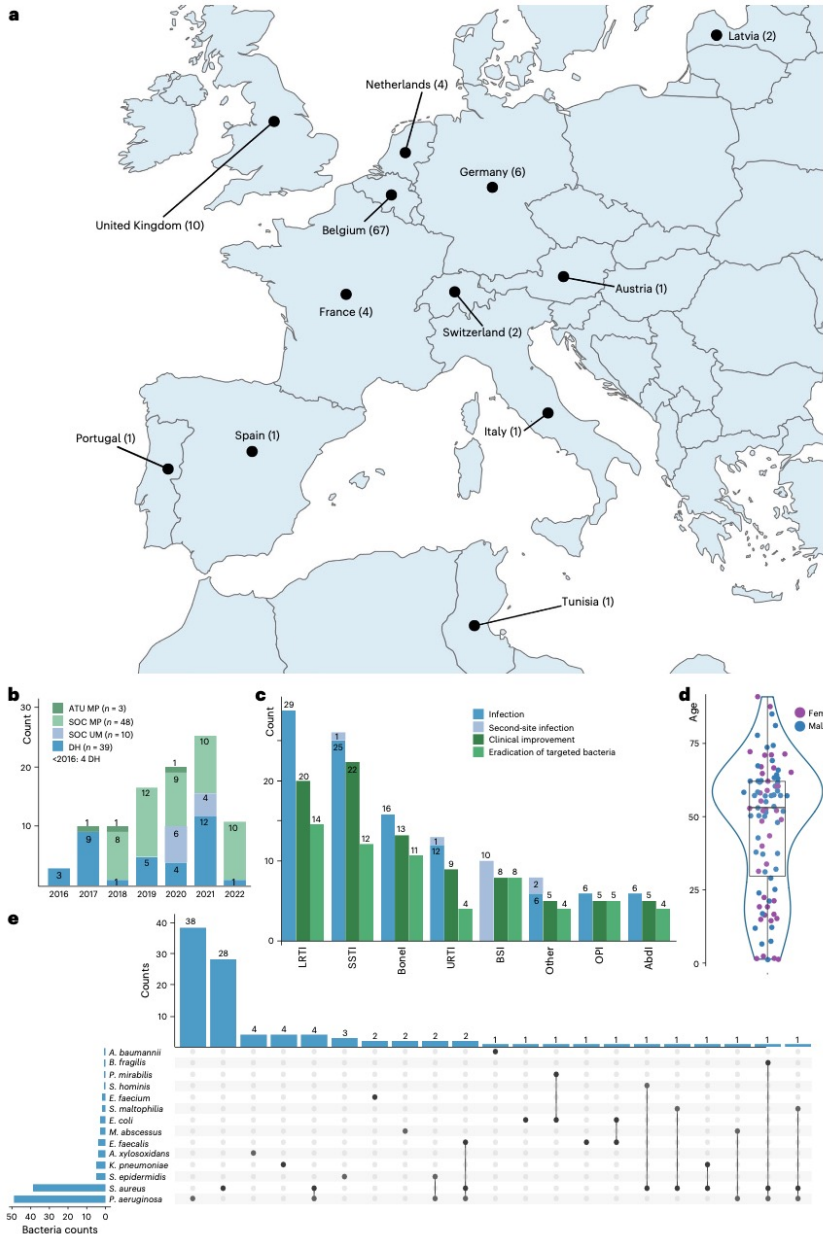
Personalized bacteriophage therapy
outcomes for 100 consecutive cases: a
multicentre, multinational, retrospective
observational study

- Objavljeno 2024 (2008-2022)
- 100 primerov personalizirane uporabe v Evropi
- **77.2% ozdravitev oz. 61.3% eradikacija**
- **sinergizem z antibiotiki!**

Table 1 | General overview of bacteriophage therapy protocols according to the main infection types

Infection type	Application route	Bacteriophage carrier	Volume (ml)	Concentration (p.f.u.sml ⁻¹)	Dose	Duration
Lower respiratory tract infections	Nebulization	NaCl 0.9%	2–4	10 ⁷ –10 ⁸	q6h	5 days–6 weeks
Bone and orthopaedic prostheses infections	Intralesional	NaCl 0.9%	2–70	10 ⁷ –10 ⁸	q24h	5 days–3 weeks
Skin and soft tissue infections	Topical	NaCl 0.9% or Flaminal Hydro	In excess	10 ⁷ –10 ⁹	q24h	5 days–3 weeks
Upper respiratory tract infections	Nasal spray	NaCl 0.9%	1–15	10 ⁷	q8h	1–3 weeks
Bloodstream infections or other infection types ^a	Intravenous	NaCl 0.9%	50–100	10 ⁶ –10 ⁷	q24h	5–10 days

^aWhen the treating physician considered it was necessary to apply bacteriophages systemically. p.f.u.s, plaque forming units; q, every.



SOČUTNA RABA

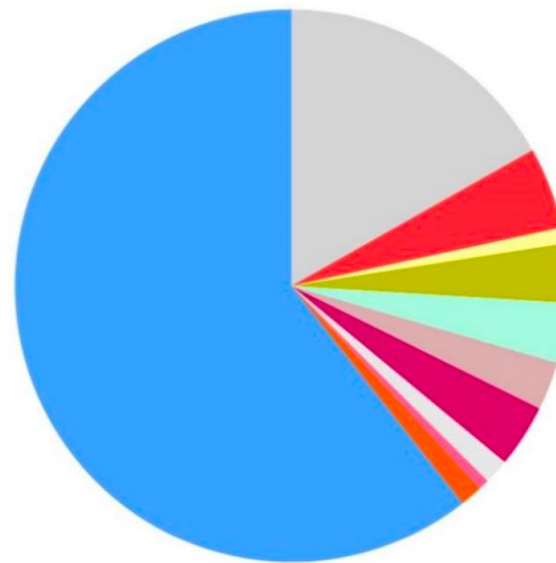
Organization/company	Country	Study	Year	Refs.
Baylor college of medicine (TAILOR)	USA	A retrospective, observational study. Device-related or systemic infections, 12 patients • Evaluated 12 cases of customized phage therapy, showing a 66% favorable response rate, with 42% bacterial eradication • Phage therapy was safe, though immunological neutralization occurred in some cases	2023	[20]
Belgian Consortium Study	Belgium	A multicenter, multinational, retrospective observational study. Individualized phage therapy • Analyzed 100 cases of individualized phage therapy across 12 countries • Showed clinical improvement in 77.2% of cases and bacterial eradication in 61.3% • The use of antibiotics alongside phage therapy increased the likelihood of success	2024	[21]
Prosthetic joint infections	Italy	A case report. Pa53 (anti- <i>P. aeruginosa</i> phage) • A 62-year-old patient with chronic <i>P. aeruginosa</i> infection was successfully treated with customized phage therapy and meropenem, showing no infection recurrence over 2 years	2023	[22]
Diabetic foot infection	UK	A case series. Anti- <i>S. aureus</i> therapy • Tested anti-<i>S. aureus</i> phage therapy on 10 patients at high risk of amputation • Nine out of 10 patients benefited, though one patient showed no response to treatment	2023	[23]
LVAD infection study	Israel/ USA	A case series. Anti- <i>P. aeruginosa</i> therapy • Phage therapy in combination with antibiotics for LVAD-related <i>P. aeruginosa</i> infections had limited success, with breakthrough bacteremia and immune reactions hindering effectiveness	2023	[24]



National Library of Medicine
National Center for Biotechnology Information

PubMed®

bacteriophage therapy|



USA	(16.82%)
China	(4.79%)
Russia	(0.93%)
Japan	(3.43%)
UK	(3.52%)
France	(2.82%)
Germany	(3.69%)
India	(1.46%)
Brazil	(0.63%)
Italy	(1.30%)
Other	(60.61%)

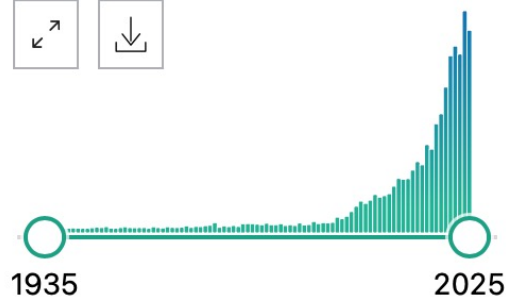
Figure 2. Percentage of articles published on the National Center for Biotechnology Information (NCBI) database.

MY CUSTOM FILTERS

9,072 results

Page 1 of 908

RESULTS BY YEAR



Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles.

1

Furfaro LL, Payne MS, Chang BJ.

Cite

Front Cell Infect Microbiol. 2018 Oct 23;8:376. doi: 10.3389/fcimb.2018.00376. eCollection 2018.

PMID: 30406049 [Free PMC article.](#) [Review.](#)

Phage therapy is rapidly evolving and has resulted in cases of life-saving therapeutic use and multiple clinical trials. ...This review discusses the multi-drug resistant Gram-negative pathogens of highest critical priority and summarizes the current state-of-the-ar ...

Evaluation of Bacteriophage Cocktail on Septicemia Caused by Colistin-Resistant *Klebsiella pneumoniae* in Mice Model

Aprajita Singh^{1†}, Alakh Narayan Singh^{1†}, Nisha Rathor², Rama Chaudhry
Sudhir Kumar Singh¹ and Gopal Nath^{1*}

Conclusion: Our study showed that the optimized relatively lower and multiple dosages of phage cocktails with the strict monitoring of vitals in clinical settings might cure septicemia caused by MDR bacteria with different severity of infection.

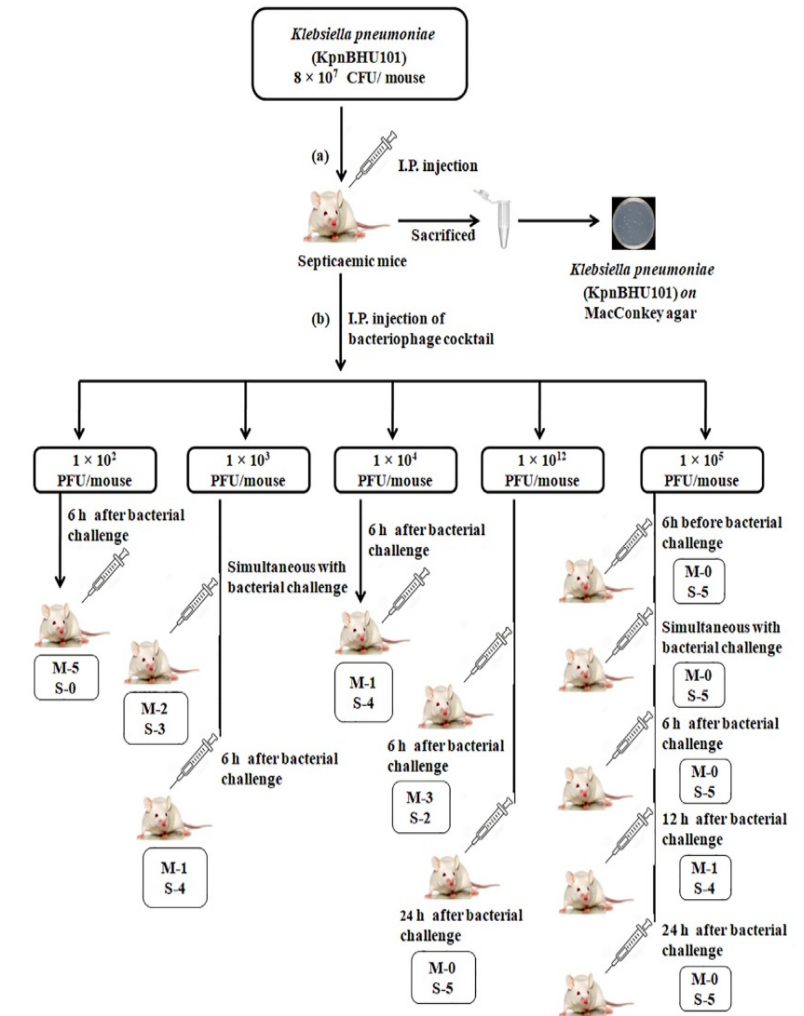


FIGURE 4 | Mouse experiment flowchart **(A)** Depicts the induction of septicemia through intraperitoneal route with *K. pneumoniae* at the dose of 8×10^7 CFU/mouse of KpnBHU101. After 24 h, mice were killed, and organs were homogenized and cultured on MacConkey plates to see the presence of bacteria. **(B)** Outline of the efficacy of the bacteriophage cocktail dosage with varying concentrations at a different time point after the initial bacterial challenge. Each group has 5 mice; (M) depicts the number of dead mice and (S) depicts the number of mice surviving.



Phage-induced protection against lethal bacterial reinfection

Yikun Xing^{a,b,1} , Haroldo J. Hernandez Santos^{a,b,1} , Ling Qiu^b , Samantha R. Ritter^{a,b} , Jacob J. Zulk^{a,b} , Rachel Lahowetz^b , Kathryn A. Patras^{b,c} , Austen L. Terwilliger^{a,b} , and Anthony W. Maresso^{a,b,2}

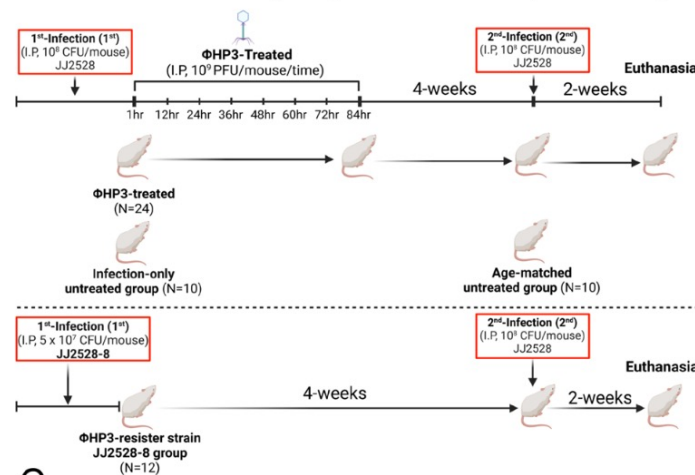
Affiliations are included on p. 9.

Edited by Bruce Levin, Emory University, Atlanta, GA; received November 8, 2024; accepted April 1, 2025

- Študija na miših
- Poleg lize bakterijskih celic – dolgotrajna zaščita pred ponovno okužbo
- Terapevtsko – vakcinacijski učinek

A

Murine model of sepsis (Φ HP3-treatment, JJ2528-8)



New Phage cocktail against infantile Sepsis bacteria

Sadeq Abdulridha Gatea Kaabi , Hadeel Kareem Musafer  

[Show more](#) 

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- In vitro raziskava (2020)
- 54 kliničnih izolatov (HK) pediatričnih bolnikov s sepso: *E. coli*, *K. pneumoniae*, *H. influenzae*, *P. aeruginosa*, *C. freundii*, *M. catarrhalis*
- Fagi izolirani iz odpadnih vod
- Fagni cocatail – in vitro 100 % aktivnost proti izolatom

REVIEW

Open Access

Bacteriophages and antibiotic interactions in clinical practice: what we have learned so far



Marzanna Łusiak-Szelachowska^{1*} , Ryszard Międzybrodzki^{1,2,3}, Zuzanna Drulis-Kawa⁴, Kathryn Cater⁵, Petar Knežević⁶, Cyprian Winogradow⁷, Karolina Amaro⁸, Ewa Jończyk-Matysiak¹, Beata Weber-Dąbrowska^{1,2}, Justyna Rękas¹ and Andrzej Górski^{1,2,9}

- Biofilmi – fagno–antibiotični sinergizem (PAS)
- Fagno-antibiotični antagonizem - v nekaterih primerih lahko opazimo tudi kot zmanjšano učinkovitost zdravljenja v primerjavi z enim od posameznih zdravljenj

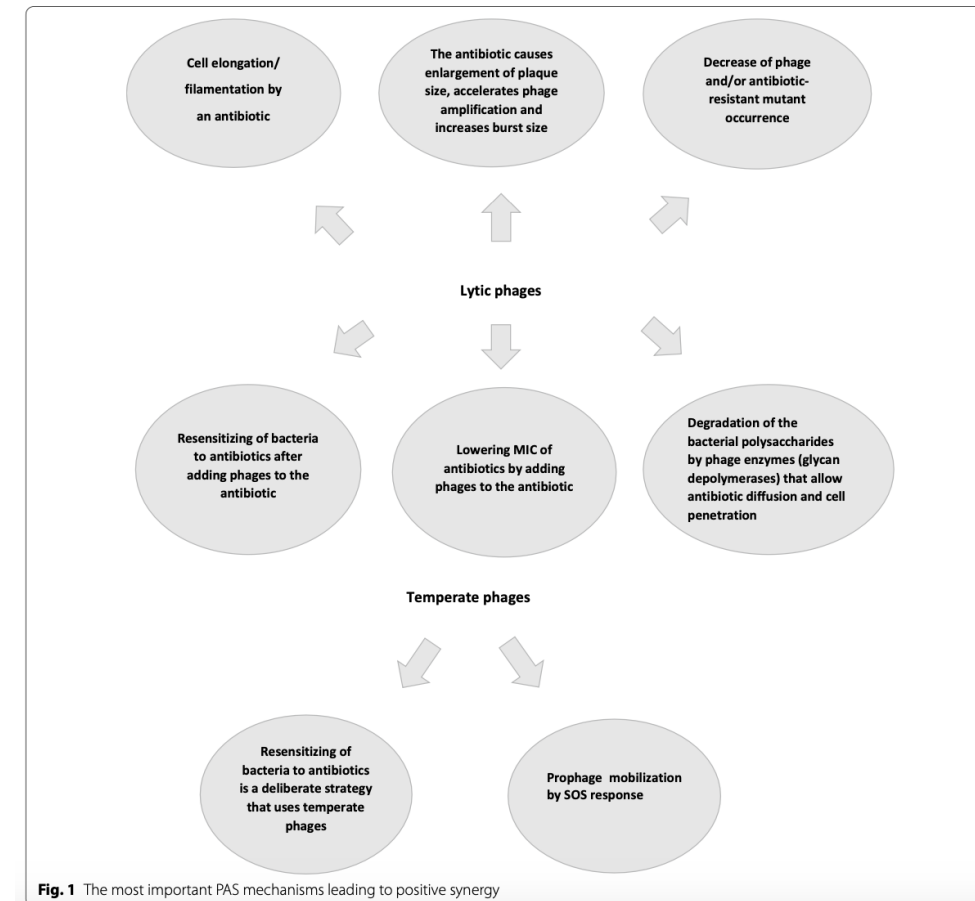


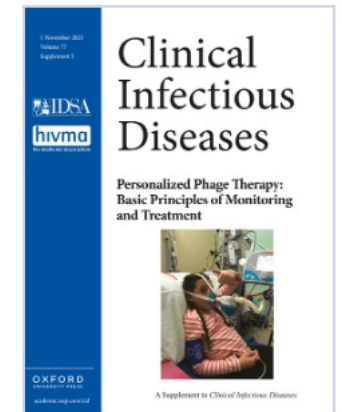
Fig. 1 The most important PAS mechanisms leading to positive synergy

Phage Therapy as a Novel Therapeutic for the Treatment of Bone and Joint Infections

Gina A. Suh,¹ Tristan Ferry,² and Matthew P. Abdel³

¹Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester Minnesota, USA; ²Department of Infectious and Tropical Diseases, Hospital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, France; and ³Department of Orthopedic Surgery, Mayo Clinic, Rochester Minnesota, USA

- 2010-2023: 33 opisov uporabe pri okužbah kosti in sklepov
- Sistemska in lokalna aplikacija
- **87 % ozdravitev**
- 24 % - blagi neželeni učinki (najpogosteje povišani jetrni testi!)



Volume 77, Issue
Supplement_5
1 November 2023

INSTRUCTIONAL LECTURE: GENERAL ORTHOPAEDICS

Bacteriophage therapy in musculoskeletal infections: from basic science to clinical application

Tristan Ferry^{1,2,3,4,5}, Jolien Onsea^{6,7}, Tiphaine Roussel-Gaillard⁸, Cécile Batailler⁹,
 Thomas Fintan Moriarty¹⁰ and Willem-Jan Metsemakers^{6,7}

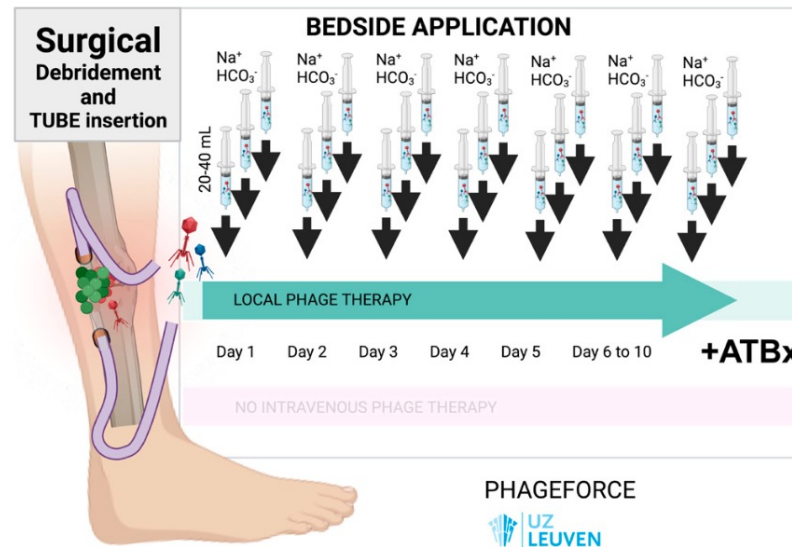


Figure 2

Typical phage treatment course in patients with FRI included in the PHAGEFORCE study, with surgical debridement, surgical tube (drain) insertion, and then administration of 20–40 mL of active phages three times per day for 7–10 days. Prior to each phage application, the drain is rinsed with sodium bicarbonate (NaHCO₃) 1.4%. All patients receive concomitant antibiotics.

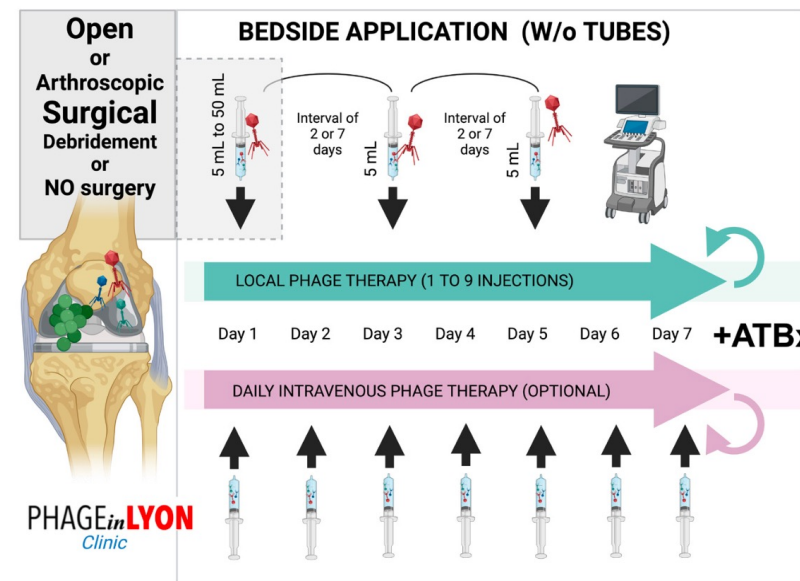
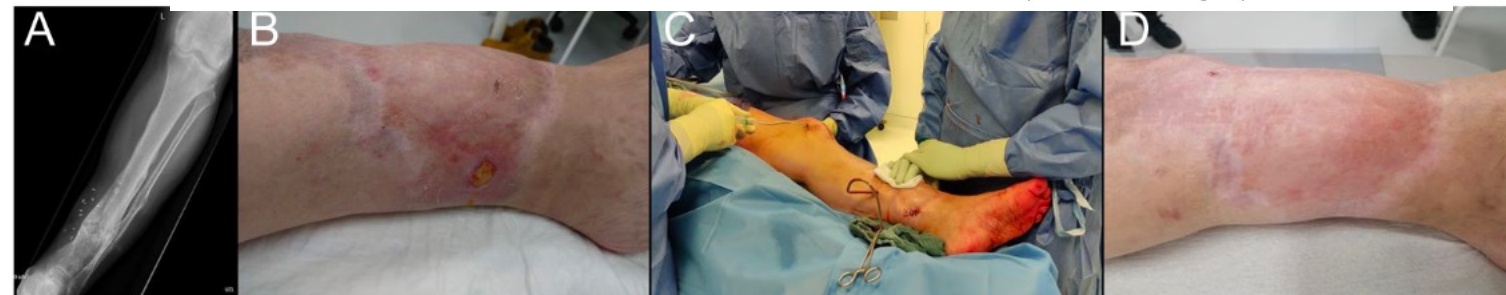


Figure 4

Typical phage treatment course in patients with PJI included in the PHAGEinLYON Clinic programme. As adjuvant treatment to antibiotics, based on susceptibility testing, the patient received a first local injection of phages in the joint cavity during surgery (volume 30–50 mL) or after the surgery under sonography (5 mL), and then subsequent injections under sonography exclusively (5 mL), without using tubes. The number of injections and the time interval between each injection are depending on the clinical presentation. Daily intravenous injections of phages could be prescribed in non-surgical patients.





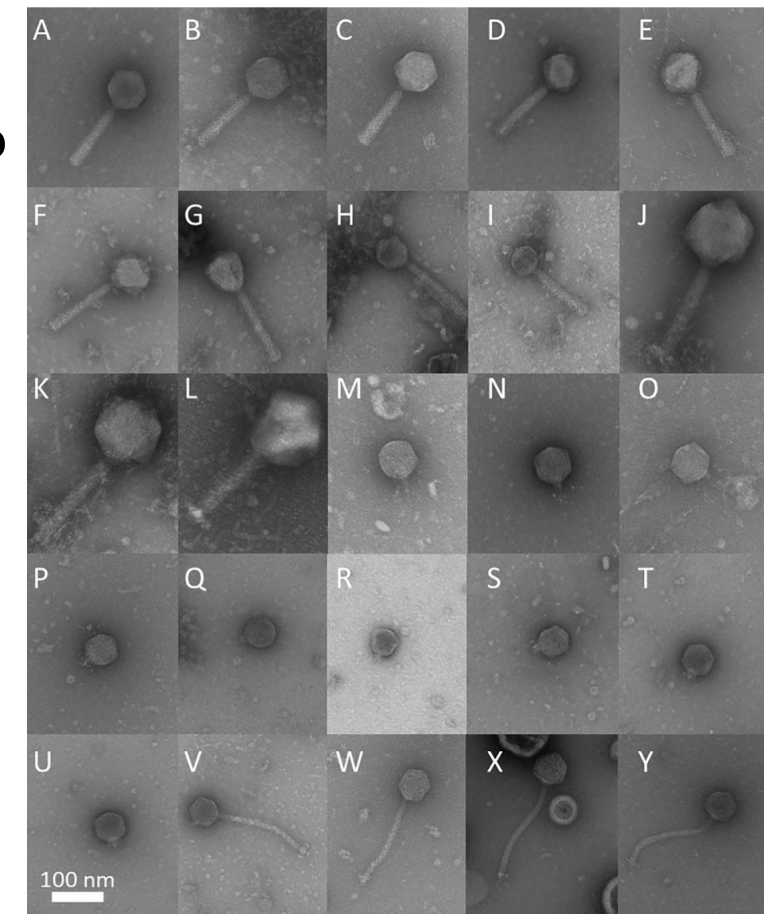
OPEN ACCESS

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Mercedes Gonzalez Moreno,
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and Infection Biology, Hans Knoll Institute,
Germany

REVIEWED BY
Yunxue Guo,
Chinese Academy of Sciences (CAS), China
Robert Ramirez-Garcia,
Imperial College London, United Kingdom

Systematic bacteriophage selection for the lysis of multiple *Pseudomonas aeruginosa* strains

Finja Rieper^{1,2}, Johannes Wittmann³, Boyke Bunk³,
Cathrin Spröer³, Melanie Häfner⁴, Christian Willy⁴,
Mathias Müsken⁵, Holger Ziehr¹, Imke H.E. Korf^{1*†}
and Dieter Jahn^{2,6*†}



- 6 fagov
- testirana učinkovitost na 141 izolatih (klinični vzorci od bolnikov) – liza pri 92 % vzorcih

5 Conclusion

25 *P. aeruginosa* phages were characterized and compared showing a great diversity within all morphotypes. A combination of six phages could theoretically lyse 92% of clinical *P. aeruginosa* strains. Further, we showed that especially *Pakpunavirus*, *Pbunavirus*, *Pawinskivirus*, *Elvirus* (all myoviruses), *Litunavirus* and *Bruynoghevirus* (all podoviruses) showed the greatest potential in the proposed ranking and should be employed with priority, as they have already been used for phage therapy. Siphoviruses were less suitable. Phages with a lysogenic cycle also have less potential due to their narrow host range and efficiency (low vp value), as well as safety concerns regarding phage therapy.

TRENUITNO POTEKAJOČE RCT

Organization/company	Country	Study	Year	Refs.
Pherecydes Pharma (Phaxiam Therapeutics)	France	A randomized, controlled, double-blind phase 1/2 trial • PhagoBurn <i>E.coli</i> , and <i>P aeruginosa</i> infections	NCT02116010 2019	[25] , [26]
Armata Pharmaceuticals	USA	A multi-center, double-blind, randomized, placebo-controlled, single and multiple ascending dose phase 1/ 2 trial • AP-PA02, <i>P. aeruginosa</i> for severe respiratory infections • Evaluating safety and tolerability of inhaled AP-PA02 in chronic lung infections and cystic fibrosis (SWARM-Pa)	NCT04596319 2024–	[27]
Adaptive phage therapeutics	USA	A randomized, parallel, double-blind, placebo-controlled, repeat dose, multi-site phase 1/2 trial • DANCE™; diabetic foot osteomyelitis	NCT05177107 2022–	[28]
Israeli phage therapy center	Israel	A pre-phase-1 cohorts trial • PASA16; <i>P. aeruginosa</i> infection	– 2023	[29]
TechnoPhage	Portugal	A randomized, parallel, open label, phase 1/2a trial • TP-122A, ventilator-associated pneumonia • Assess safety and tolerability	NCT06370598 2024–	[30]
MB pharma	Czech Republic	A randomized, double-blind, placebo-controlled phase 1/2a trial • DUOFAG®; phage cocktail against <i>S. aureus</i> and <i>P. aeruginosa</i> for surgical wound infection	NCT06319235 2022–	[31]
Locus biosciences	USA	A double-blind, randomized, active-controlled phase 2/3 trial • LBP-EC01; <i>E. coli</i> -induced UTIs • Treatment of acute uncomplicated UTI caused by drug-resistant <i>E. coli</i> (ELIMINATE Trial)	NCT05488340 2024	[32]

UPORABA V SLOVENIJI

- Zaenkrat še ne
- Ortopedska bolnišnica Valdortra – in vitro študije
- Pomlad 2025; PeK
- Raziskovalno (TP v UKC)

ZANIMIVOST

JAFRAL is **world's biggest phage manufacturer (CDMO)**, which primary focus is development and production of phage-based products for various (GMP and non-GMP) applications:

- **Human therapeutics**
- **Food industry**
- **Cosmetics**
- **Veterinary**
- **Agriculture**

Other biomolecules: pDNA, mRNA, OMVs, rProteins, Fill & Finish



SKLEPI

- “Virusi, ki uničujejo bakterije” (tudi VOB)
- Selektivno delovanje, ni vpliva na normalno mikrobioto
- Relativno poceni proizvodnja
- Redki stranski učinki
- Primerni za zdravljenje bakterijskih okužb, tudi sepse in tistih povzročenih z VOB
- Sinergizem z antibiotiki, prodor v biofilm
- Zaenkrat pomanjkanje RCT – možna je sočutna raba

HVALA ZA POZORNOST

