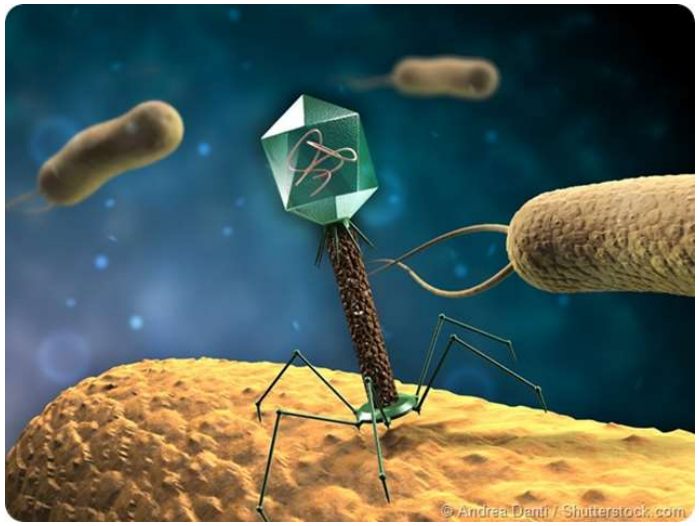


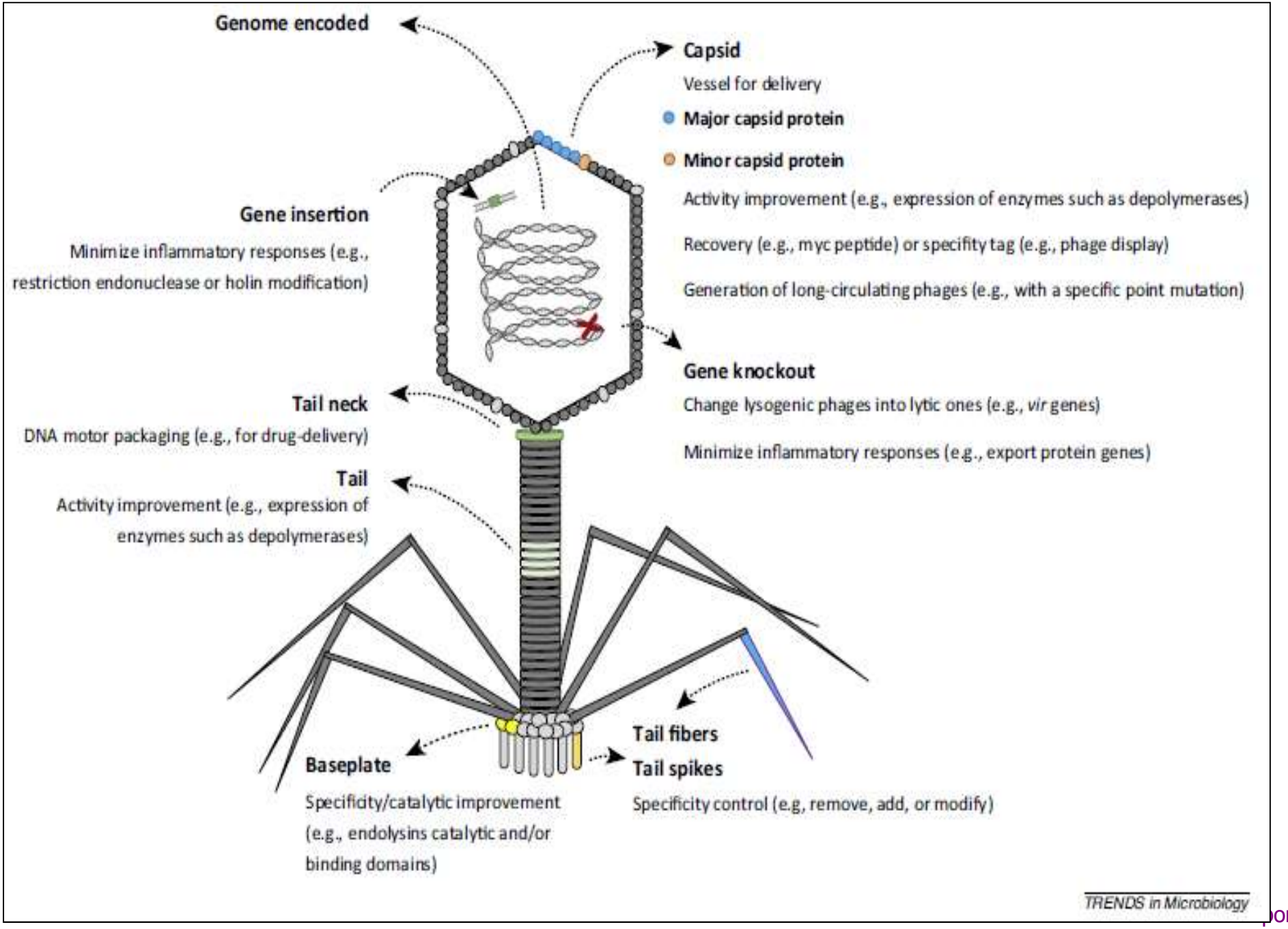
Phagothérapie dans les IOA

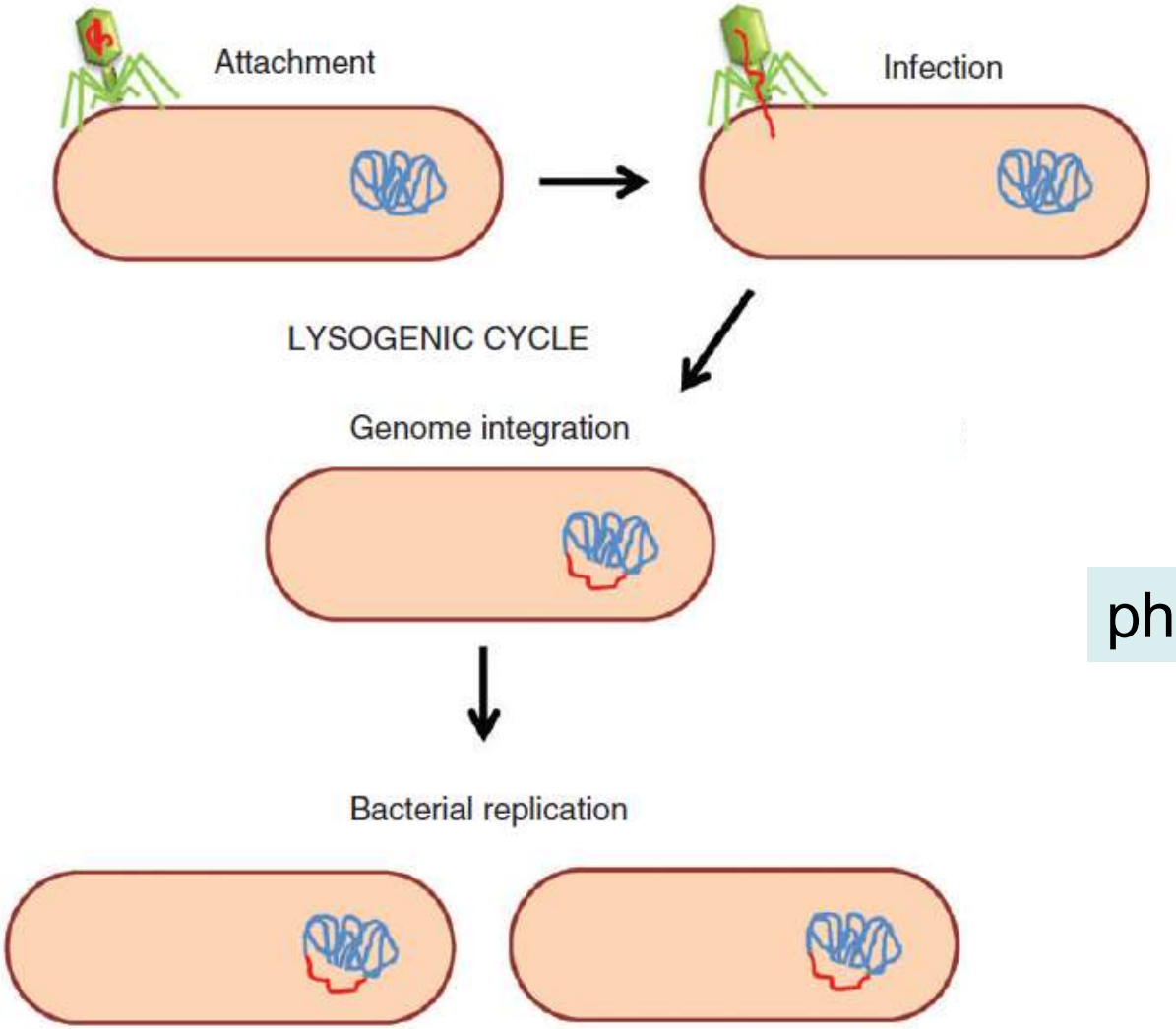
Pr M Dupon

Service de Maladies infectieuses et Tropicales
Centre correspondant de prise en charge
des IOA complexes. CRIOAC-GSO.
SMIT - Hôpital Pellegrin - Bordeaux

DESC 13/10/16

Centre de Recherche Inserm-
Université de Bordeaux U1219
« Bordeaux population health »

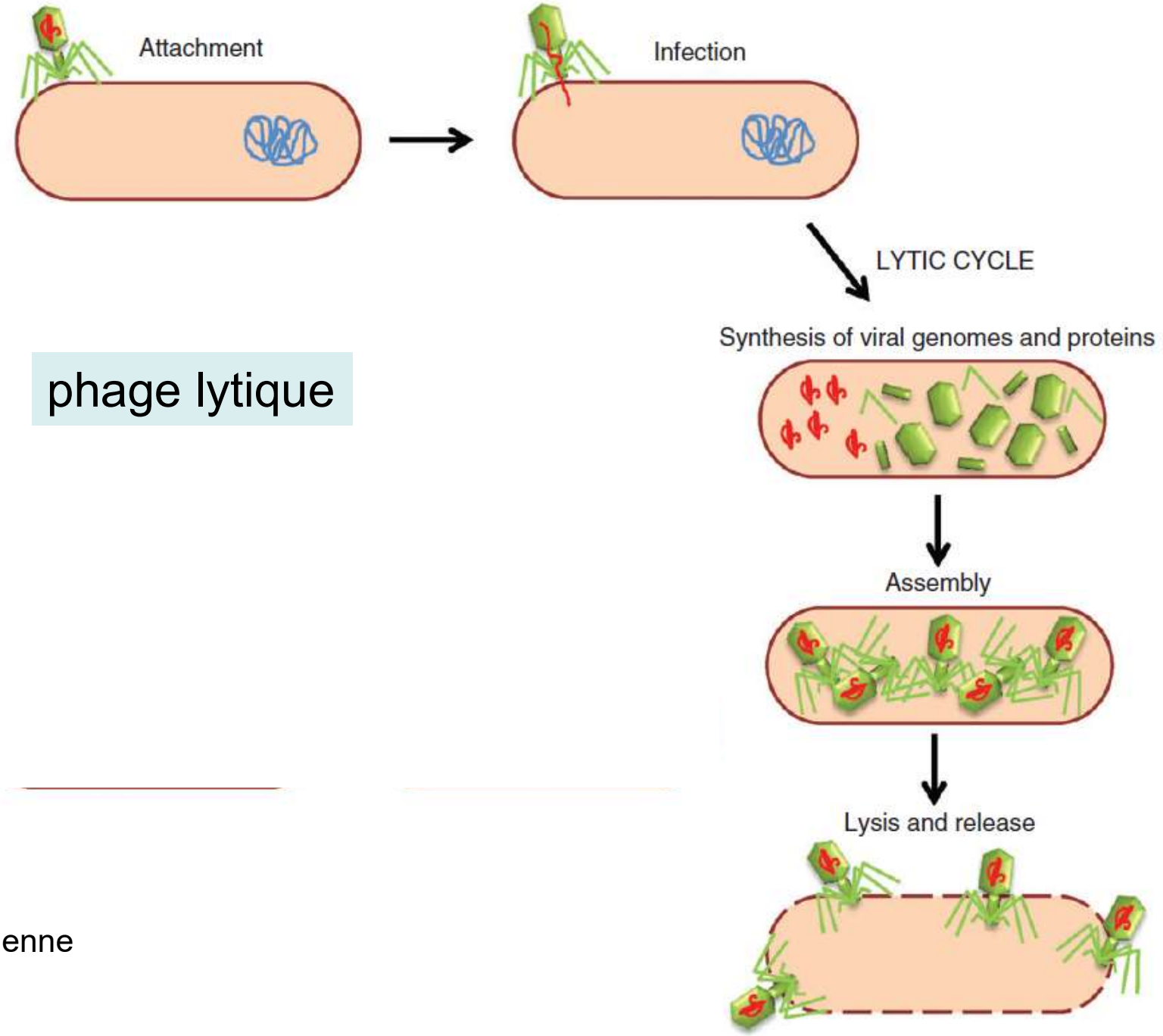

Genome encoded
Capsid
Vessel for delivery
Major capsid protein
Minor capsid protein
Activity improvement (e.g., expression of enzymes such as depolymerases)
Recovery (e.g., myc peptide) or specifity tag (e.g., phage display)
Generation of long-circulating phages (e.g., with a specific point mutation)
Gene insertion
Minimize inflammatory responses (e.g., restriction endonuclease or holin modification)
Gene knockout
Change lysogenic phages into lytic ones (e.g., vir genes)
Minimize inflammatory responses (e.g., export protein genes)
Tail neck
DNA motor packaging (e.g., for drug-delivery)
Tail
Activity improvement (e.g., expression of enzymes such as depolymerases)
Tail fibers
Tail spikes
Specificity control (e.g, remove, add, or modify)
Baseplate
Specificity/catalytic improvement (e.g., endolysins catalytic and/or binding domains)
TRENDS in Microbiology



phage tempéré

- diffusion des facteurs de résistance aux antibiotiques (transduction)
- ajout de nouvelles fonctions au génome de la bactérie, « conversion lysogénique ». Ex : *Vibrio* qui, lysogénisée par un phage, cause le choléra
- Usine à produire des protéines dans E. coli

P. Domingo-Calap et al. HLA, 2016, 87, 133–140



phage lytique

- Lyse bactérienne

P. Domingo-Calap et al. HLA, 2016, 87, 133–140

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GEEPhage
Phagothérapie

Hankin observed *V. cholerae* antibacterial activity in Indian river water (1896)

Bacteriophages characterized and named by d'Hérelle (1917)

Use of phages as molecular biology tools begins (1950s) and continues to present day

Phage genome sequencing begins (1980s) and continues to present day

1890 1900 1920 1940 1960 1980 2000 2010


Ancienues fioles contenant des phages à visée thérapeutique. [8]

Phage institute set up in Tbilisi, Georgia (1923)

Phage lambda (λ) isolated (1951)

Animal studies of Smith and Huggins revitalize phage therapy research in West (1980s)

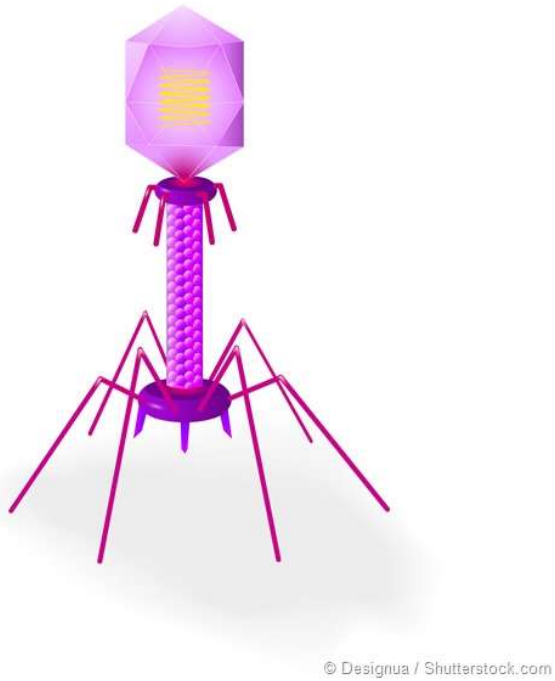
Fischetti's group demonstrate in vivo activity of phage lysins (2001)

Phage cocktail for biocontrol of *Listeria* in ready-to-eat meats approved by FDA (2006)

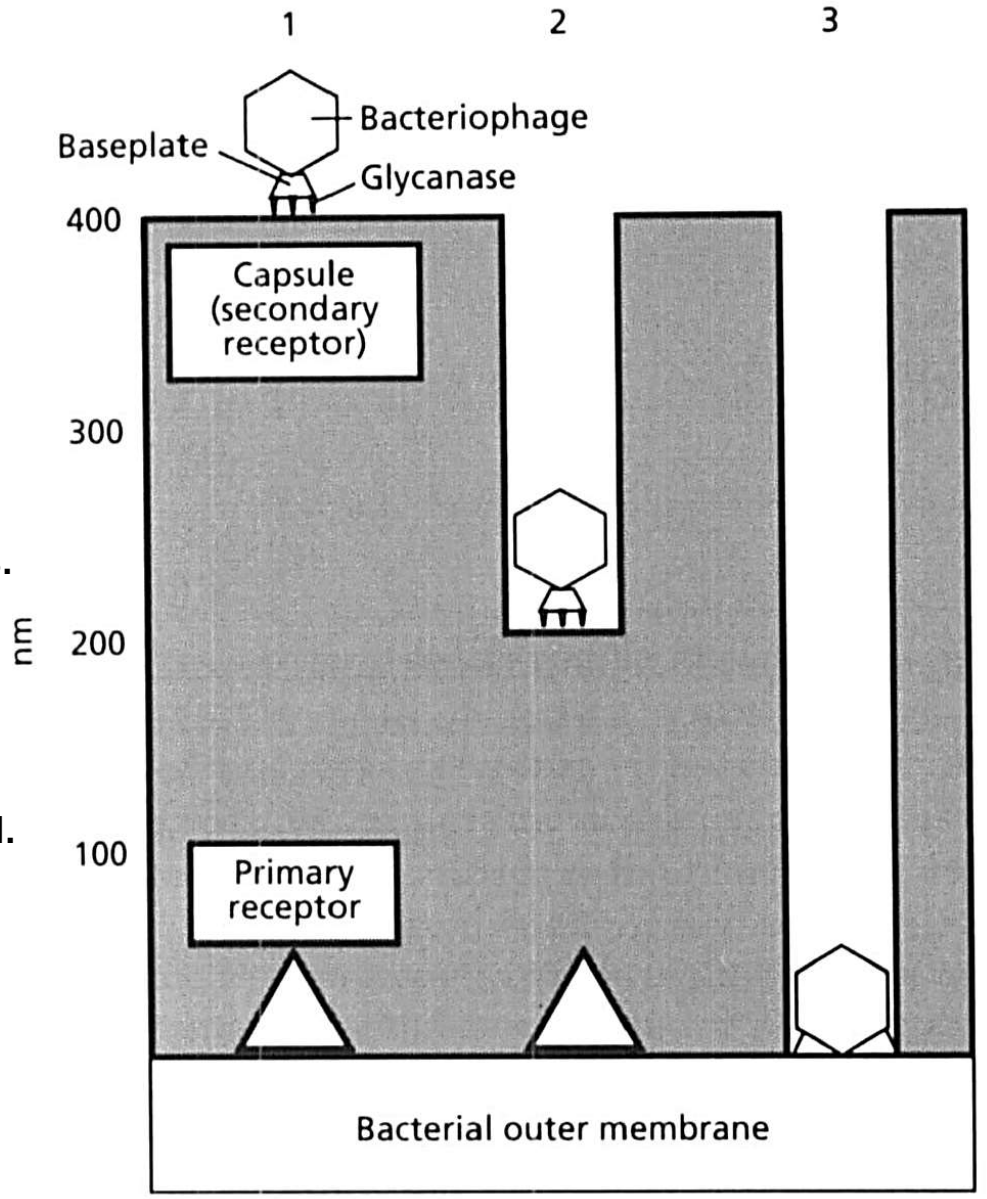
Antibacterial activity against *S. aureus* published by Twort (1915)

Major timelines in th py.

In vitro



Biofilm susceptibility to bacteriophage attack : the role of phage-borne polysaccharide depolymerase.



Degradation of bacterial capsular polysaccharide by a phage-borne glycanase occurs in three stages.
(1) Phage binds to capsular polysaccharide (secondary receptor).
(2) Phageborne glycanase degrades polymer until phage reaches cell surface.
(3) Phage binds to primary receptor and infects cell.
Adapted from Lindberg (1977).

Hughes K..Microbiology (1 998), 144,3039-3047

Synergie ATB-linezolide in vitro
broches métalliques (Kirschner, acier, diamètre 1.5 mm)

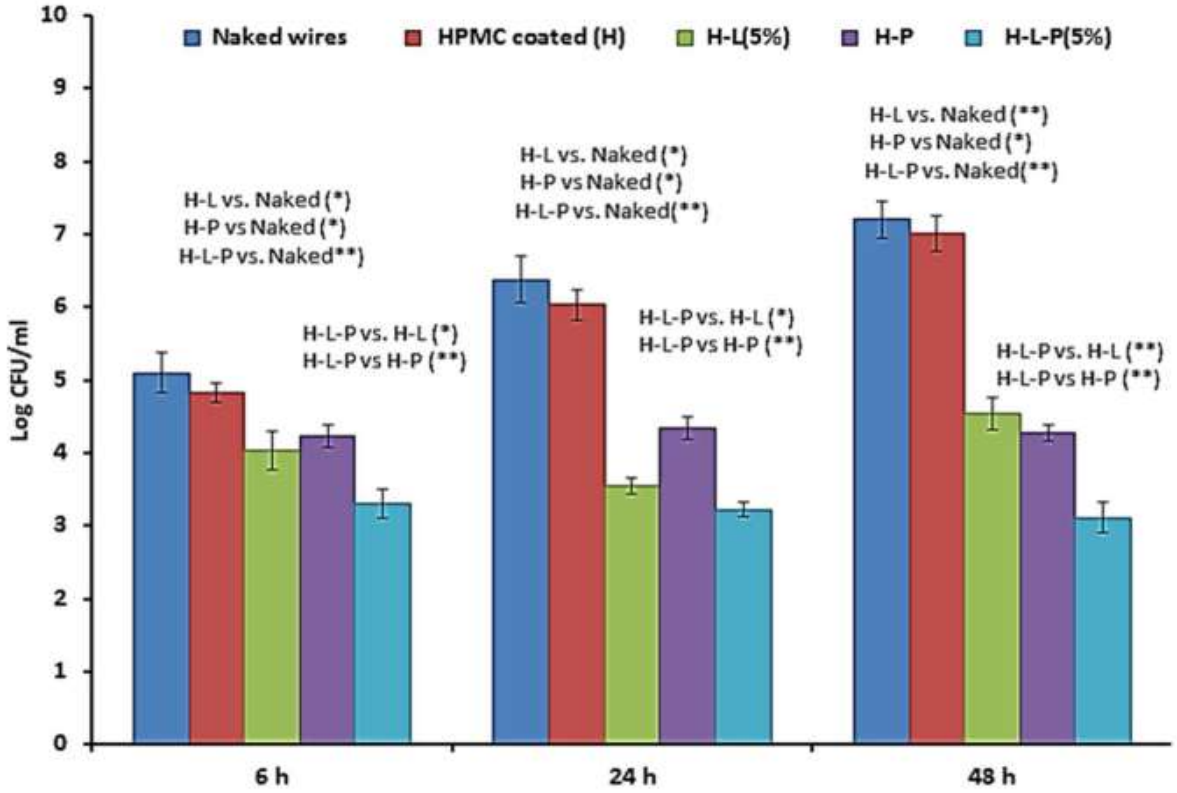


Figure 1. Total biomass of *S.aureus* ATCC 43300 (in terms of Log CFU/ml) adhering to either naked as well as HPMC coated K-wires (n = 4 per group per time point). Error bars represent S.D. p values among groups have been determined where (*) represent $p<0.05$ and (**) represent $p<0.01$.

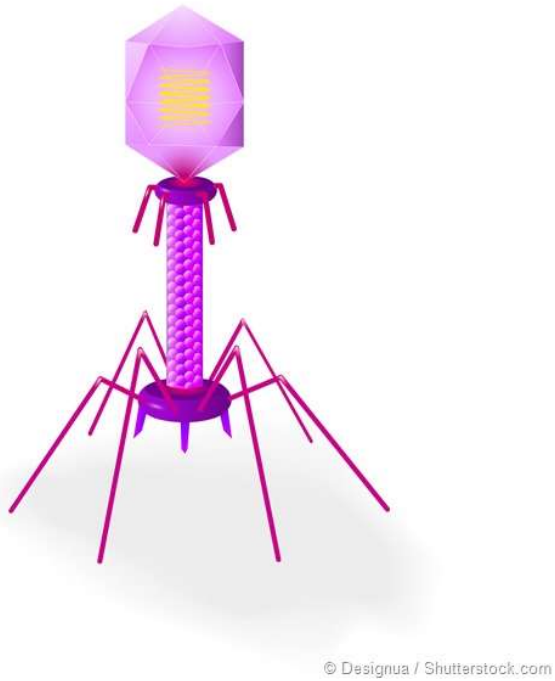
Table 4. Mutation frequencies to resistance for linezolid and phage in *S. aureus* 43300 (MRSA).

Phage (MR-5) MOI-1*	Phage (MR-5) MOI-10**	Linezolid at (8 μg/ml)	Phage + linezolid (MOI-1+8 μg/ml)	Phage + linezolid (MOI-10+8 μg/ml)
$(7.5\pm1.1)\times10^{-6}$	$(1\pm0.31)\times10^{-7}$	$(5\pm1.2)\times10^{-9}$	$<10^{-9}$	$<10^{-9}$

MOI-1*: phage added at a multiplicity of 1 i.e 10^9 PFU of phage added.
MOI-10**: phage added at a multiplicity of 10 i.e 10^{10} PFU of phage added.

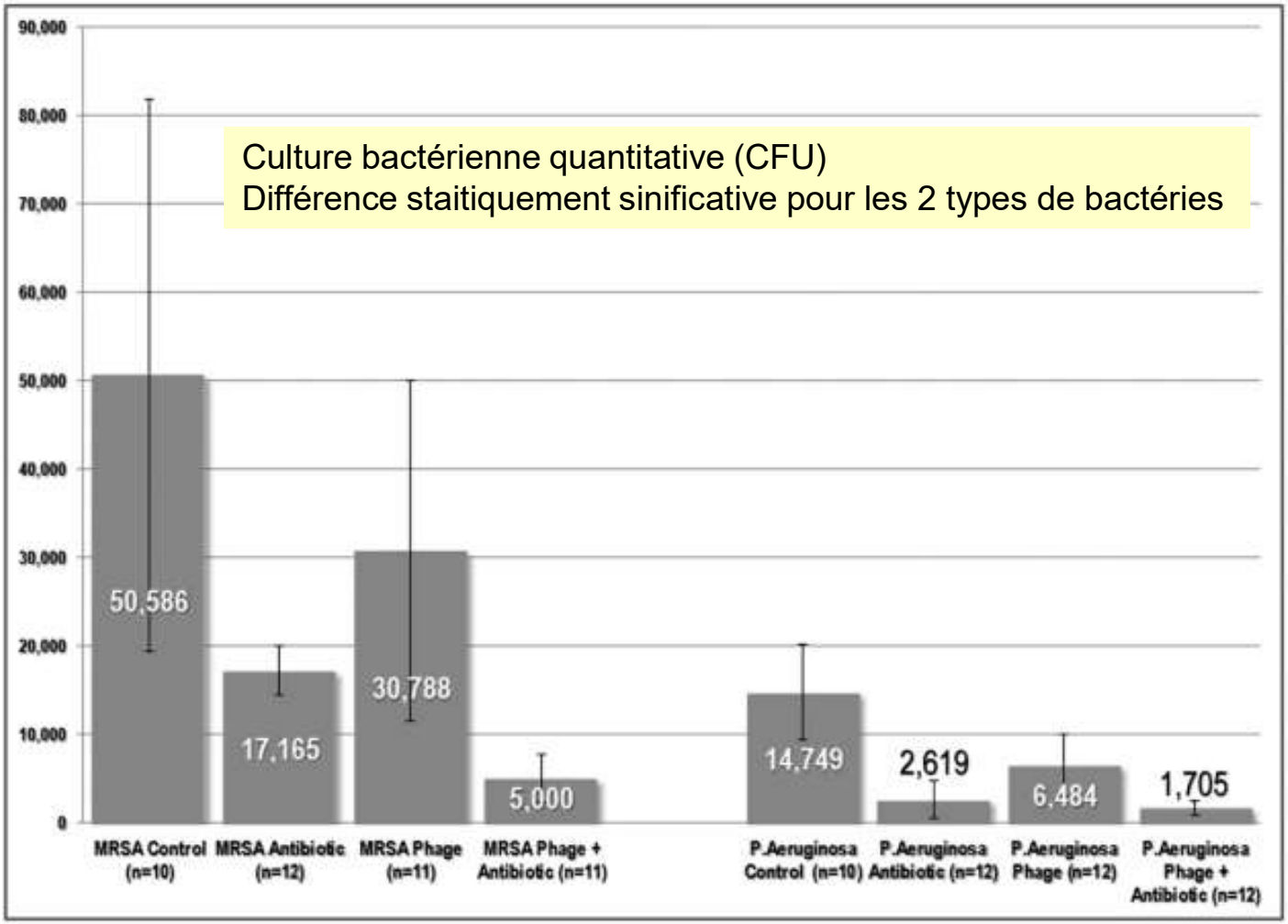
Kaur S, Harjai K, Chhibber S (2014) Bacteriophage Mediated Killing of Staphylococcus aureus In Vitro on Orthopaedic K Wires in Presence of Linezolid Prevents Implant Colonization. PLoS ONE 9(3): e90411. doi:10.1371/journal.pone.0090411

Animal



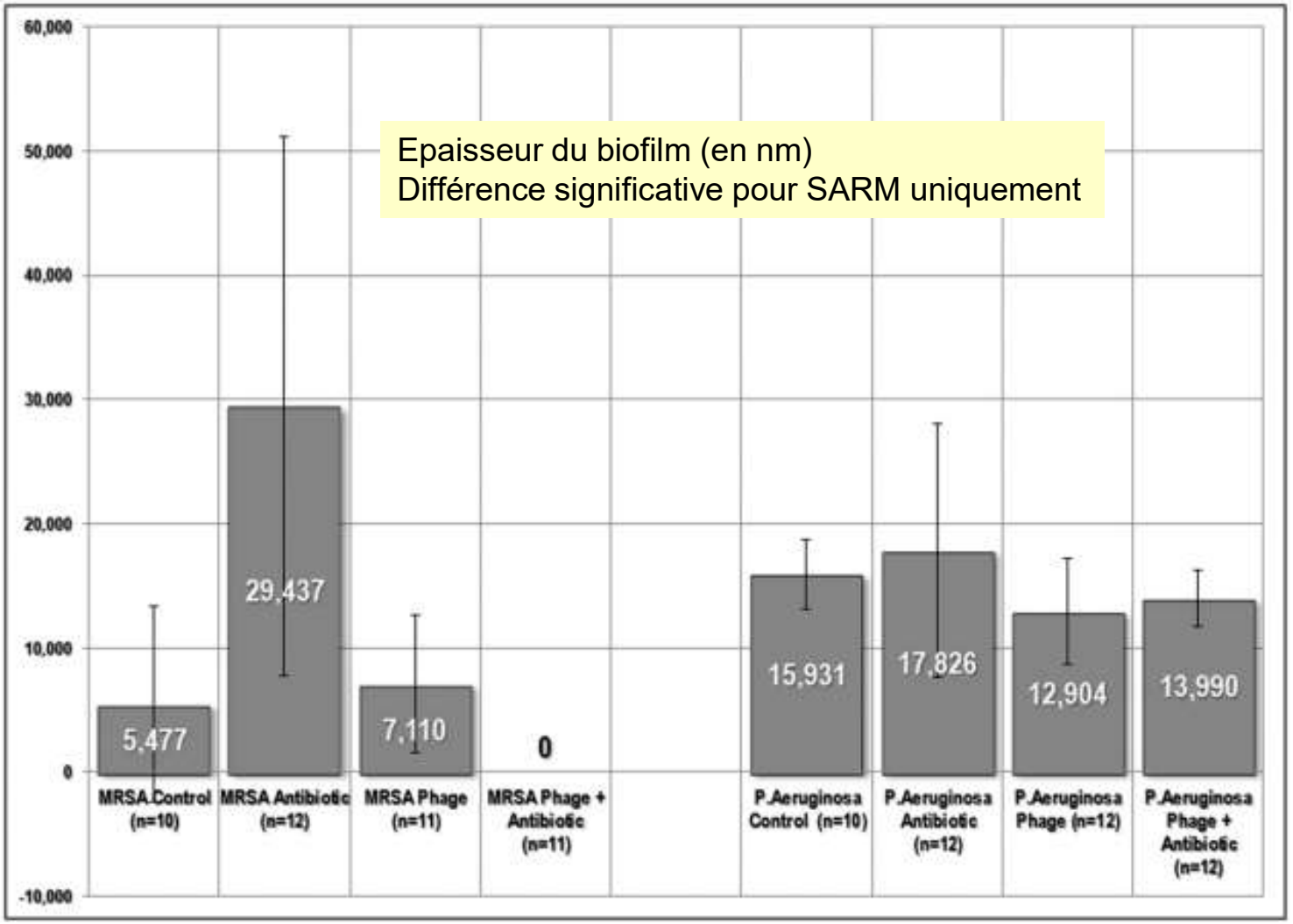
M Dupon

Modèle animal : ostéite tibiale sur matériel chez le rat



Yilmaz J. Bone Joint Surg Am. 2013;95:117-25

Modèle animal : ostéite tibiale sur matériel chez le rat



Yilmaz J. Bone Joint Surg Am. 2013;95:117-25

Homme

Bactériophages et chirurgie orthopédique

A propos de sept cas.

G. Lang, P. Kehr, H. Mathevon, J. M. Clavert, P. Séjourne et J. Pointu *

(*Strasbourg*)

RÉSUMÉ

Les auteurs rapportent sept observations où furent utilisés les bactériophages en chirurgie orthopédique. Ils soulignent l'intérêt de cette thérapeutique dans les cas d'infections chroniques à germes polyrésistants. Ce sont toujours des phages adaptés qui ont été utilisés. Le protocole d'utilisation est précisé. Il doit être rigoureux tant dans la chronologie que dans l'exécution des différents gestes. Les résultats obtenus sont très encourageants. Évidemment le bactériophage reste une thérapeutique d'exception mais il peut rendre de très grands services surtout en chirurgie orthopédique quand on connaît la chronicité désespérante des ostéites.

CONCLUSION

L'utilisation de bactériophages adaptés dans le traitement des infections osseuses chroniques polyrésistantes aux antibiotiques nous paraît être une solution thérapeutique de secours intéressante. Nos résultats nous encouragent pleinement à poursuivre dans ce sens.

Revue de Chirurgie Orthopédique, 1979, *65*, 33-37.

G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

Table 1. Effectiveness of staphylococcal phage preparation against staphylococcal sepsis, septic infection of the lungs and osteomyelitis[a]

Diagnosis	Phage therapy only				Phage with antibiotics				Antibiotics only			
	Total number (*N*)	Complete recovery	Improvement	No effect	Total number (*N*)	Complete recovery	Improvement	No effect	Total number (*N*)	Complete recovery	Improvement	No effect
Sepsis	46	19	6	21	40	31	4	5	96	22	22	52
Lung infection	60	21	25	14	61	31	24	6	55	10	21	24
Osteomyelitis and arthritis	9	9	–	–	51	51	–	–	60	60	–	–

[a]Soviet data on phage therapy trials from the Eliava Institute [22]. In a series of clinical trials in the 1970s, the therapeutic effectiveness of the staphylococcal phage preparation against different infectious diseases was evaluated, from which some results are listed here.

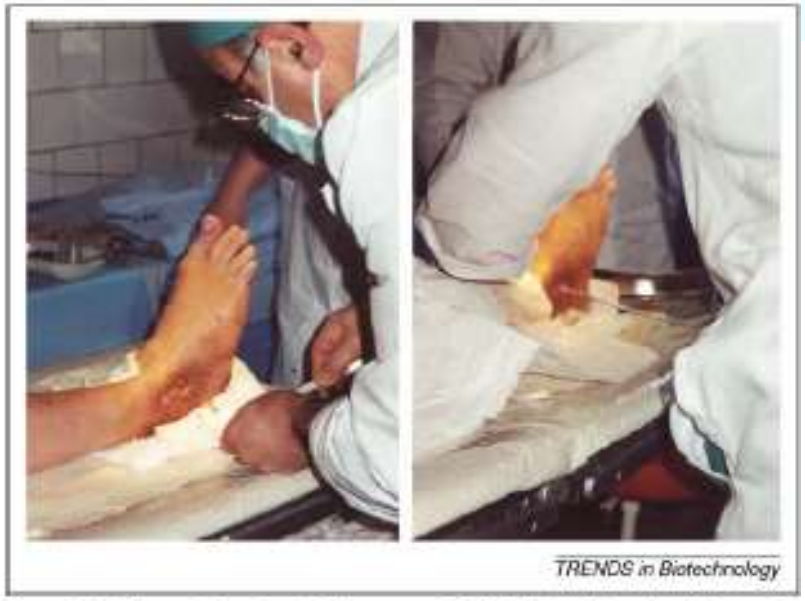


Figure 1. Dr Guram Gvasalia, chief surgeon of Tbilisi Republican Hospital, applies bacteriophage therapy for joint inflammation caused by *S. aureus*.

Kutateladze M. Trends in Biotechnology 2010; 28 ;12:592-5

Bacteriophage Laboratory, Ludwik Hirszfeld Institute of Immunology Experimental Therapy, Wrocław, Poland

TABLE VIII Detailed evaluation of results of phage therapy in patients with different disorders (does not include one patient with recurrent bacteremia included in general analysis)

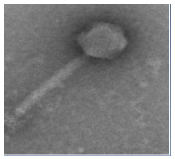
	Genital and urinary tract infections in men[a] (*n* = 29)		Genital and urinary tract infections in women[b] (*n* = 22)		Soft tissue infections[c] (*n* = 30)		Skin infections[d] (*n* = 10)		Orthopedic infections[e] (*n* = 37)		Respiratory tract infections[f] (*n* = 24)	
Category of response to treatment	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
A - pathogen eradication and/or recovery	11	37.9	3	13.6	5	16.7	0	0.0	7	18.9	2	8.3
B - good clinical result	2	6.9	0	0.0	2	6.7	2	20.0	3	8.1	3	12.5
C - clinical improvement	1	3.4	5	22.7	4	13.3	1	10.0	7	18.9	2	8.3
D - questionable clinical improvement	2	6.9	0	0.0	2	6.7	0	0.0	3	8.1	3	12.5
E - transient clinical improvement	5	17.2	4	18.2	8	26.7	5	50.0	8	21.6	3	12.5
F - no response to treatment	8	27.6	10	45.5	6	20.0	1	10.0	7	18.9	7	29.2
G - clinical deterioration	0	0.0	0	0.0	3	10.0	1	10.0	2	5.4	4	16.7
Good response (total A–C):	**14**	**48.3**	**8**	**36.4**	**11**	**36.7**	**3**	**30.0**	**17**	**45.9**	**7**	**29.2**
Inadequate response (total D–G):	**15**	**51.7**	**14**	**63.6**	**19**	**63.3**	**7**	**70.0**	**20**	**54.1**	**17**	**70.8**

[e] Including prosthetic joint infection (n=8), osteomyelitis (n=21), joint infection (n= 5), osteomyelitis/joint infection (n=2), and discitis (n=1).

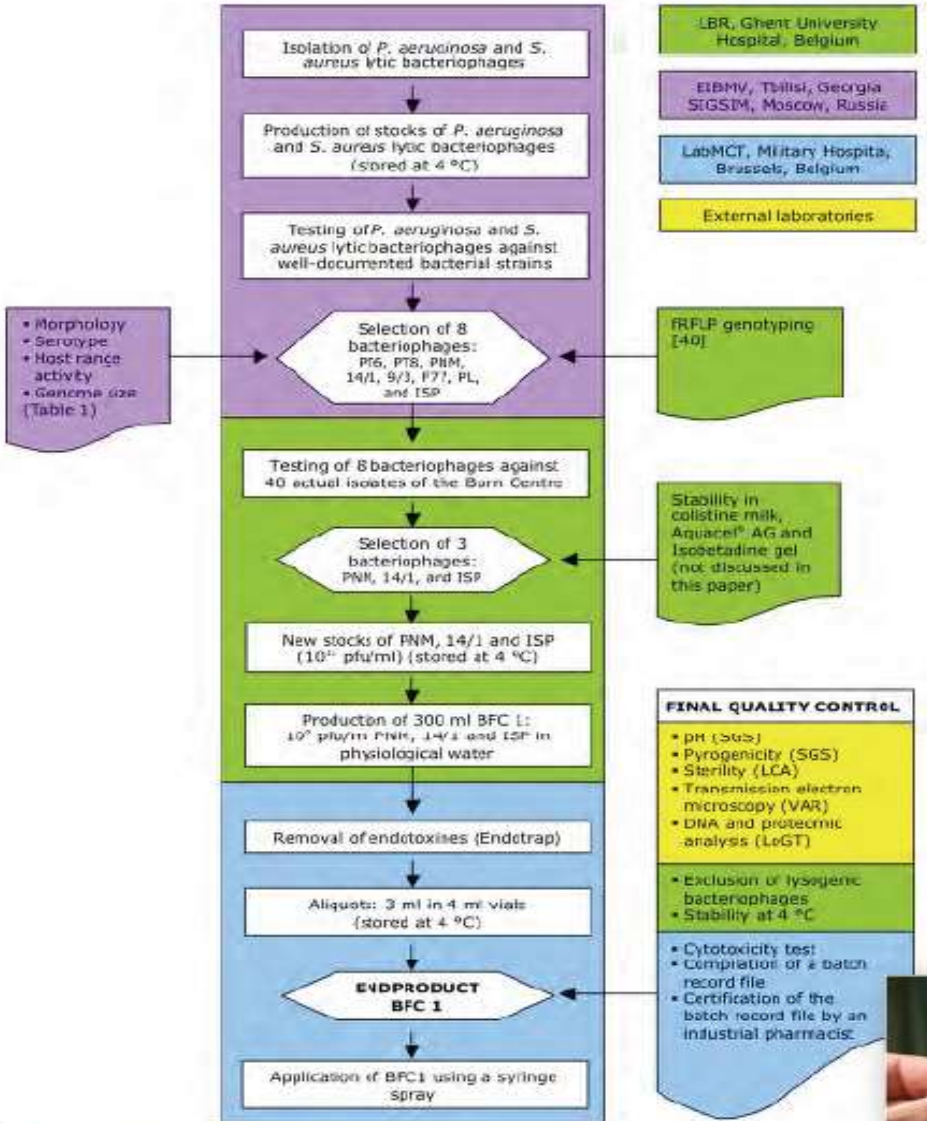
Miedzybrodzki R. et al. Clinical Aspects of PhageTherapy. Advances in Virus Research, Volume 83,73-121

GEEPhage
Phagothérapie

Quality-Controlled Small-Scale Production of a Well-Defined Bacteriophage Cocktail for Use in Human Clinical Trials



Maya Merabishvili[1,2,8], Jean-Paul Pirnay[2]*, Gilbert Verbeken[2], Nina Chanishvili[1], Marina Tediashvili[1], Nino Lashkhi[1], Thea Glonti[1], Victor Krylov[3], Jan Mast[4], Luc Van Parys[5], Rob Lavigne[6], Guido Volckaert[6], Wesley Mattheus[6], Gunther Verween[2], Peter De Corte[2], Thomas Rose[2], Serge Jennes[2], Martin Zizi[5,7], Daniel De Vos[2], Mario Vaneechoutte[8]



Un cocktail de phages (BFC-1) avec contrôle de qualité

- Un manque de produits bien définis et caractérisés
- Souvent l'interaction récepteur-phage entre la bactérie et les phages n'était pas optimale (empirisme)

  Présence d'endotoxines.
- Une production "GMP-like"

Sécurité PT chez l'homme

Virology 443 (2013) 187–196

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Safety analysis of a Russian phage cocktail: From MetaGenomic analysis to oral application in healthy human subjects

Shawna McCallin [a], Shafiqul Alam Sarker [b], Caroline Barretto [a], Shamima Sultana [b], Bernard Berger [a], Sayeda Huq [b], Lutz Krause [a,1], Rodrigo Bibiloni [a,2], Bertrand Schmitt [a], Gloria Reuteler [a], Harald Brüssow [a,*]

Bioinformatic analysis did not reveal undesired genes and a small human volunteer trial did not associate adverse effects with oral phage exposure.

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Sécurité et tolérance de la PT chez l'homme

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2005, p. 2874–2878 Vol. 49, No. 7
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.7.2874–2878.2005

Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy

Anne Bruttin and Harald Brüssow*

Nestlé Research Center, Nestec Ltd., Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland

Received 25 November 2004/Returned for modification 24 January 2005/Accepted 3 April 2005

Fifteen healthy adult volunteers received in their drinking water a lower *Escherichia coli* phage T4 dose (10^3 PFU/ml), a higher phage dose (10^5 PFU/ml), and placebo. Fecal coliphage was detected in a dose-dependent way in volunteers orally exposed to phage. All volunteers receiving the higher phage dose showed fecal phage 1 day after exposure; this prevalence was only 50% in subjects receiving the lower phage dose. No fecal phage was detectable a week after a 2-day course of oral phage application. Oral phage application did not cause a decrease in total fecal *E. coli* counts. In addition, no substantial phage T4 replication on the commensal *E. coli* population was observed. No adverse events related to phage application were reported. Serum transaminase levels remained in the normal range, and neither T4 phage nor T4-specific antibodies were observed in the serum of the subjects at the end of the study. This is, to our knowledge, the first safety test in the recent English literature which has measured the bioavailability of oral phage in humans and is thus a first step to the rational evaluation of phage therapy for diarrheal diseases.

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Lucie & Raymond AUBRAC

PHAGE THERAPY CENTER

GEEPhage
Phagothérapie

ELIAVA INSTITUTE
TBILISSI
GEORGIA

DSMZ

HIRZFELD INSTITUTE
WROCLAW
POLAND

EUROPEAN PHAGES BANK

DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen)
GERMANY

CHI VILLENEUVE
SAINT GEORGES
FRANCE

USA
Eliava Phages NY

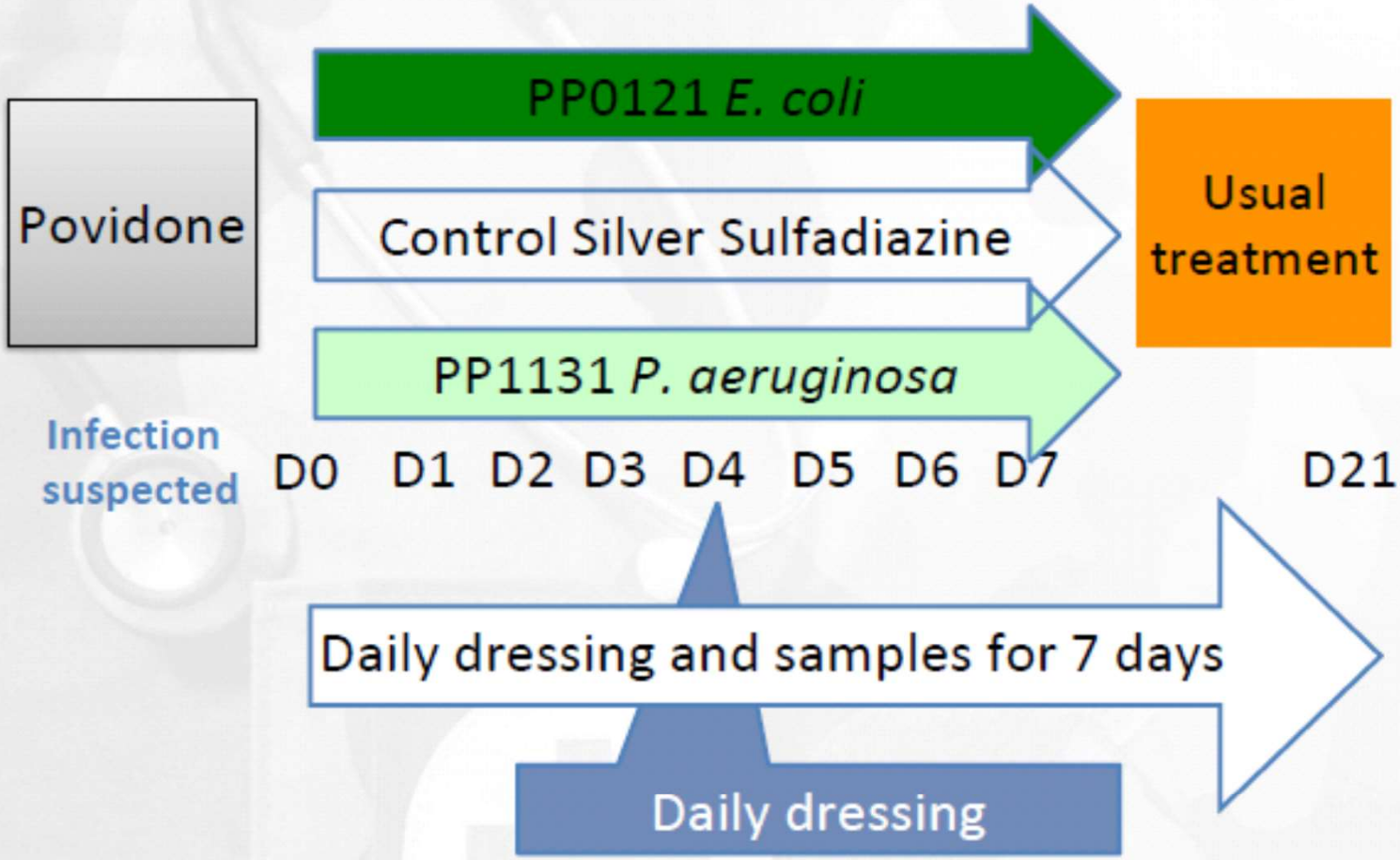
M BALS INSTITUTE
BUCAREST
ROMANIA ?

QUEEN ASTRID
MILITARY HOSPITAL
BELGIAN

PhagoBurn

- **Phagoburn** is a European Research & Development (R&D) project funded by the European Commission under the 7th Framework Programme for Research and Development.
- Phagoburn is a collaborative 27-months-project launched in June 2013 and gathering 5 partners from 3 European countries:
  - The French Ministry of Defence (**Project Coordinator**) through its Military Health Service and Percy military hospital (its reference burn treatment centre),
  - The French biotech SME Pherecydes Pharma, offering solutions based on phage therapy technology to better fight infections,
  - Clean Cells, French SME with expertise in biosafety testing and characterisation of biological products,
  - The Royal Military Academy of Belgium, through the Queen Astrid Military Hospital and more particularly its burn wound centre,
  - The Lausanne Burn Reference Centre (Switzerland), located within the Centre Hospitalier Universitaire Vaudois (CHUV).
- Phase I–II clinical trial / Burn infection with E. coli or Pseudomonas aer.

DESIGN


Povidone
PP0121 *E. coli*
Control Silver Sulfadiazine
PP1131 *P. aeruginosa*
Usual treatment
Infection suspected
D0 D1 D2 D3 D4 D5 D6 D7 D21
Daily dressing and samples for 7 days
Daily dressing

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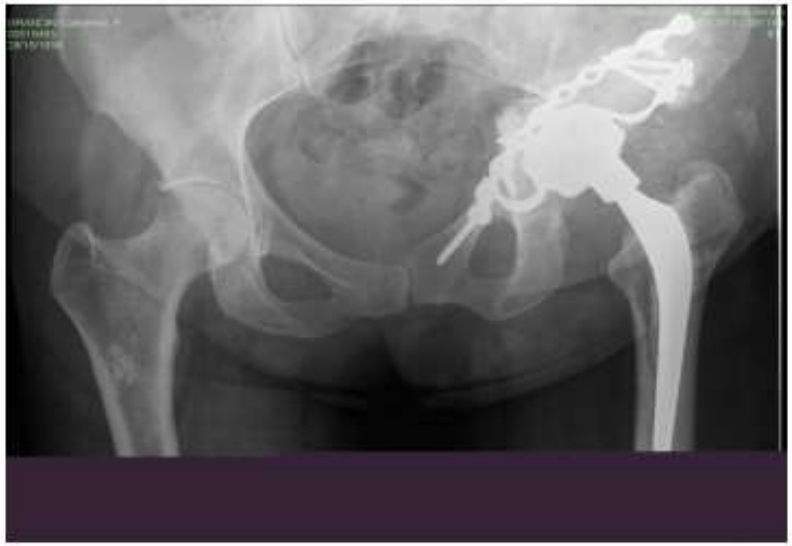
PROJETS
STAPHYLOCOQUES ET OS

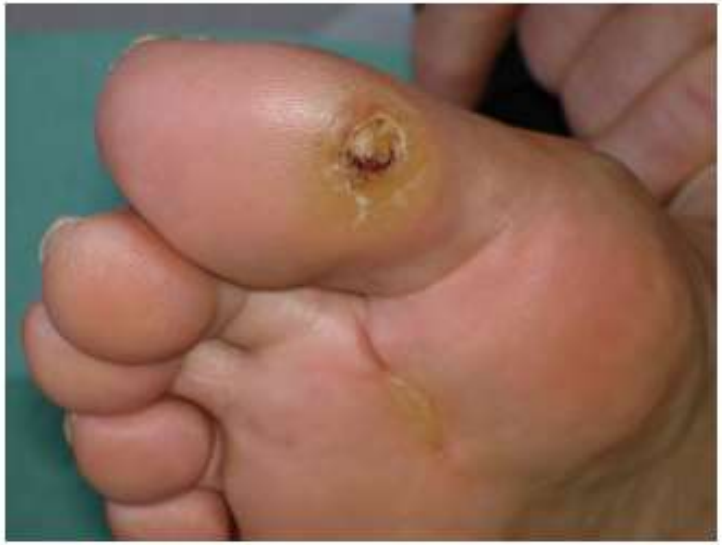
PHOSA

Cocktail de bactériophages pour lutter contre certaines infections bactériennes ostéo-articulaires provoquées par *Staphylococcus (aureus* et *epidermidis*)

PHAGOS



PHAGOPIEDS



Phase 1/2 study of tolerance and efficacy of phagotherapy added to standard treatment by surgery and antibiotics in adults with relapsing staphylococcal prosthetic joint infections of hip and knee.

PHAGOS study

CHUBX2015/XX

BIOMEDICAL STUDY PROTOCOL

PHRC national acceptation financement déc 2015 après expertise

Sponsor:
Centre Hospitalier Universitaire de Bordeaux
12 rue Dubernat
33400 Talence

Coordinating investigator:
Pr Michel DUPON
Bordeaux University Hospital. Hôpital Pellegrin
Pôle des Spécialités médicales. Infectious and tropical diseases unit (Service de Maladies Infectieuses et Tropicales : SMIT)
Place Amélie Raba Léon
33076 Bordeaux Cedex
Tel: +33(0)556795536 / Fax: +33(0)556796036
Email: michel.dupon@chu-bordeaux.fr

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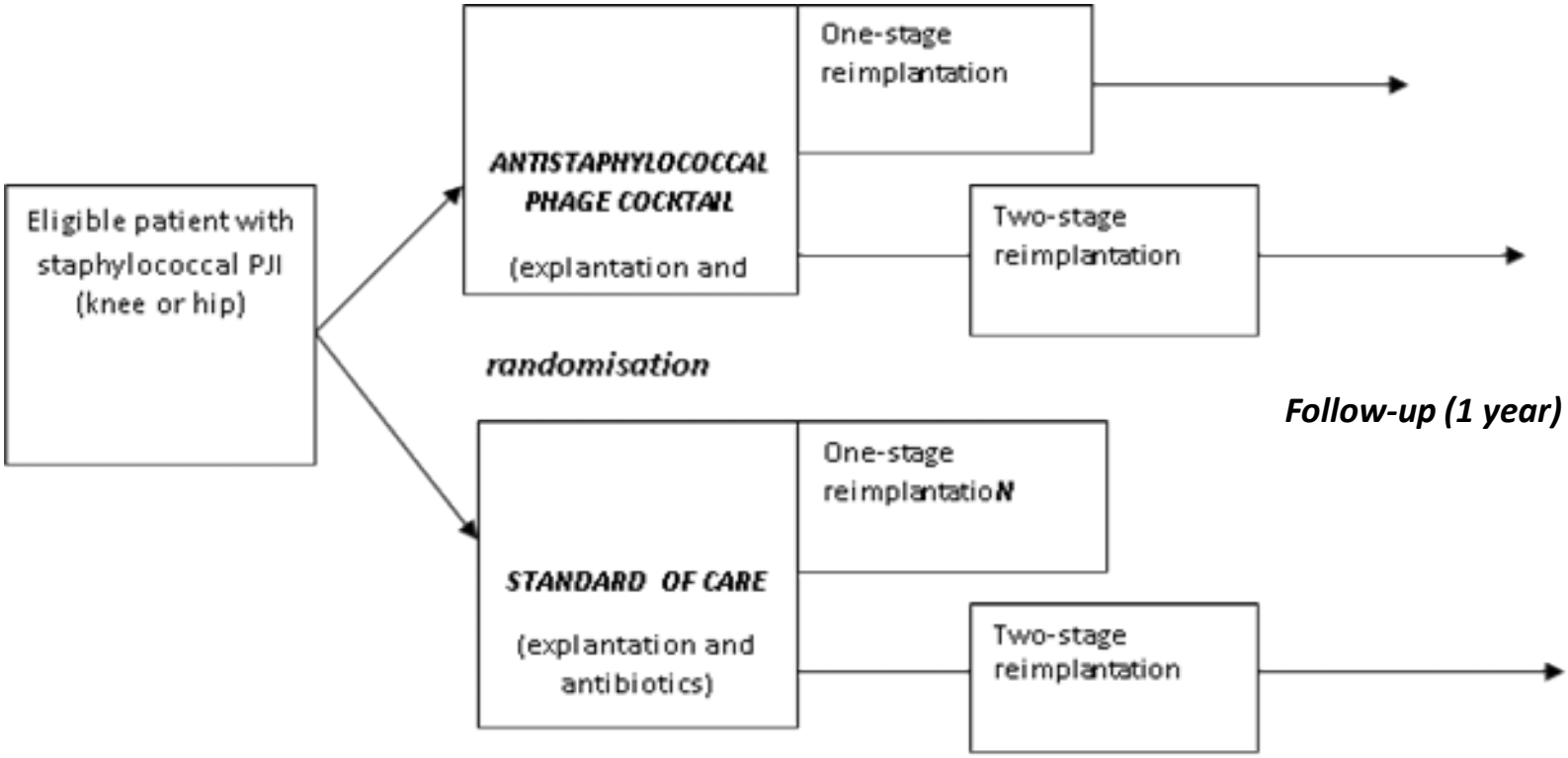
PHAGOS

- Open Randomized clinical trial
- Phase 1/2 multicenter clinical trial based on a Fleming design, to assess the safety and efficacy of phagotherapy associated to standard treatment.
- Central coordination (CHU Bordeaux)

Patients who fufilled criteria for inclusion will be randomized into two arms:



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PHAGOS

INCLUSION CRITERIA	▪ Subjects aged ≥18 years with hip, knee PJI due to staphylococcal infection in whom all prosthetic components are removed. ▪ hip, knee PJI according to current standard definition with *Staphylococci* isolation before surgical procedure assessed by blood culture, arthrocentesis, surgical biopsy. ▪ Subjects are willing to participate in the study (signed informed consent)

EXCLUSION CRITERIA	▪ Subjects treated with debridement and prosthesis retention ▪ Subjects with polymicrobial hip, knee chronic prosthetic joint infection ▪ Pregnant women

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PHAGOS

STUDY SIZE	104 patients * Experimental group, n=69. * Control group, n=35
NUMBER OF CENTRES PLANNED	20
STUDY DURATION	Duration of the inclusion period : 15 months Duration of participation of each participant : 12 months Total study duration: 27 months

Procedures	Cocktails of antistaphylococcal LP will be provided without charges by Pherecydes Pharma, 102 avenue Gaston Roussel,93230 Romainville. Efficacy of cocktail of LP against *Staphylocci* isolated from the infected prosthesis will be tested in vitro before administration and only use if active. 100ml of sterile mixture of saline serum containing 10^7 LP/ml will be scattered by the surgeon in the operative field (at the end of the explantation in the osseous barrels, the articular space and the muscular tissues. A second identical preparation will be used for a second dispersal just after the reimplantation (for 1-stage procedure) before the closure (for both procedures) within the deep plans.

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PHAGOS

Primary outcome

Proportion of patients experiencing grade 2 ,3 and/or 3 adverse events, 3 /months after surgery graded on the basis of the Common Terminology Criteria for Adverse Events.
Tolerance of phagotherapy will be assessed by :

- local tolerance assessed every day up to 21th day
- systemic tolerance
- biological tolerance

Secondary outcomes

1/ Infection-free outcome

This will be determined as the infection-free interval until one-year after end of treatment. The infection-free status is defined as absence of clinical local and systemic, laboratory and radiological signs of infection. Time (days) for wound healing will be recorded as time to standing and walking.
Functional outcome. The functional assessment will be performed using joint with specific scores and with a subjective evaluation of pain using a visual analog pain scale (1-10 points).
2/ Collection of microbial isolates in order to establish a microbio-bank , with characterization of staphylococci involved (conventional CMI of antistaphylococcal antibiotic, induction of biofilm and CMI of antibiotic in biofilm assessed by BioFilm Ring Tests and Antibiofilmogramme tests licensed by société BioFilm Control.
3/ Collection of serum samples from patients who received phagotherapy taken at day 0, 42 and 84 in order to establish if **antibodies** against bacteriophage could be induced.

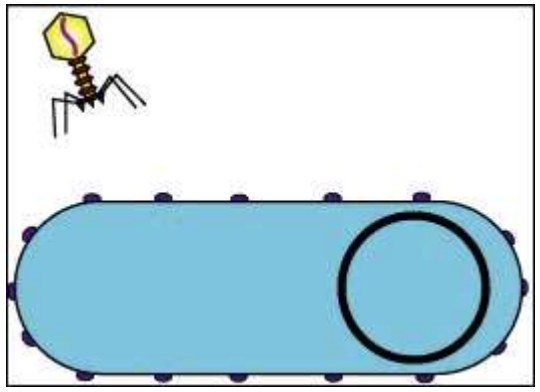
PhagoPied

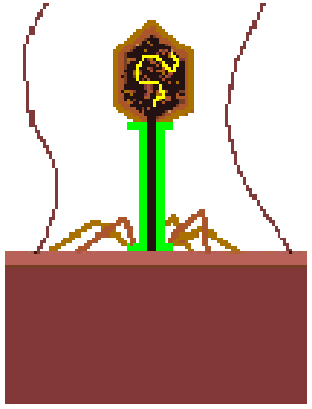
- Comparison of the Efficacy of Standard Treatment Associated With Phage Therapy Versus Standard Treatment Plus Placebo for Diabetic Foot Ulcers Monoinfected by Staphylococcus Aureus: a Randomized, Multi-centre, Controlled, 2-parallel-group, Double-blind, Superiority Trial
- Topical anti-Staphylococcus bacteriophage therapy. sterile compress dressings impregnated with a phage solution of 10^7 PFU/ml on days 0, 7 and 14 (unless the wound is already healed, i.e. phage solutions are not applied to healed wounds).
- Primary Outcome Measures: The relative reduction in wound surface area (%) [12 weeks]
- Secondary Outcome Measures: Immediate Safety [Day 0, 1 h after application of experimental dressing]
- Phase 1/2

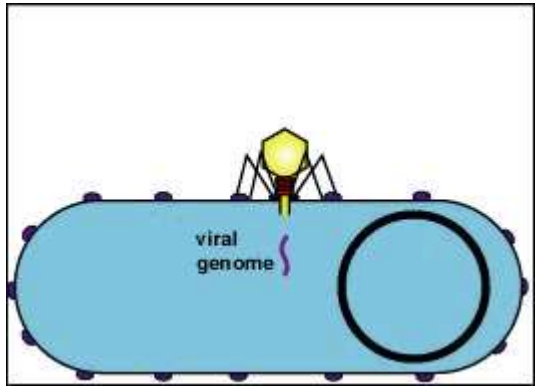
PHRC-15-239; CHU NIMES; Sotto A.

M Dupon

Merci pour votre écoute !







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