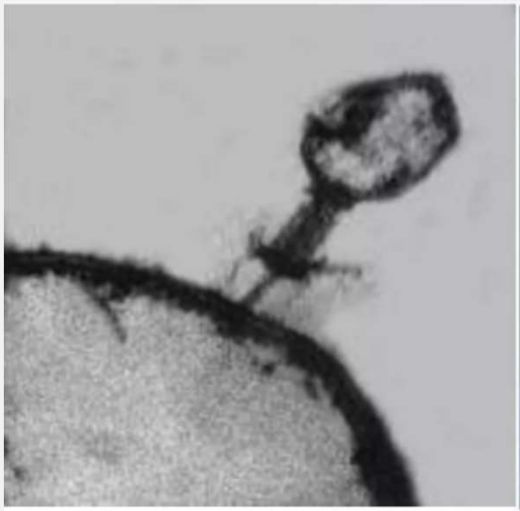
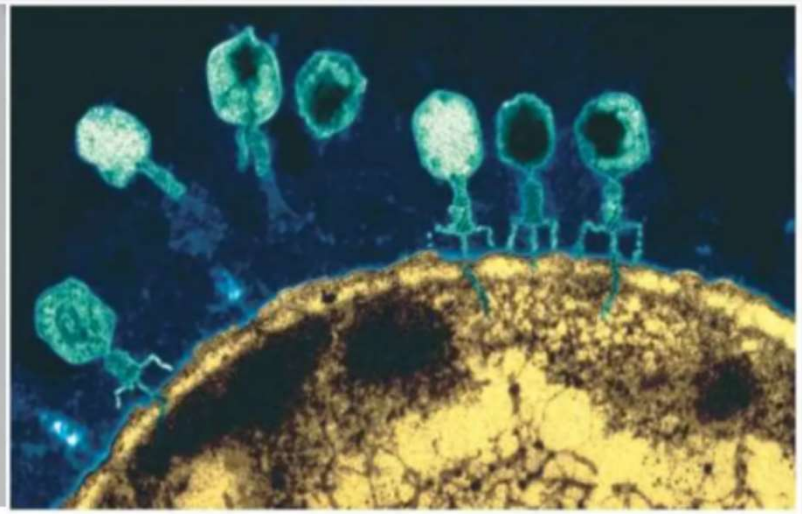
MEDICAMENTOS MANIPULADOS PARA TERAPIA FÁGICA EM CONTEXTO HOSPITALAR

DIREÇÃO DE AVALIAÇÃO DE MEDICAMENTOS

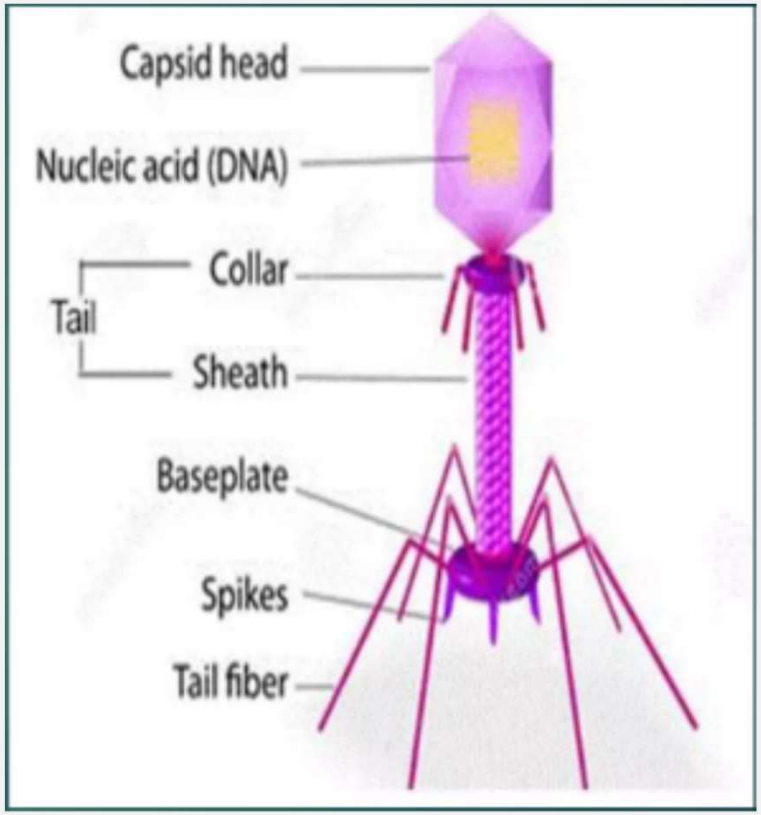
ANA PAULA MARTINS

Bacteriófagos

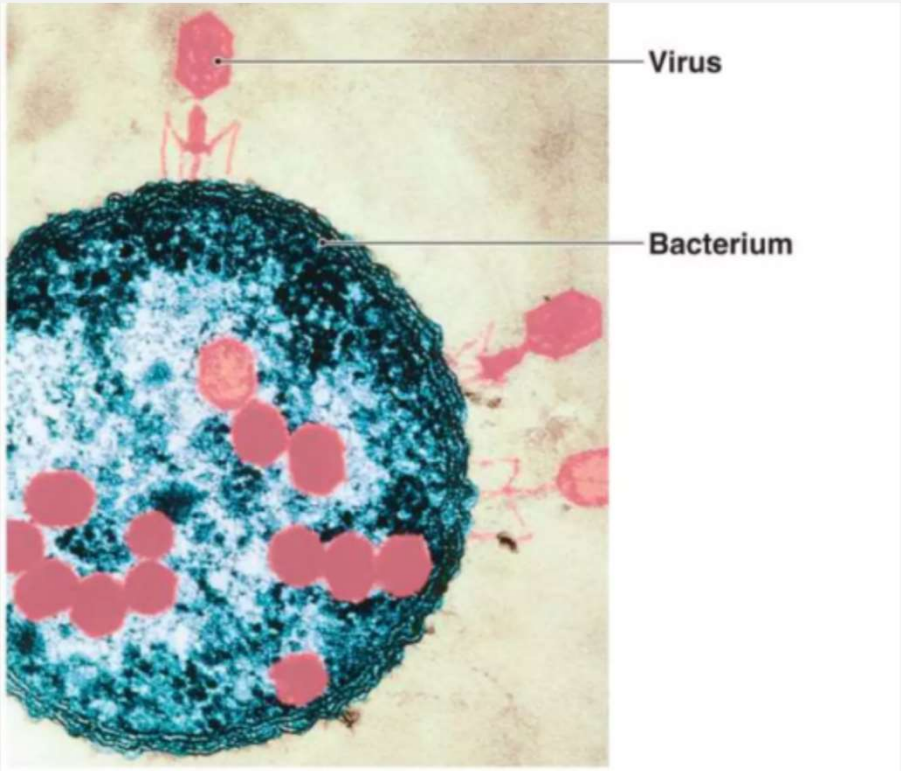




Bacteriófagos - vírus que infectam apenas bactérias e geralmente muito específicos, atacando apenas uma única estirpe bacteriana.
Esta especificidade, juntamente com a capacidade de matar, torna-os inimigos naturais das bactérias



Bacteriófagos



Bacteriófagos infectando uma bactéria

Fago:
Fago deriva da palavra grega “φαγεῖν” que significa “devorar”.

“Bacteriófagos” são “devoradores de bactérias”, tendo sido descritos pela primeira vez em 1915

O bacteriófago é um vírus que ataca as bactérias.

Ataca a membrana celular bacteriana e entra na célula bacteriana.

Introduz o seu material genético dentro da célula hospedeira.

Utiliza o mecanismo de replicação do hospedeiro (bactérias) para criar mais cópias de si próprio.

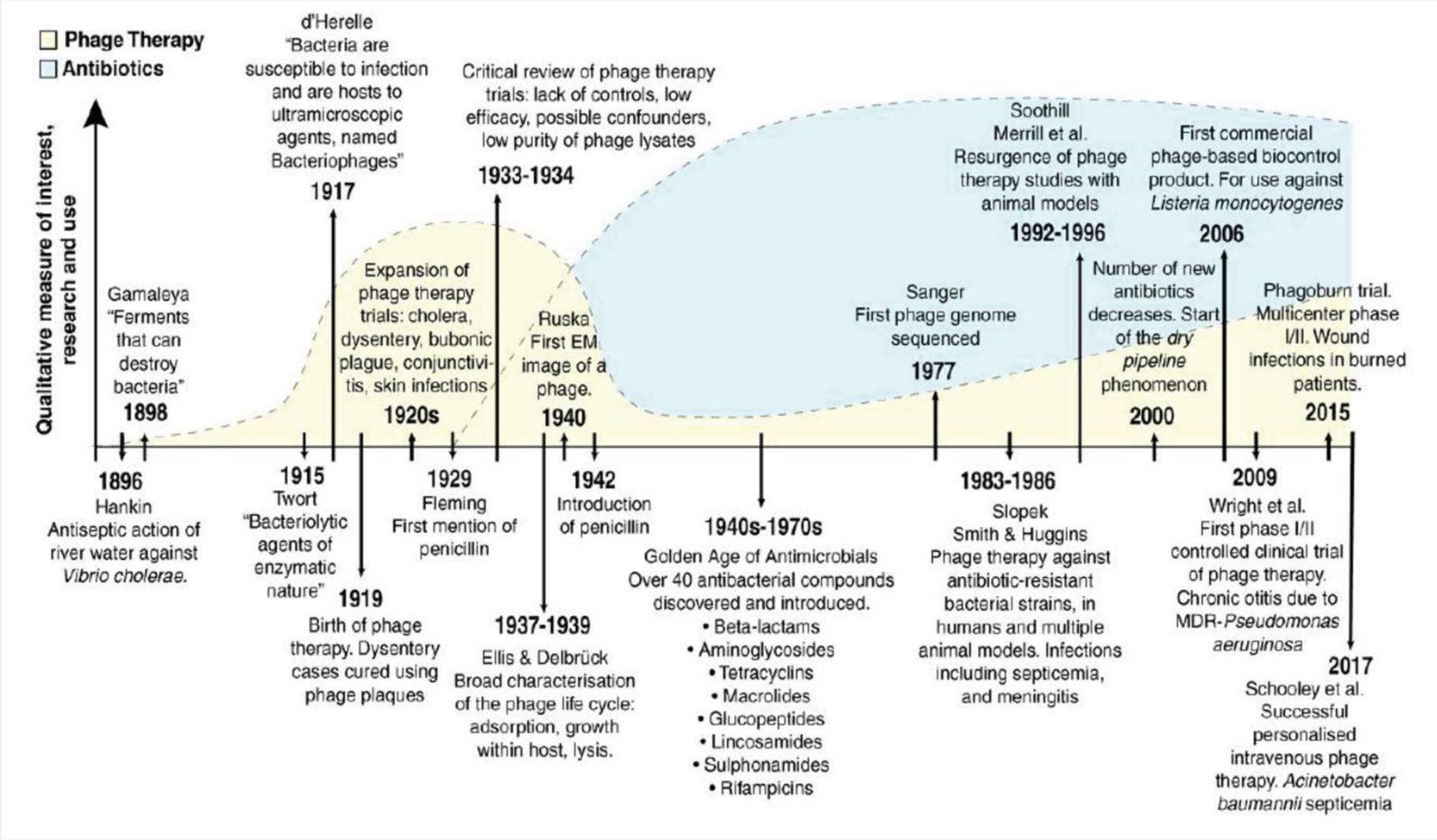
Bacteriófagos

Left panel - Frederick William Twort
Right panel - Félix d'Herelle and George Eliava (Phage therapy)
Term Bacteriophage was coined by Félix d'Hérelle

Em 1915, o bacteriologista britânico Frederick Twort, da Brown Institution de Londres, descobriu um pequeno agente que infectava e matava bactérias.

Independentemente, o microbiologista franco-canadiano Félix d' Hérelle, trabalhando no Instituto Pasteur em Paris, anunciou a 3 de Setembro de 1917, que tinha descoberto "um micróbio invisível e antagónico do bacilo da disenteria“
Foi d'Herelle quem conduziu muitas pesquisas sobre bacteriófagos e introduziu o conceito de terapia fágica

Bacteriófagos

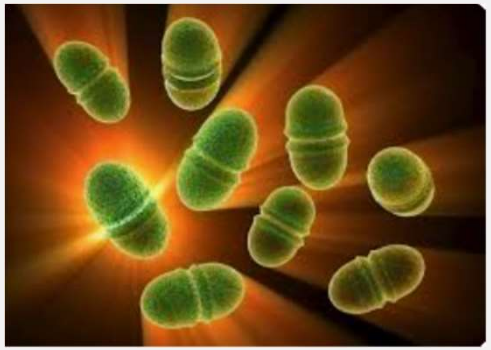


Cronologia dos principais eventos relacionados com a pesquisa de fagos, terapia fágica e antibióticos. A curva com fundo amarelo representa uma medida qualitativa do interesse geral na utilização e estudo da terapia fágica comparativamente com a dos antibióticos (curva azul)

Bacteriophage therapy: an overview and the position of Italian Society of Infectious and Tropical Diseases. September 2020Infezioni in Medicina 28(3):322-331

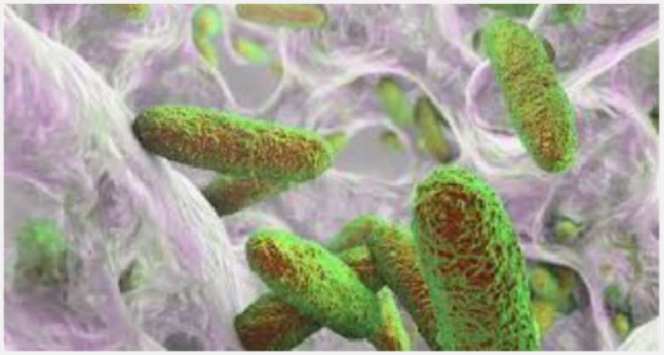
Bacteriófagos

Algumas bactérias resistentes a antibióticos muito preocupantes

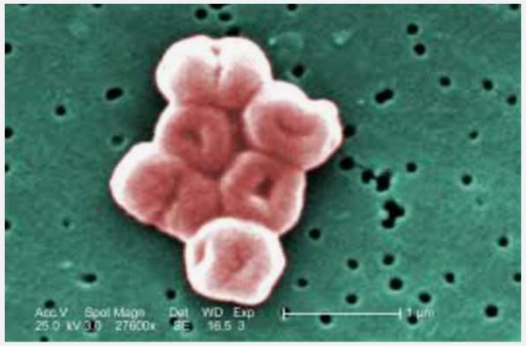


Enterococcus faecium

Staphylococcus aureus

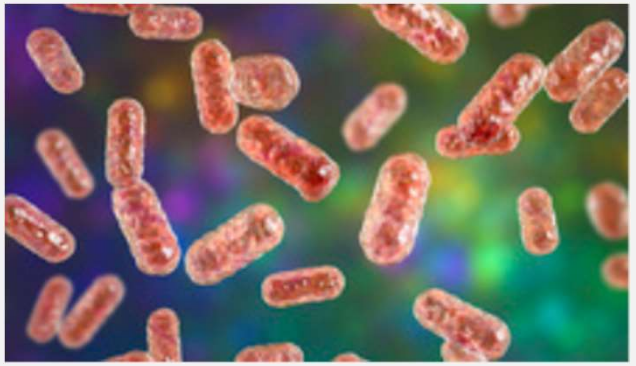


Klebsiella pneumoniae



Acinetobacter baumannii

Pseudomonas aeruginosa

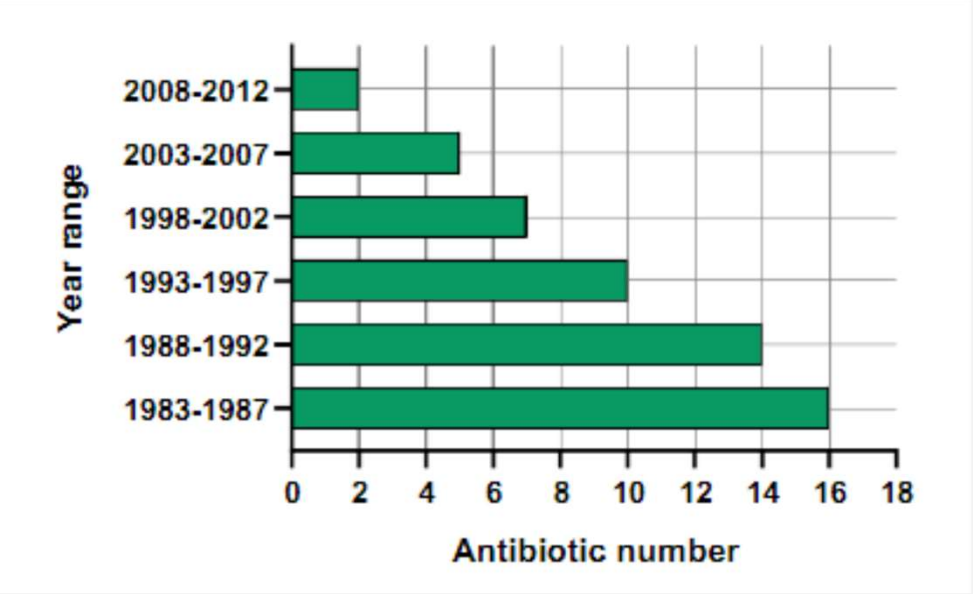


Enterobacter sp

Miller, W.R., Arias, C.A. **ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics.** Nat Rev Microbiol 22, 598–616 (2024). https://doi.org/10.1038/s41579-024-01054-w

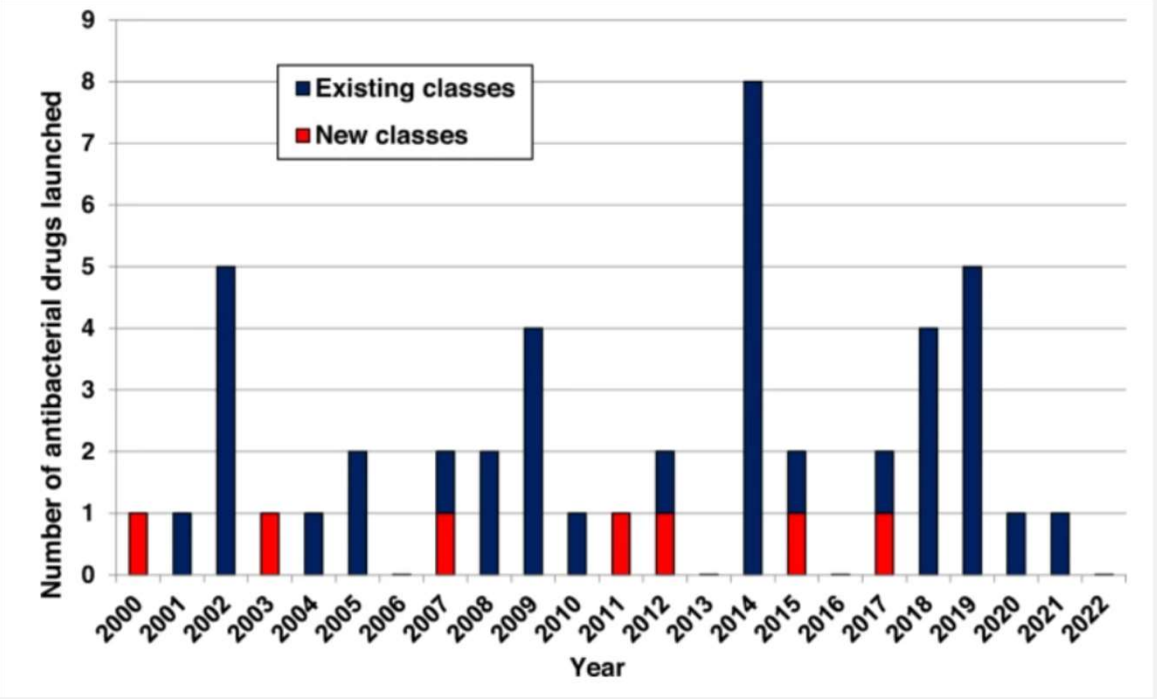
Bacteriófagos

Resistência aos antibióticos muito preocupante



Bacteriophage therapy: an overview and the position of Italian Society of Infectious and Tropical Diseases; September 2020Infezioni in Medicina 28(3):322-331

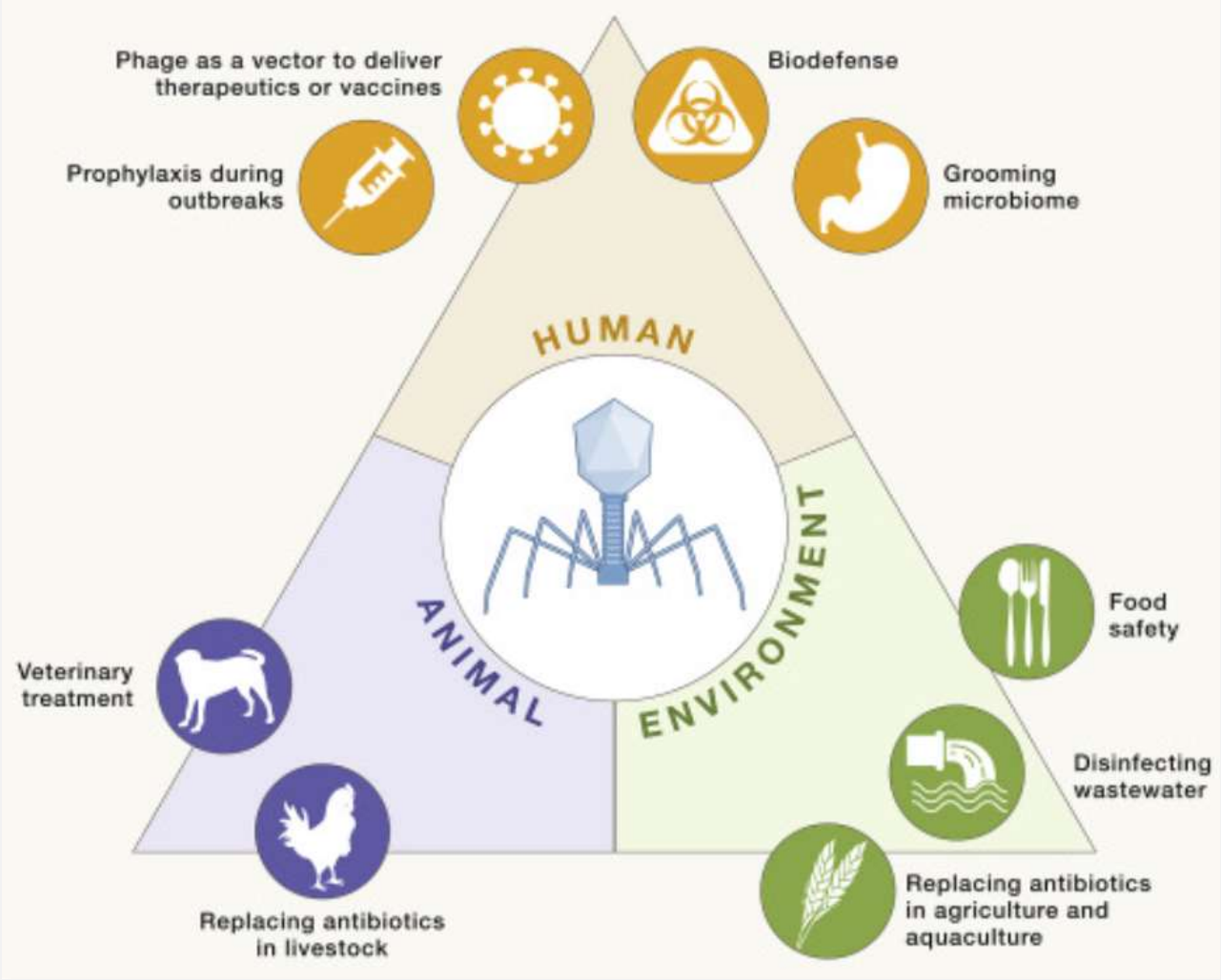
Rápido declínio do número de antibióticos desenvolvidos e aprovados ao longo dos anos. Cada histograma representa o número de novos antibióticos aprovados a cada quatro anos desde 1983 a 2012.



Butler, M.S., Henderson, I.R., Capon, R.J. et al. Antibiotics in the clinical pipeline as of December 2022. J Antibiot 76, 431–473 (2023). https://doi.org/10.1038/s41429-023-00629-8

Novos medicamentos antibacterianos e combinações BL/BLI lançados de janeiro de 2000 a dezembro de 2022 com novas classes em destaque

Bacteriófagos



Potential phage therapy applications from the One Health perspective

Phage therapy: From biological mechanisms to future directions
January 2023Cell 186(1):17-31
DOI:10.1016/j.cell.2022.11.017

Bacteriófagos

Sandbox regulamentar

Artigo 113.º

Ambiente de testagem da regulamentação

1. A Comissão pode criar um ambiente de testagem da regulamentação de acordo com um plano específico, com base numa recomendação da Agência e em conformidade com o procedimento estabelecido nos n.ºs 4 a 7, se estiverem preenchidas todas as condições seguintes:

   a) Não é possível desenvolver o medicamento ou a categoria de medicamentos em conformidade com os requisitos aplicáveis aos medicamentos devido a dificuldades científicas ou regulamentares decorrentes das características ou métodos inerentes ao medicamento;

   b) As características ou os métodos referidos na alínea a) contribuem de forma positiva e distinta para a qualidade, a segurança ou a eficácia do medicamento ou da categoria de medicamentos ou proporcionam uma vantagem significativa para o acesso dos doentes ao tratamento.

(90) Reconhece-se que o desenvolvimento de produtos farmacêuticos é um domínio em que nem a ciência nem a tecnologia estão paradas. Nas últimas décadas, assistiu-se à emergência de novas categorias de medicamentos, desde os medicamentos biológicos aos biossimilares e aos medicamentos de terapia avançada ou, no futuro, à fagoterapia. Estas categorias de produtos podem, em alguns casos, exigir regras adaptadas que tenham plenamente em conta as suas características específicas. Por esse motivo, um quadro jurídico preparado para o futuro deve incluir disposições que permitam esses quadros adaptados, sujeitos a critérios rigorosos e no âmbito de uma habilitação da Comissão orientada pelo contributo científico da Agência Europeia de Medicamentos.

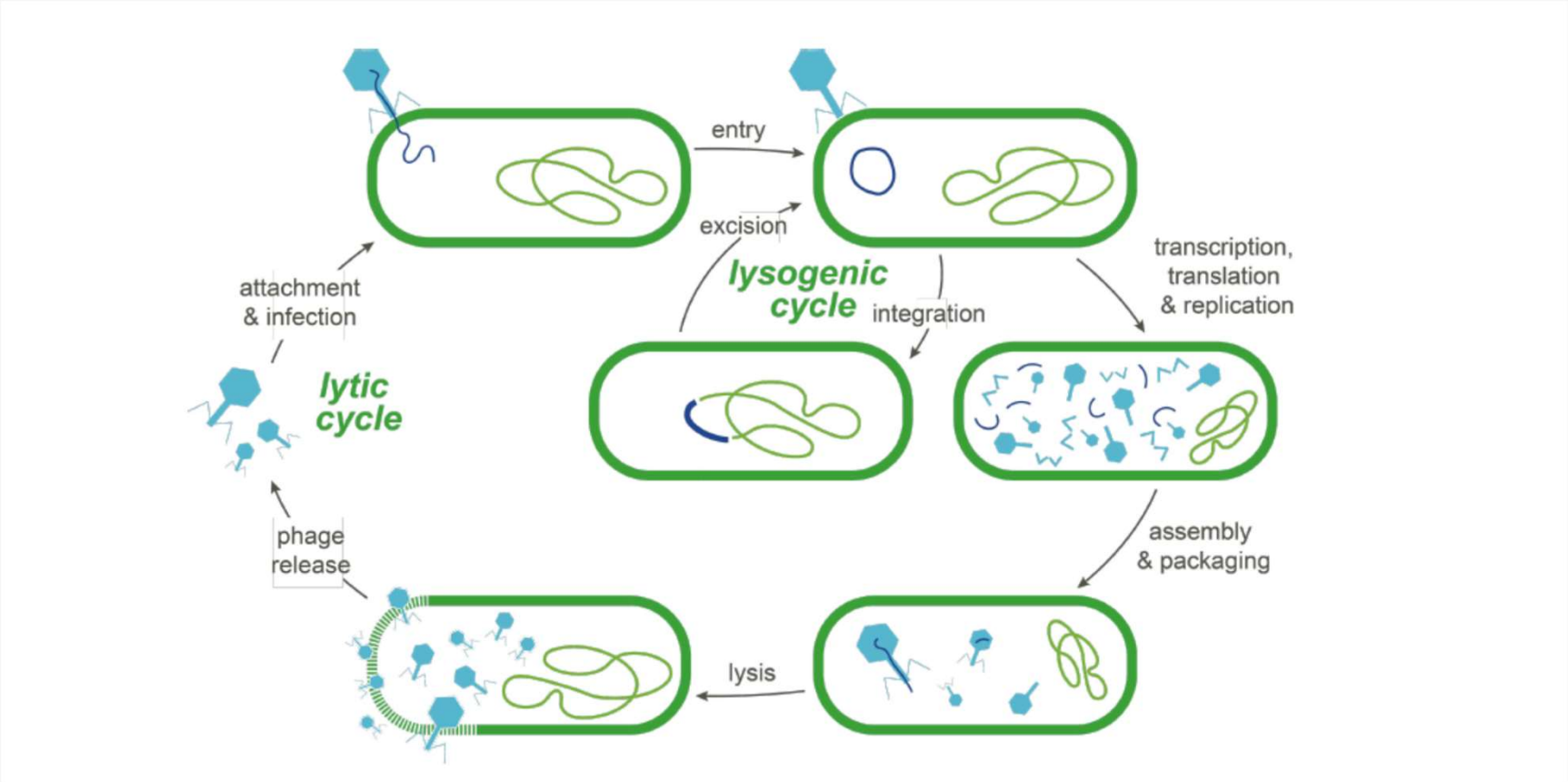
ANEXO VII

DOMÍNIOS PARA OS QUADROS ADAPTADOS REFERIDOS NO ARTIGO 28.º

Medicamentos que contenham fagos, nos casos em que o medicamento tenha uma composição variável em função do contexto clínico específico.

Propostas de revisão da legislação farmacêutica (Regulamento e Diretiva Europeias):
https://health.ec.europa.eu/medicinal-products/pharmaceutical-strategy-europe/reform-eu-pharmaceutical-legislation_en

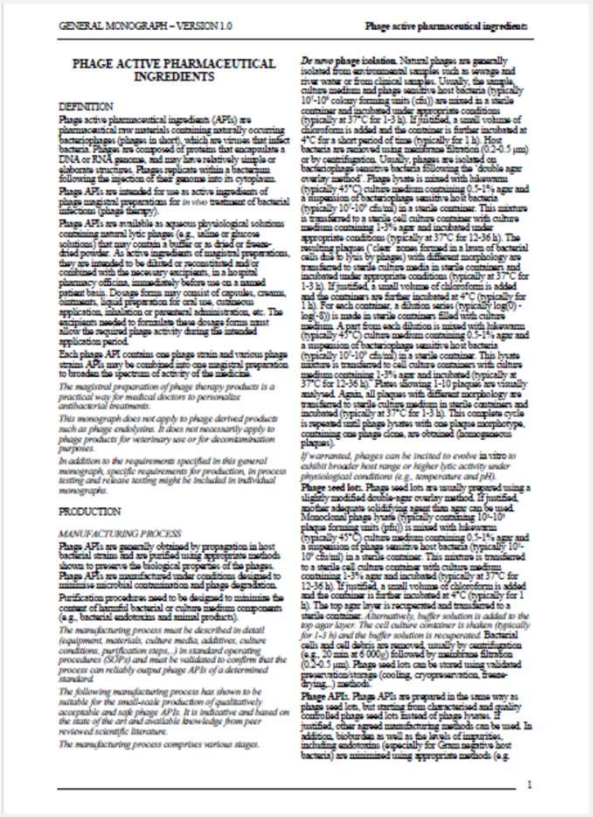
Bacteriófagos



Ciclo biológico dos bacteriófagos. Com base no seu ciclo, os fagos podem ser distinguidos em duas classes: **lisogénicos** (vírus que integram o seu genoma no genoma bacteriano) e **líticos** (vírus que não são capazes de realizar lisogenia). Em ambos os casos, os fagos têm de induzir a lise da célula bacteriana para infetar outro hospedeiro.

Bacteriophage therapy: an overview and the position of Italian Society of Infectious and Tropical Diseases. September 2020Infezioni in Medicina 28(3):322-331

Bacteriófagos
Garantia da qualidade



GENERAL MONOGRAPH – VERSION 1.0 **Phage active pharmaceutical ingredients**

PHAGE ACTIVE PHARMACEUTICAL INGREDIENTS

DEFINITION

Phage active pharmaceutical ingredients (APIs) are pharmaceutical raw materials containing naturally occurring bacteriophages (phages in short), which are viruses that infect bacteria. Phages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Phages replicate within a bacterium following the injection of their genome into its cytoplasm.

Phage APIs are intended for use as active ingredients of phage magistral preparations for *in vivo* treatment of bacterial infections (phage therapy).

Phage APIs are available as aqueous physiological solutions containing natural lytic phages (e.g., saline or glucose solutions) that may contain a buffer or as dried or freeze-dried powder. As active ingredients of magistral preparations, they are intended to be diluted or reconstituted and/or combined with the necessary excipients, in a hospital pharmacy officina, immediately before use on a named patient basis. Dosage forms may consist of capsules, creams, ointments, liquid preparation for oral use, cutaneous application, inhalation or parenteral administration, etc. The excipients needed to formulate these dosage forms must allow the required phage activity during the intended application period.

Each phage API contains one phage strain and various phage strains APIs may be combined into one magistral preparation to broaden the spectrum of activity of the medicine.

The magistral preparation of phage therapy products is a practical way for medical doctors to personalize antibacterial treatments.

This monograph does not apply to phage derived products such as phage endolysins. It does not necessarily apply to phage products for veterinary use or for decontamination purposes.

In addition to the requirements specified in this general monograph, specific requirements for production, in process testing and release testing might be included in individual monographs.

PRODUCTION

MANUFACTURING PROCESS

Phage APIs are generally obtained by propagation in host bacterial strains and are purified using appropriate methods shown to preserve the biological properties of the phages. Phage APIs are manufactured under conditions designed to minimise microbial contamination and phage degradation.

Purification procedures need to be designed to minimise the content of harmful bacterial or culture medium components (e.g., bacterial endotoxins and animal products).

The manufacturing process must be described in detail (equipment, materials, culture media, additives, culture conditions, purification steps...) in standard operating procedures (SOPs) and must be validated to confirm that the process can reliably output phage APIs of a determined standard.

The following manufacturing process has shown to be suitable for the small-scale production of qualitatively acceptable and safe phage APIs. It is indicative and based on the state of the art and available knowledge from peer reviewed scientific literature.

The manufacturing process comprises various stages.

De novo **phage isolation.** Natural phages are generally isolated from environmental samples such as sewage and river water or from clinical samples. Usually, the sample, culture medium and phage sensitive host bacteria (typically 10^7-10^9 colony forming units (cfu)) are mixed in a sterile container and incubated under appropriate conditions (typically at 37°C for 1-3 h). If justified, a small volume of chloroform is added and the container is further incubated at 4°C for a short period of time (typically for 1 h). Host bacteria are removed using membrane filtration (0.2-0.5 µm) or by centrifugation. Usually, phages are isolated on bacteriophage sensitive bacteria following the 'double agar overlay method'. Phage lysate is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of bacteriophage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This mixture is transferred to a sterile cell culture container with culture medium containing 1-3% agar and incubated under appropriate conditions (typically at 37°C for 12-36 h). The resulting plaques ('clear' zones formed in a lawn of bacterial cells due to lysis by phages) with different morphology are transferred to sterile culture media in sterile containers and incubated under appropriate conditions (typically at 37°C for 1-3 h). If justified, a small volume of chloroform is added and the containers are further incubated at 4°C (typically for 1 h). For each container, a dilution series (typically log(0) - log(-8)) is made in sterile containers filled with culture medium. A part from each dilution is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of bacteriophage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This lysate mixture is transferred to cell culture containers with culture medium containing 1-3% agar and incubated (typically at 37°C for 12-36 h). Plates showing 1-10 plaques are visually analysed. Again, all plaques with different morphology are transferred to sterile culture medium in sterile containers and incubated (typically at 37°C for 1-3 h). This complete cycle is repeated until phage lysates with one plaque morphotype, containing one phage clone, are obtained (homogeneous plaques).

If warranted, phages can be incited to evolve in vitro to exhibit broader host range or higher lytic activity under physiological conditions (e.g., temperature and pH).

Phage seed lot. Phage seed lots are usually prepared using a slightly modified double-agar overlay method. If justified, another adequate solidifying agent than agar can be used. Monoclonal phage lysate (typically containing 10^9-10^{10} plaque forming units (pfu)) is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of phage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This mixture is transferred to a sterile cell culture container with culture medium containing 1-3% agar and incubated (typically at 37°C for 12-36 h). If justified, a small volume of chloroform is added and the container is further incubated at 4°C (typically for 1 h). The top agar layer is recuperated and transferred to a sterile container. *Alternatively, buffer solution is added to the top agar layer. The cell culture container is shaken (typically for 1-3 h) and the buffer solution is recuperated.* Bacterial cells and cell debris are removed, usually by centrifugation (e.g., 20 min at 6 000*g*) followed by membrane filtration (0.2-0.5 µm). Phage seed lots can be stored using validated preservation means (cooling, cryopreservation, freeze-drying...) methods.

Phage APIs. Phage APIs are prepared in the same way as phage seed lots, but starting from characterised and quality controlled phage seed lots instead of phage lysates. If justified, other agreed manufacturing methods can be used. In addition, bioburden as well as the levels of impurities, including endotoxins (especially for Gram negative host bacteria) are minimised using appropriate methods (e.g.

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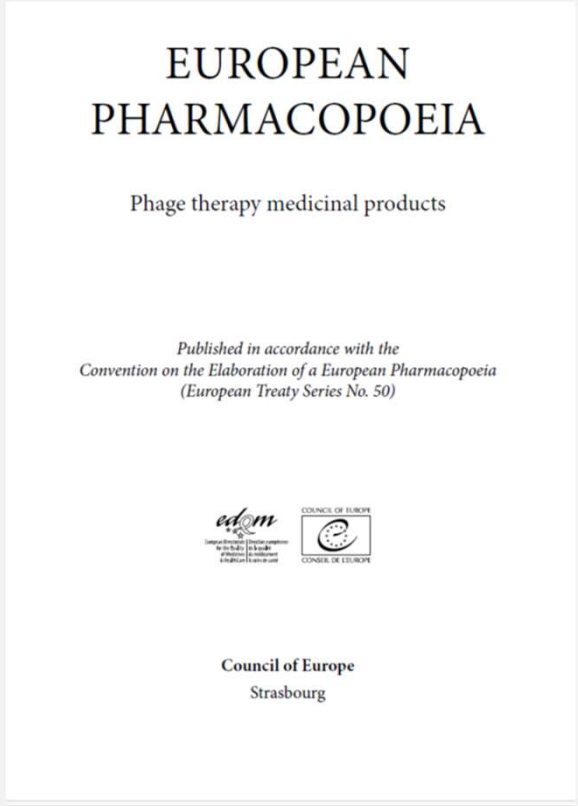


EUROPEAN PHARMACOPOEIA

Phage therapy medicinal products

Published in accordance with the Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No. 50)

Council of Europe
Strasbourg

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 October 2023
EMA/CVMP/NTWP/32862/2022
Committee for Veterinary Medicinal Products (CVMP)

Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy

Draft agreed by Novel Therapies and Technologies Working Party (NTWP)	14 November 2022
Adopted by CVMP for release for consultation	18 January 2023
Start of public consultation	27 January 2023

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

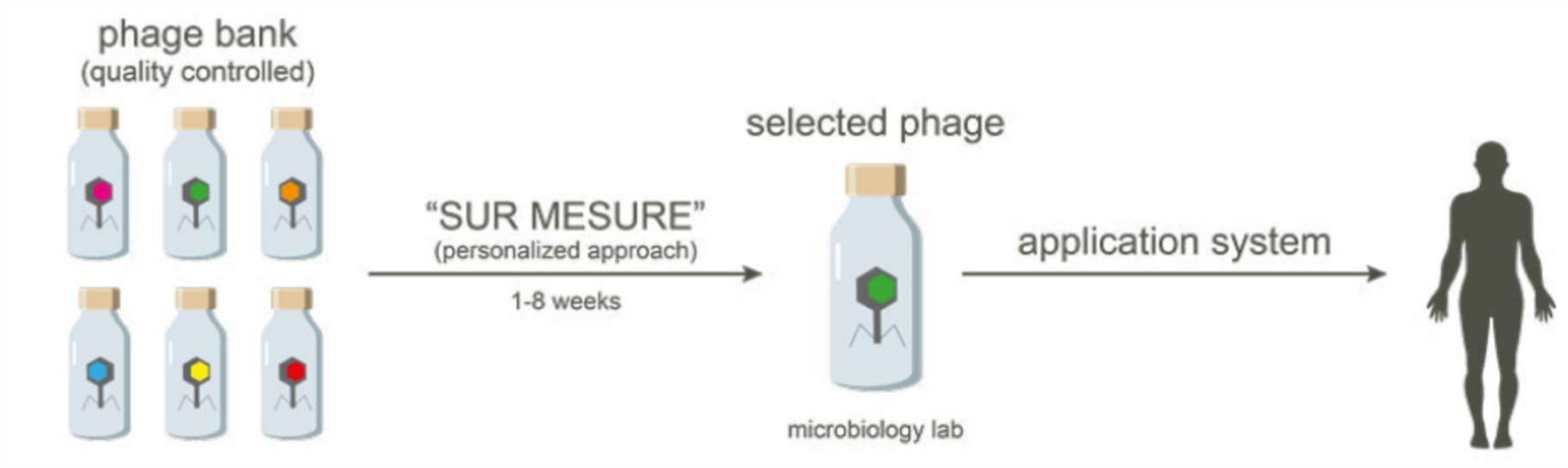
4 December 2023
EMA/CHMP/BWP/486838/2023
Committee for Medicinal Products for Human Use (CHMP)

Concept paper on the establishment of a Guideline on the development and manufacture of human medicinal products specifically designed for phage therapy

Agreed by Biologics Working Party	31 October 2023
Adopted by CHMP for release for consultation	4 December 2023
Start of public consultation	22 December 2023
End of consultation (deadline for comments)	31 March 2024

Bacteriófagos

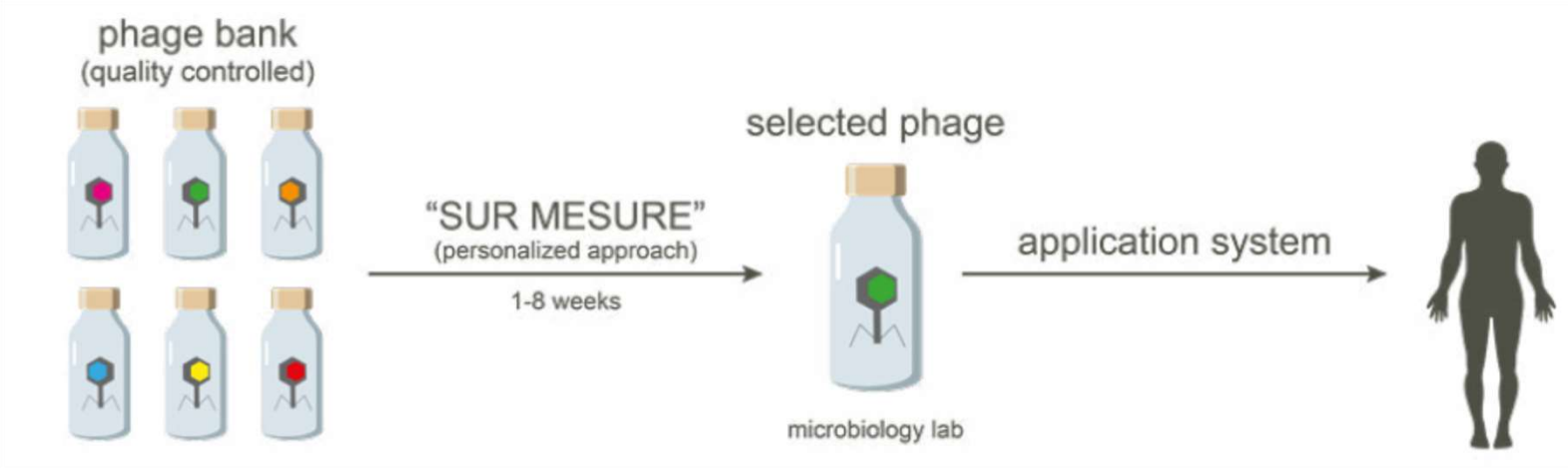
Norma orientadora sobre a utilização de medicamentos manipulados para terapia fágica em contexto hospitalar– preparações magistrais de bacteriófagos



- Restrição a **contexto hospitalar**
- Unicamente aplicável a **bacteriófagos que não tenham sido geneticamente modificados**
- Decisão de iniciar o tratamento com bacteriófagos deve ser tomada por uma equipa hospitalar multidisciplinar validada através de **parecer da Comissão de Farmácia e Terapêutica e Comissão de Ética** respetiva, garantindo integração com o PPCIRA (Programa de Prevenção e Controlo de Infeções e de Resistência aos Antimicrobianos)
- Sendo os bacteriófagos considerados substâncias activas (API'S) não existe obrigatoriedade de emissão de certificados GMP para os produtores, mas têm de disponibilizar um boletim de análise completo alinhado com o **capítulo geral da Farmacopeia Europeia e com a Monografia Belga**

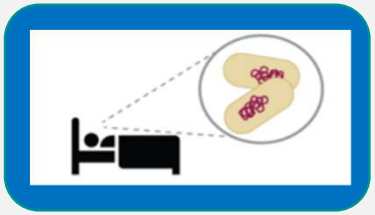
Bacteriófagos

Norma orientadora sobre a utilização de medicamentos manipulados para terapia fágica em contexto hospitalar– preparações magistrais de bacteriófagos

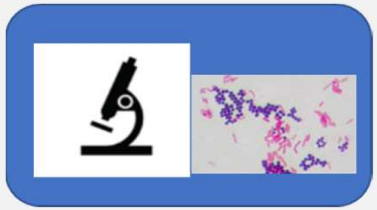


- Preparações magistrais devem ser preparadas, nos serviços farmacêuticos hospitalares, para situações clínicas para um doente individual, respeitando as **Boas Práticas de Preparação de Medicamentos Manipulados** assim como de acordo com o **Guia Orientador PIC/S *Guide to Good Practices for the Preparation of Medicinal Products in Healthcare Establishments***
- Monitorização do uso e dos resultados clínicos mediante questionário online a ser preenchido por Hospital

Norma orientadora sobre a utilização de medicamentos manipulados para terapia fágica



Colheita e isolamento da bactéria patogénica do doente



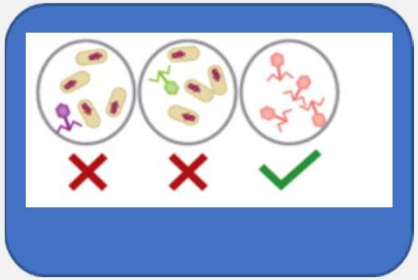
Coloração de Gram e identificação taxonómica e morfológica da bactéria do doente

Antibiograma

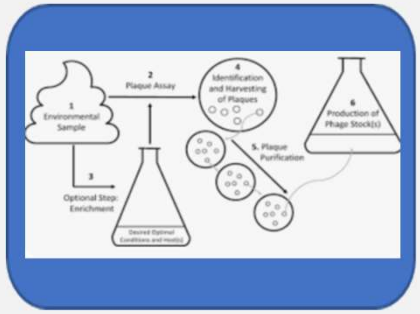
Avaliação clínica

Discussão pela Comissão de Farmácia e Terapêutica e Comissão de Ética

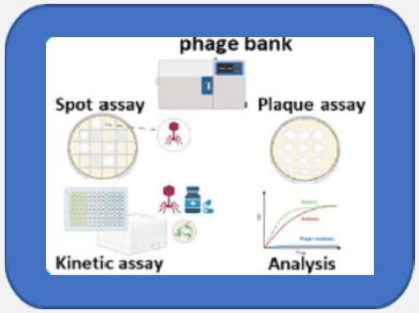
Escolha do fornecedor de fagos e envio da bactéria isolada do doente



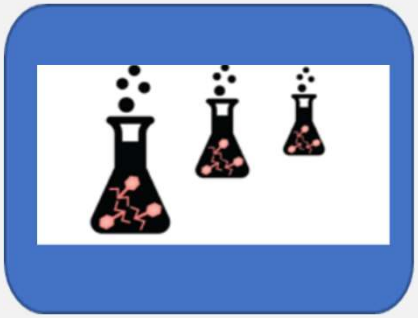
Rastreio da biblioteca de fagos para determinação da suscetibilidade bacteriana individual



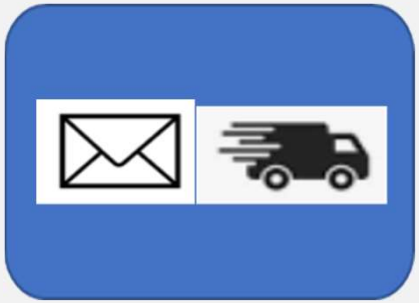
Caso não existam fagos ativos contra será necessário tentar encontrar fagos atvos a partir de amostras ambientais (ou estender a pesquisa a outros fornecedores)



Após a identificação de um (ou mais) fago ativo contra a bactéria em causa é necessário proceder à sua caracterização



Amplificação e purificação dos fagos selecionados



Envio dos fagos para a Farmácia Hospitalar

Preparação da fórmula magistral para administração na Farmácia Hospitalar

Administração ao doente e seguimento do tratamento

OBRIGADA

Infarmed
Autoridade Nacional do Medicamento
e Produtos de Saúde, I.P.