Phagothérapie en clinique : état des lieux et perspective 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

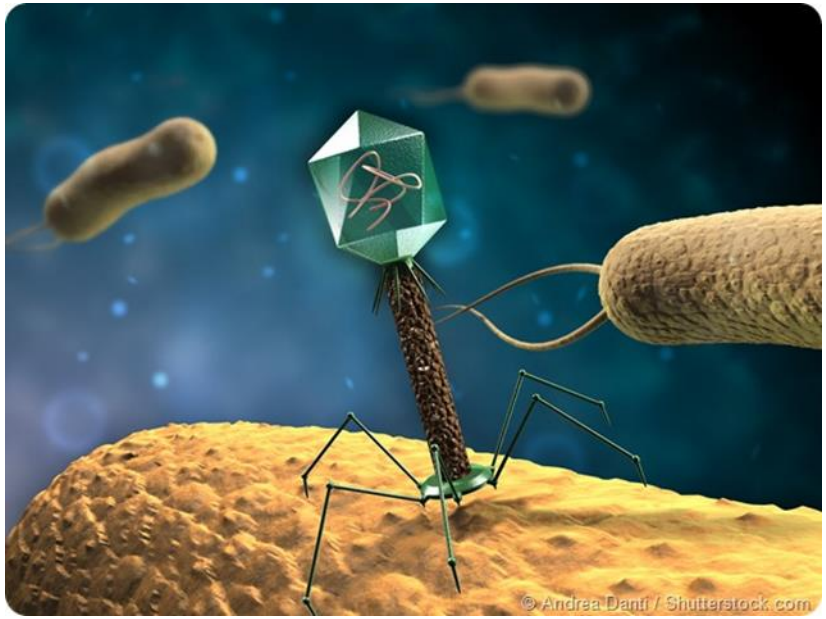
5ème journée régionale scientifique, de formation et d'échange du CRIOAc Sud Est
Faculté de Médecine Rockefeller. Lyon. 22/03/2016

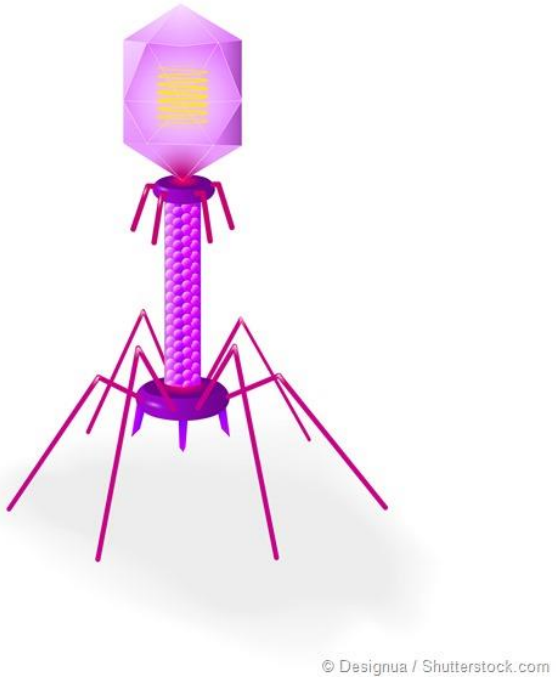
Centre de Recherche Inserm-Université de Bordeaux U1219
« Bordeaux population health »

Déclaration de liens d'intérêts

Pas de conflits d'intérêts pour cette présentation sauf :

- investigateur principal protocole PHAGOS PHRC national





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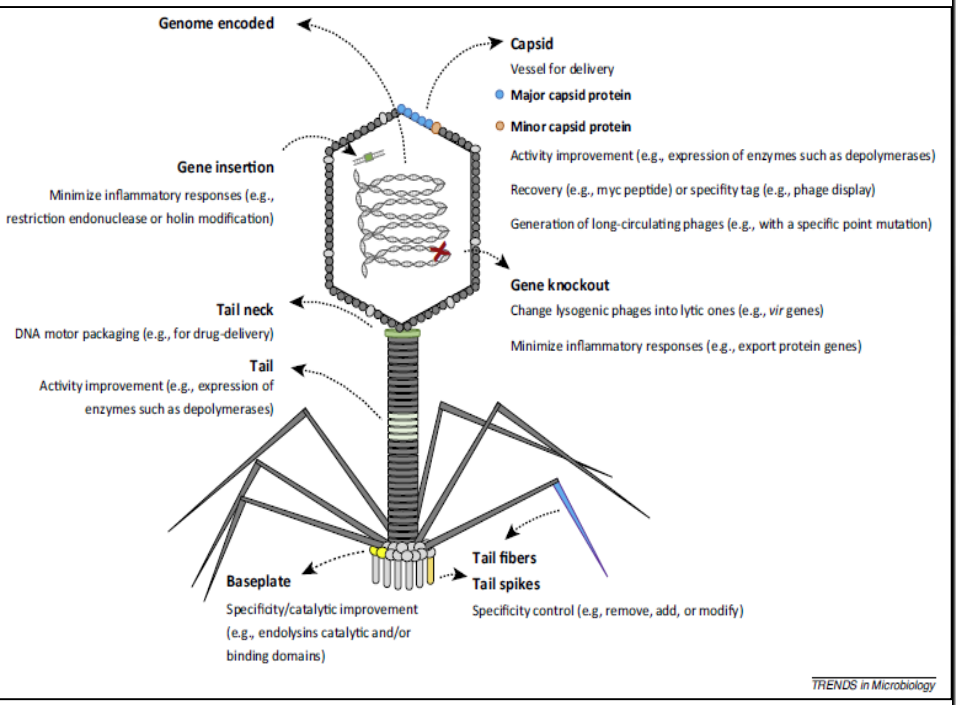
PHAGE LYTIC CYCLE



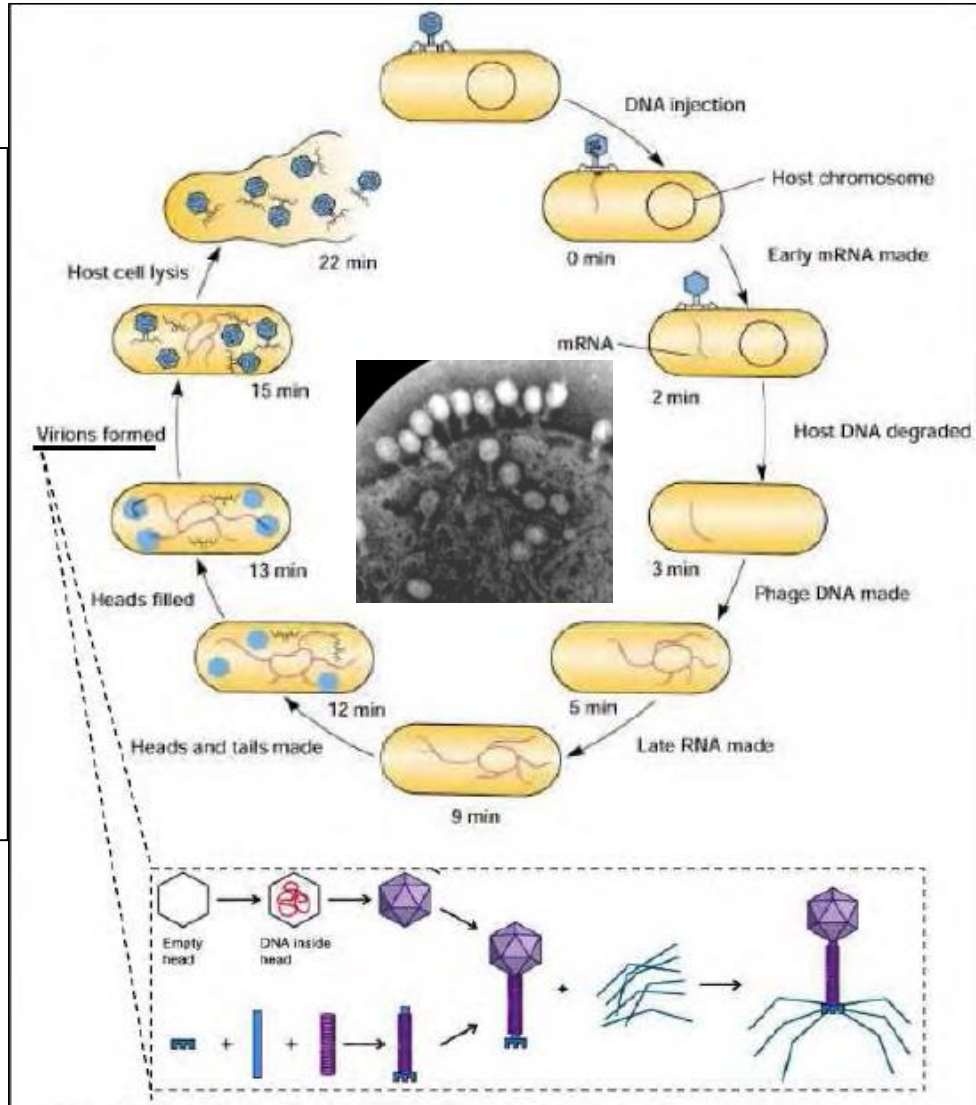
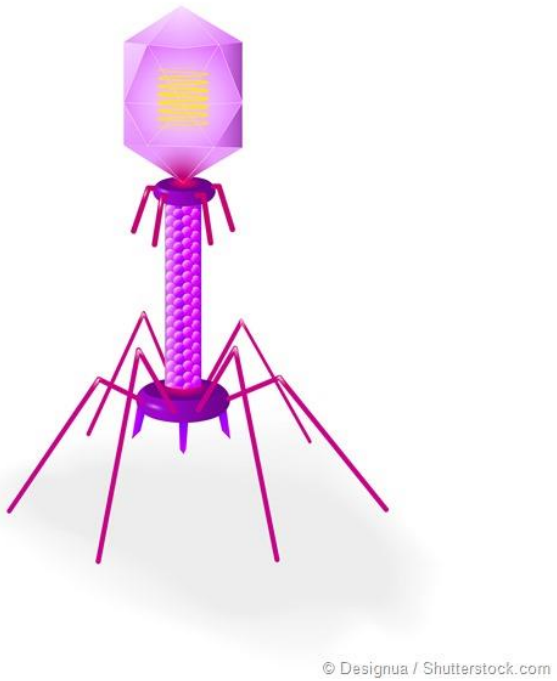


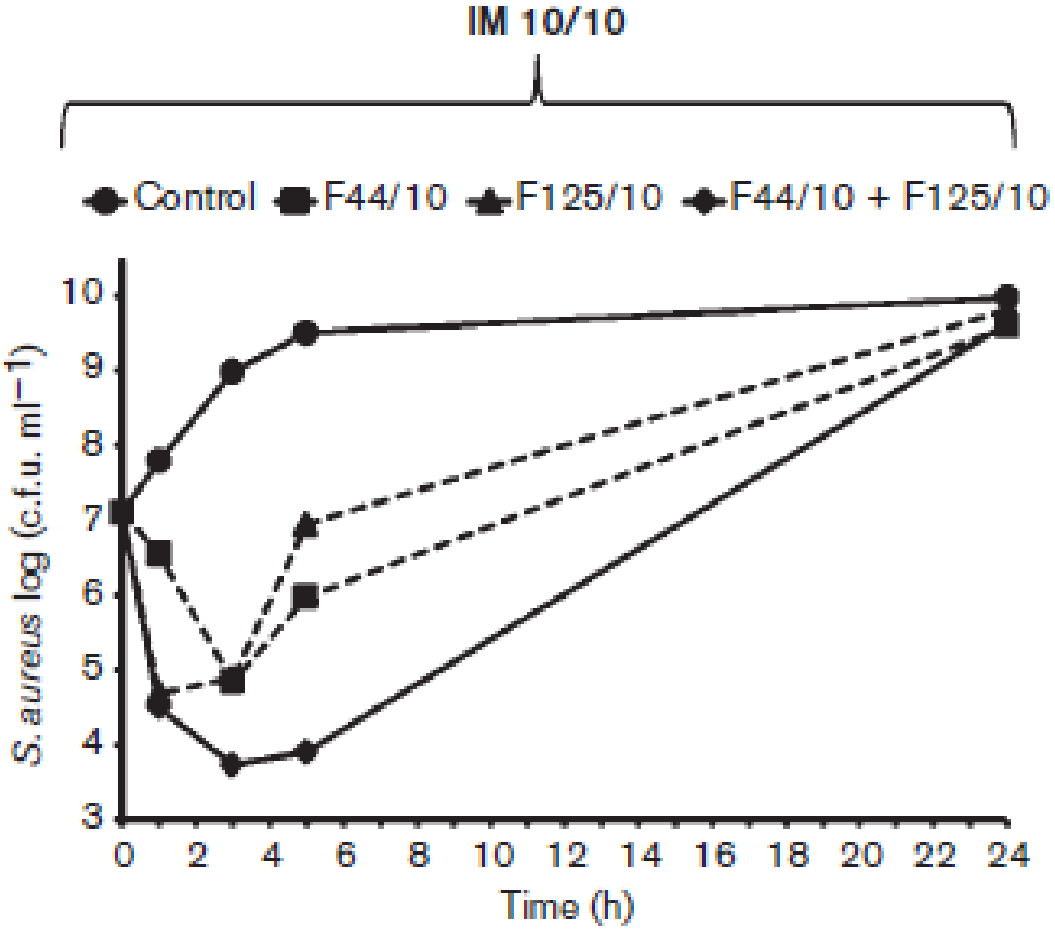
Figure 7. Cycle de développement de T4 en fonction du temps.

In vitro



In vitro design of a novel lytic bacteriophage cocktail with therapeutic potential against organisms causing diabetic foot infections

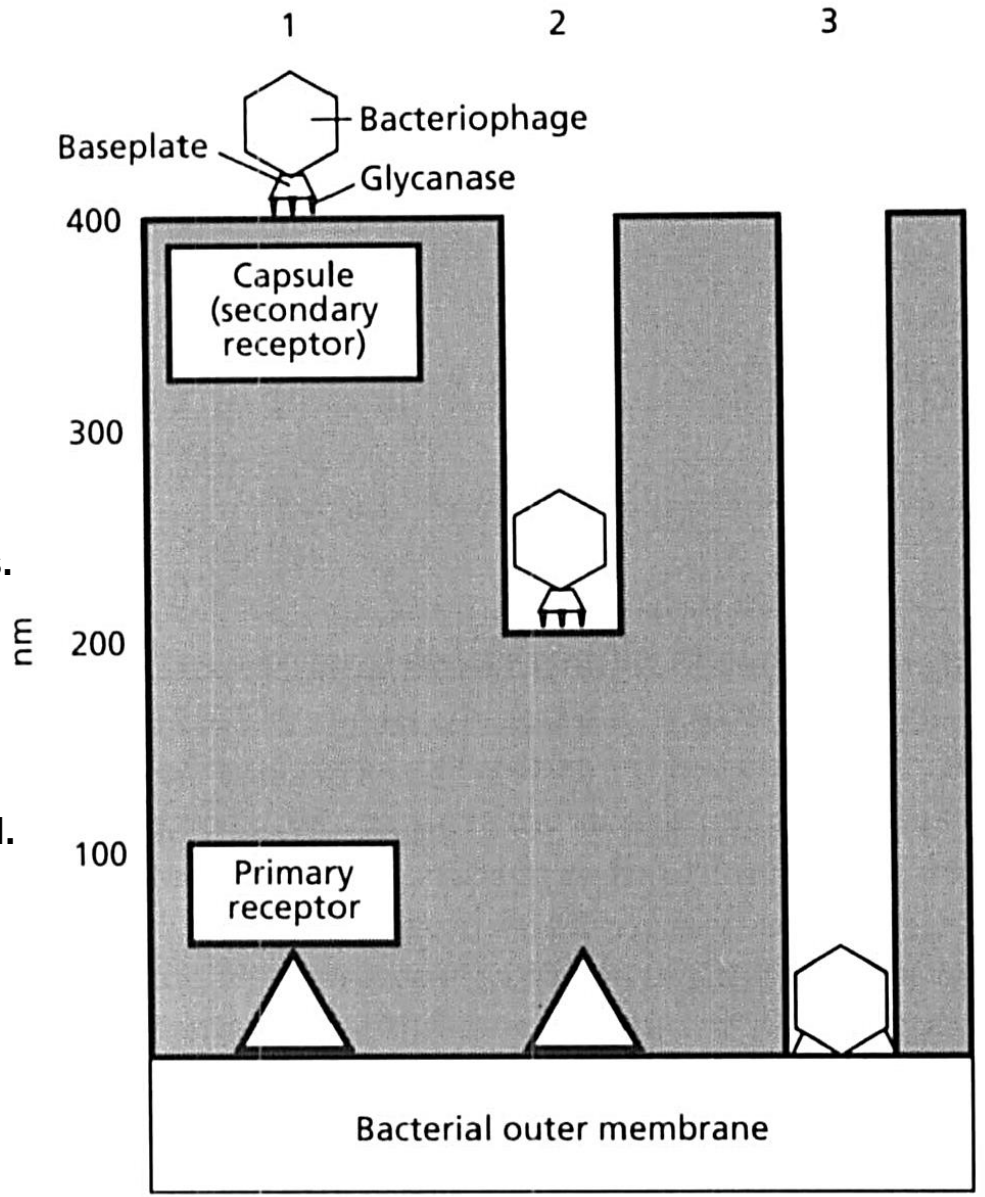
	S. aureus		*P. aeruginosa*			*A. baumannii*
	F44/10	F125/10	F770/05	F510/08	F770/05 + F510/08	F1245/05
No. bacterial strains tested	132		93			103
Bacterial strain susceptibility (%)	100	100	63.4	68.8	80.6*	74.8



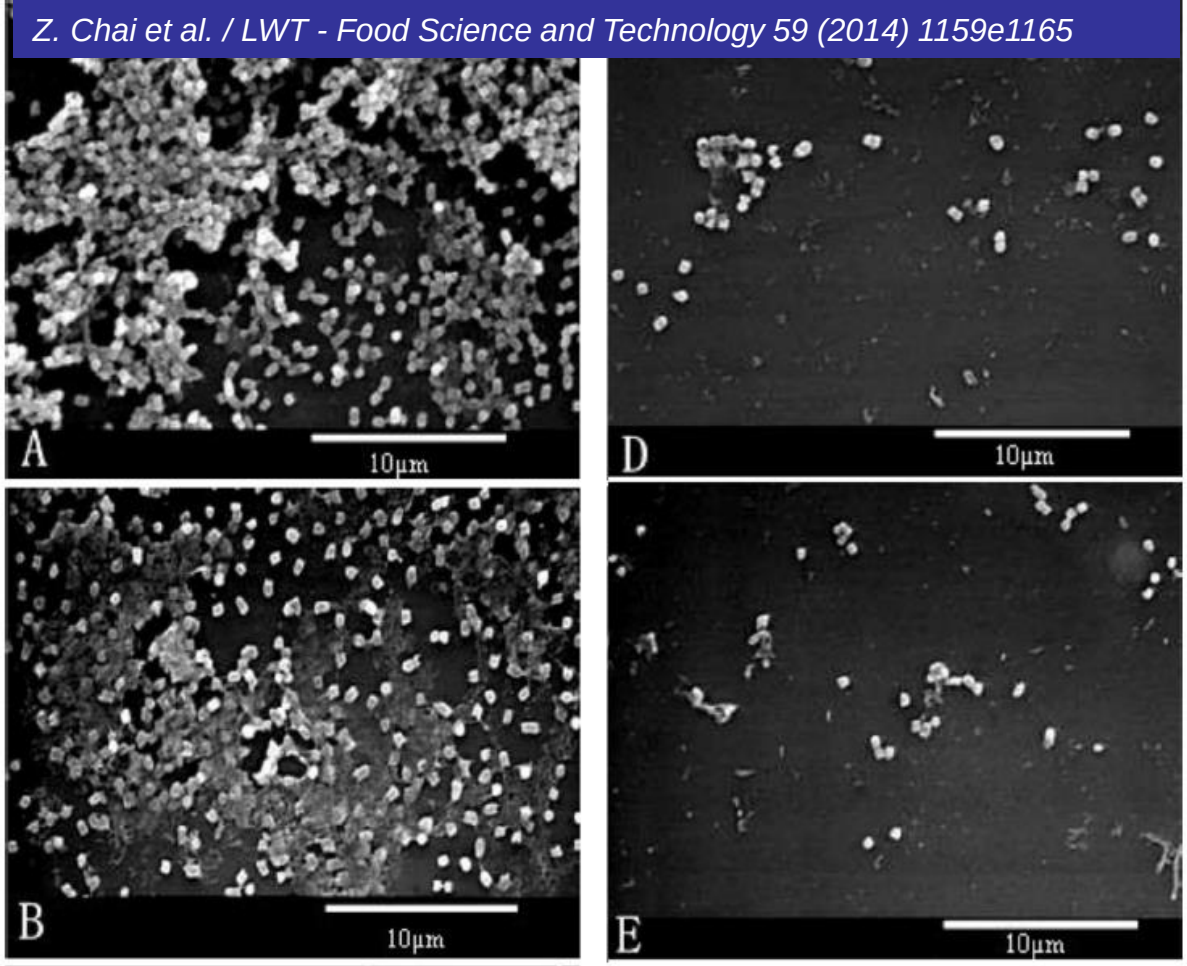
Mendes JJ, Leandro C, Mottola C, et al. J Med Microbiol 2014; 6: 1055–65.

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



Structural details of biofilm and bacterial cells under SEM. The biofilm was constructed by growing the Klebsiella cells on the glass coupons for 24 h, showing the intercellular materials combining the cells together (A) and massive extracellular polymeric substances (B). To analyze the structural changes after different treatments, biofilm was exposed to phage enzyme for 6 h (C), phage enzyme for 4 h followed by treatment of disinfection ClO2 (D), and crude phage suspension for 1 h (E), respectively.

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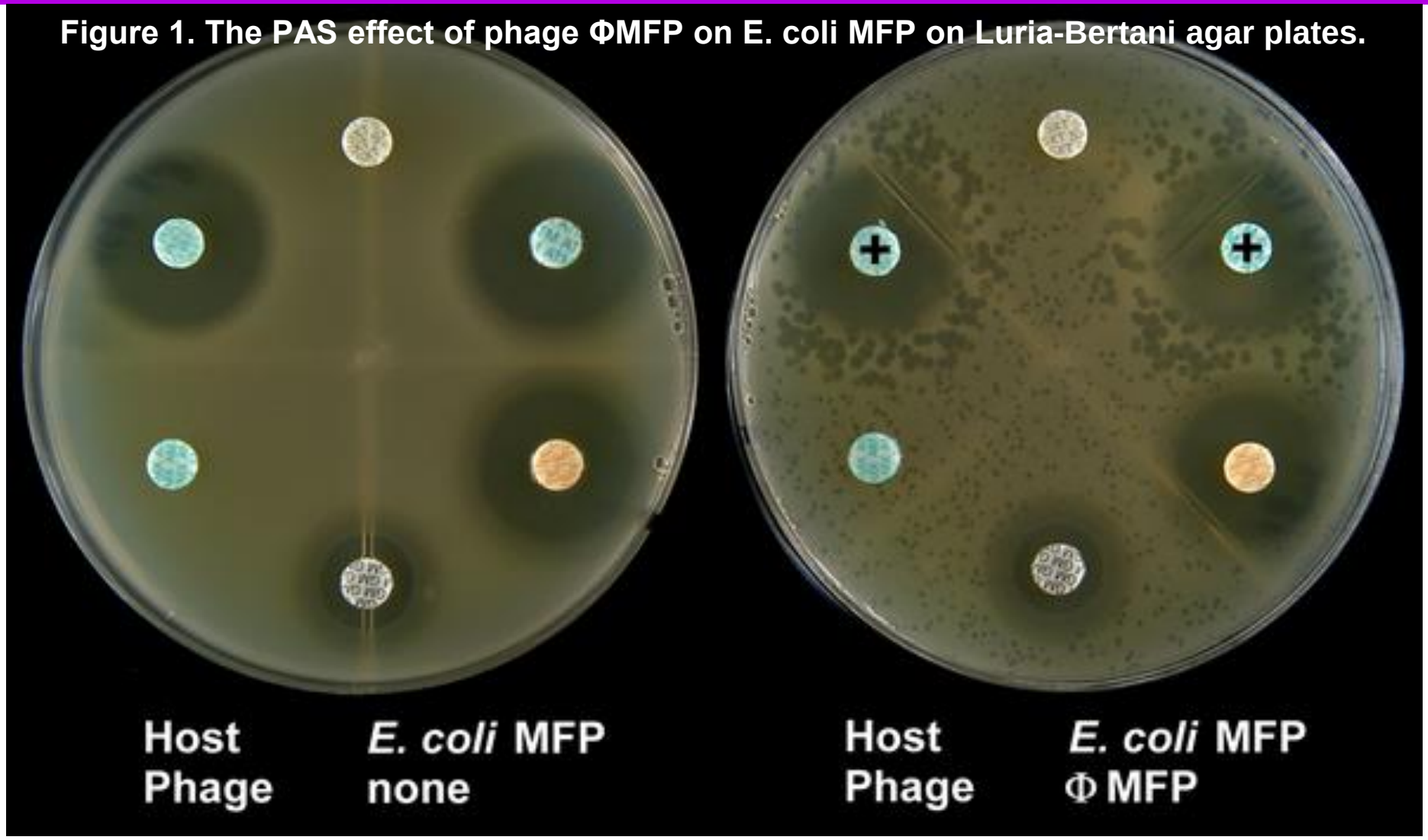
Phage-Antibiotic Synergy (PAS): b-Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth

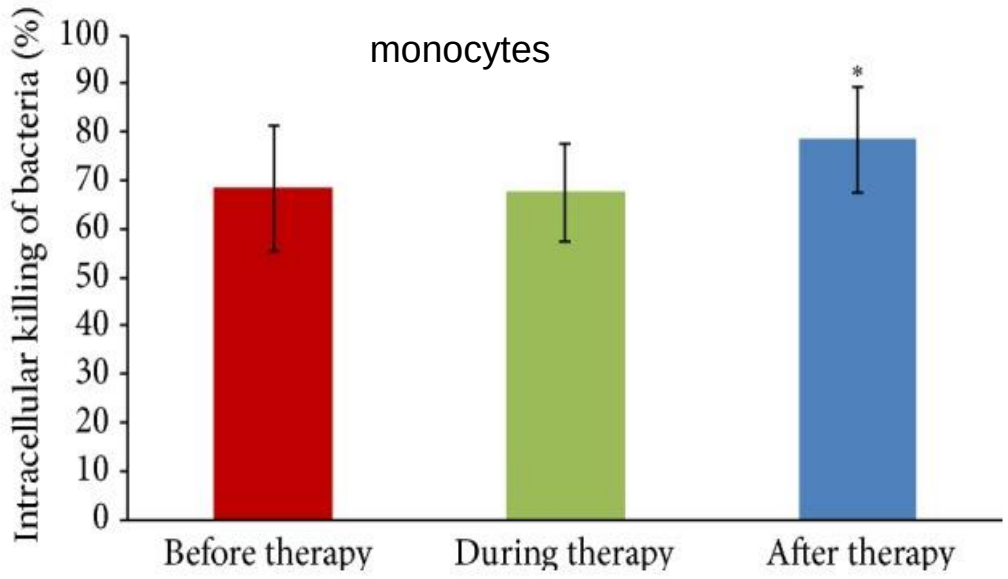
Figure 1. The PAS effect of phage ΦMFP on E. coli MFP on Luria-Bertani agar plates.

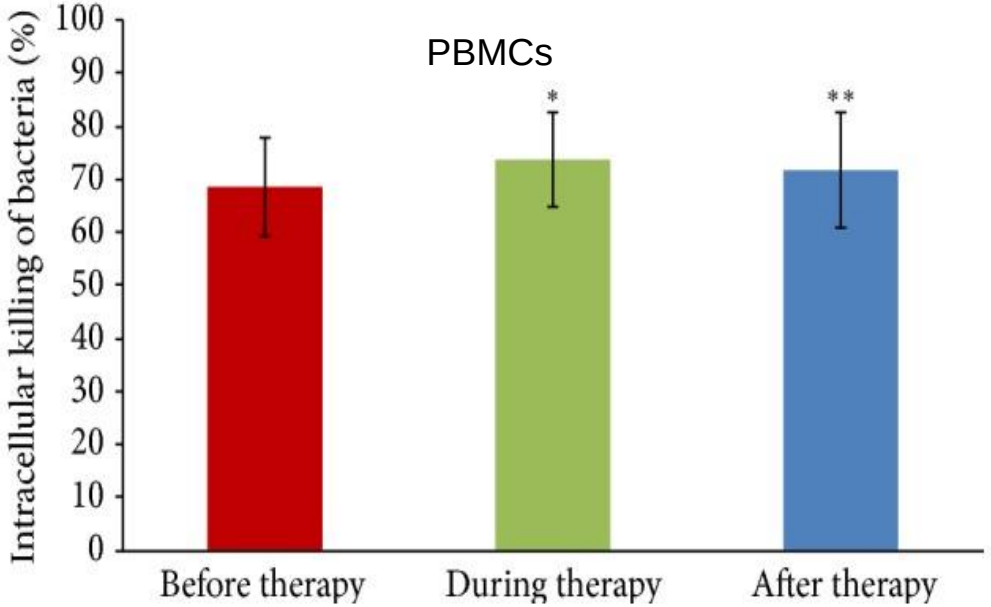


Comeau AM, Tétart F, Trojet SN, Prère MF, Krisch HM (2007) Phage-Antibiotic Synergy (PAS): β-Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. PLoS ONE 2(8): e799. doi:10.1371/journal.pone.0000799
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0000799

PLOS | ONE

The Effect of Bacteriophage Preparations on Intracellular Killing of Bacteria by Phagocytes





- 51 patients with chronic Gram-<0 and Gram >0 l infections (6 pts with diabetes)
- *Staphylococcus aureus* (n = 23), *Pseudomonas aeruginosa* (n = 11), *Escherichia coli* (n = 7), *Enterococcus faecalis* (n = 7), *K. oxytoca* (n = 2), *S. marcescens* (n = 1)
- Chronic urinary tract infection (n = 18, including chronic bacterial prostatitis, n = 5), ulceration (n = 13), fistula (n = 12), and respiratory tract infection (n = 8).
- Phage lysates used topically (n = 27), rectally (n = 12), orally (n = 5), and in combination topical and oral route (n=7)

control group 39 healthy volunteers.

We observed that phage therapy does not reduce patients' phagocytes' ability to kill bacteria, and it does not affect the activity of phagocytes in patients with initially reduced ability to kill bacteria intracellularly.

Jończyk-Matysiak E. The Effect of Bacteriophage Preparations on Intracellular Killing of Bacteria by Phagocytes. J Immunol Res. 2015;2015:482863

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

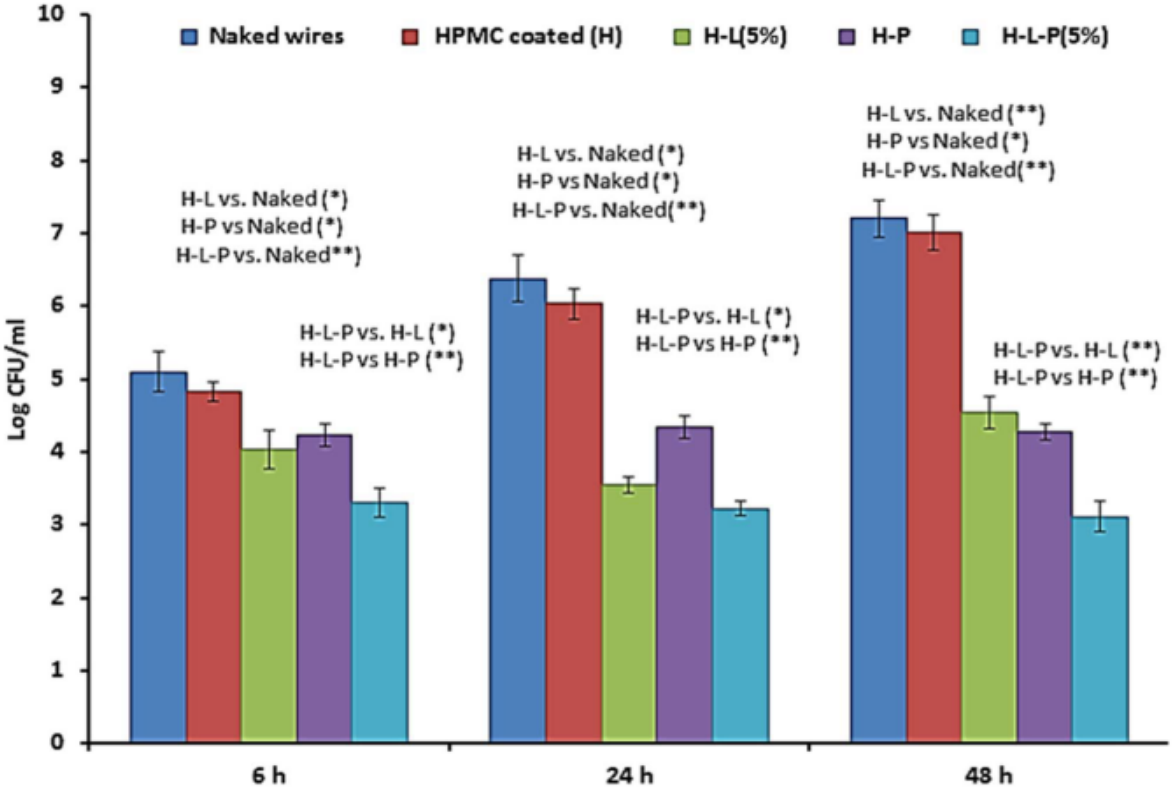


Figure 1. Total biomass of *S.aureus* ATCC 43300 (in terms of Log CFU/ml) adhering to either naked as well as HPMC coated K-wires (n = 4 per group per time point]. Error bars represent S.D. p values among groups have been determined where (*) represent $p<0.05$ and (**) represent $p<0.01$.

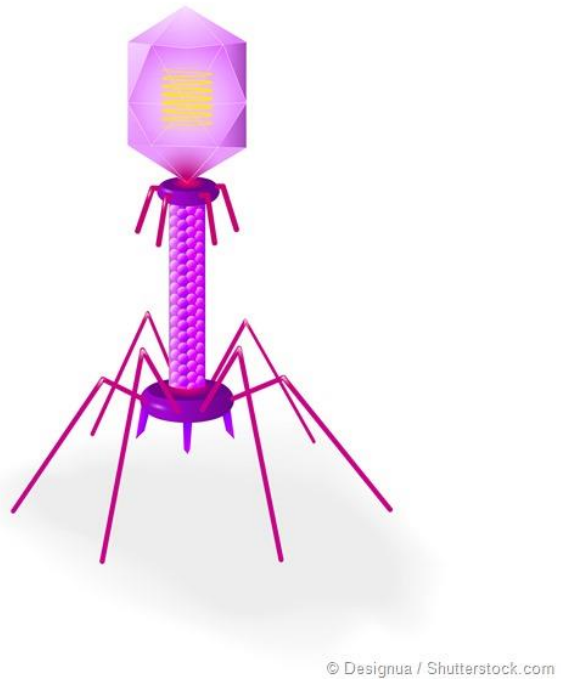
Table 4. Mutation frequencies to resistance for linezolid and phage in *S. aureus* 43300 (MRSA).

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)
$(7.5\pm1.1)\times10^{-6}$	$(1\pm0.31)\times10^{-7}$	$(5\pm1.2)\times10^{-9}$	$<10^{-9}$	$<10^{-9}$

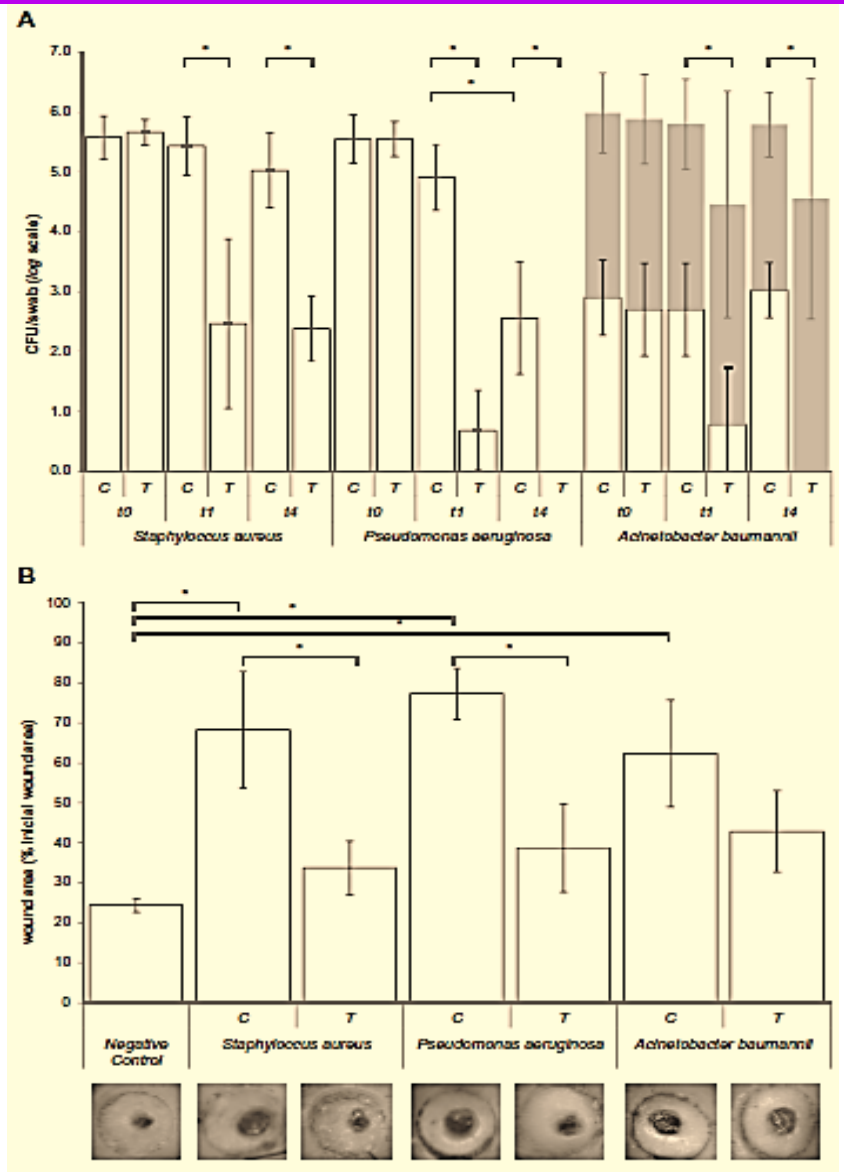
MOI-1*: phage added at a multiplicity of 1 i.e 10^9 PFU of phage added.
MOI-10**: phage added at a multiplicity of 10 i.e 10^{10} PFU of phage added.

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

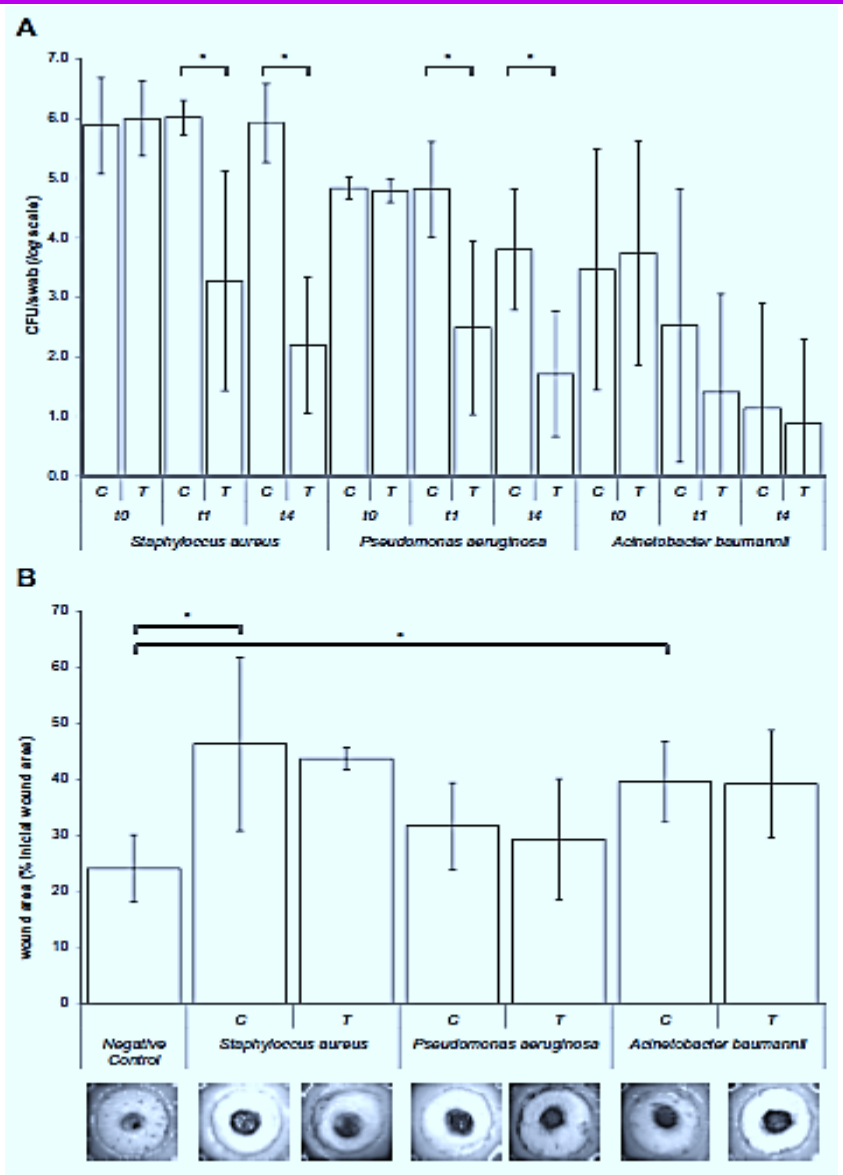
Animal



Modèle animal diabétique : bactériophages et plaies infectées



Average swab colony count in infected rats



Average swab colony count in infected pigs.

Mendes J. Bacteriophage therapy for cutaneous wounds . Wound Rep Reg (2013) 21 595–603

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Therapeutic Potential of Staphylococcal Bacteriophages for Nasal Decolonization of *S.aureus* in Mice

The *in vivo* efficacy of a phage cocktail was evaluated in an experimental murine nasal colonization model, which showed that the phage cocktail was efficacious

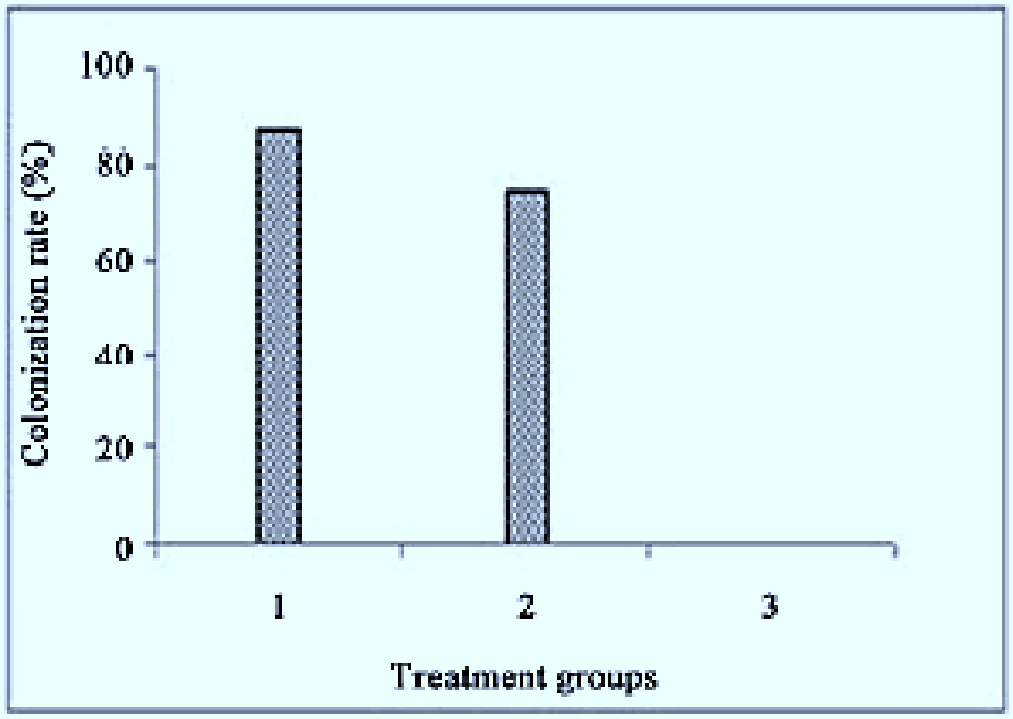

Figure 4. ***In vivo*** **efficacy of phages 44AHJD and K at the end of eight day. Treatment group no. 1: colonization control; treatment group no. 2: placebo treated; treatment group no. 3: phage cocktail treated.**

Narasimhaiah M. H. et al. Advances in microbiology, 2013, 3, 52-60

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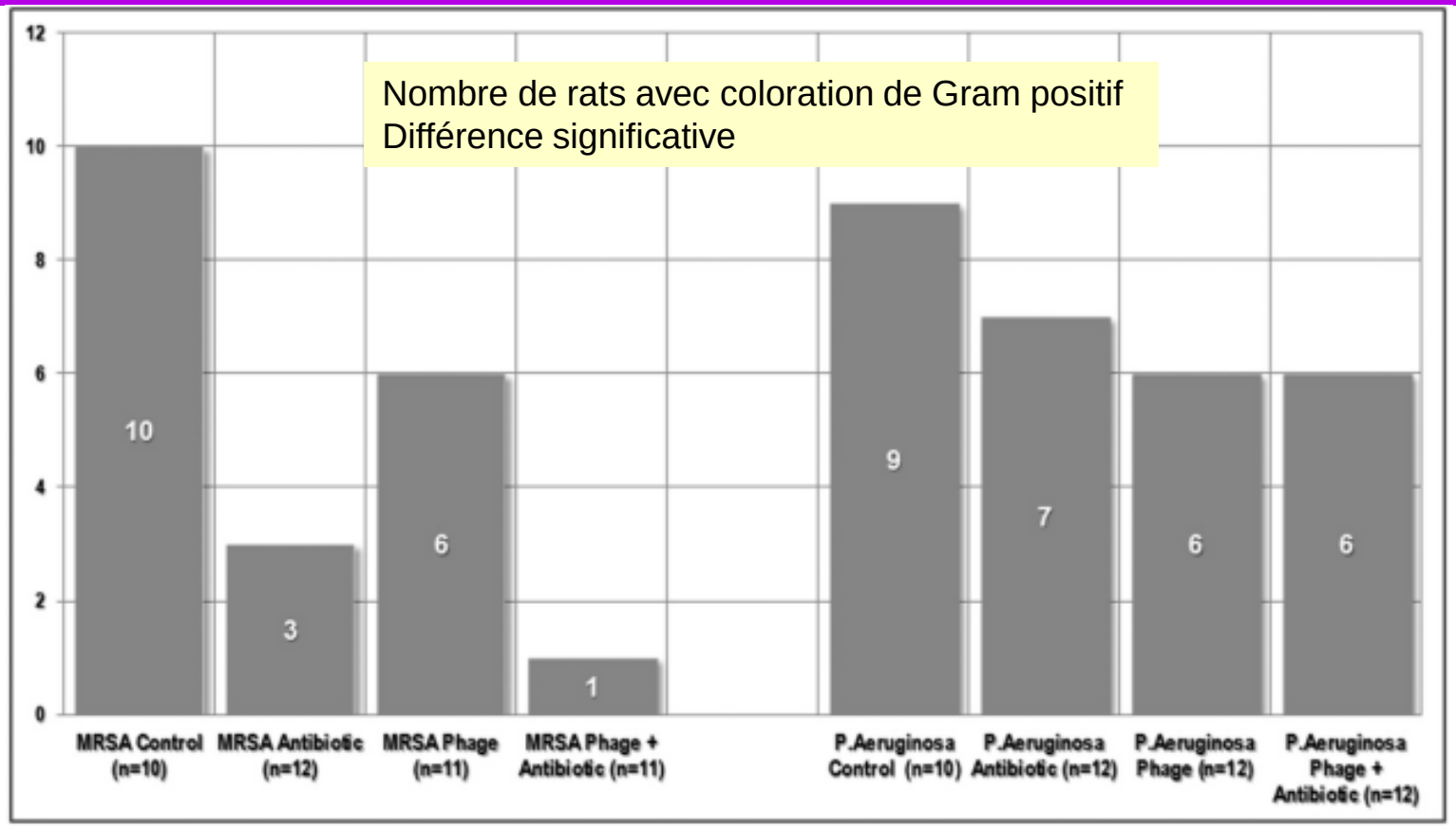
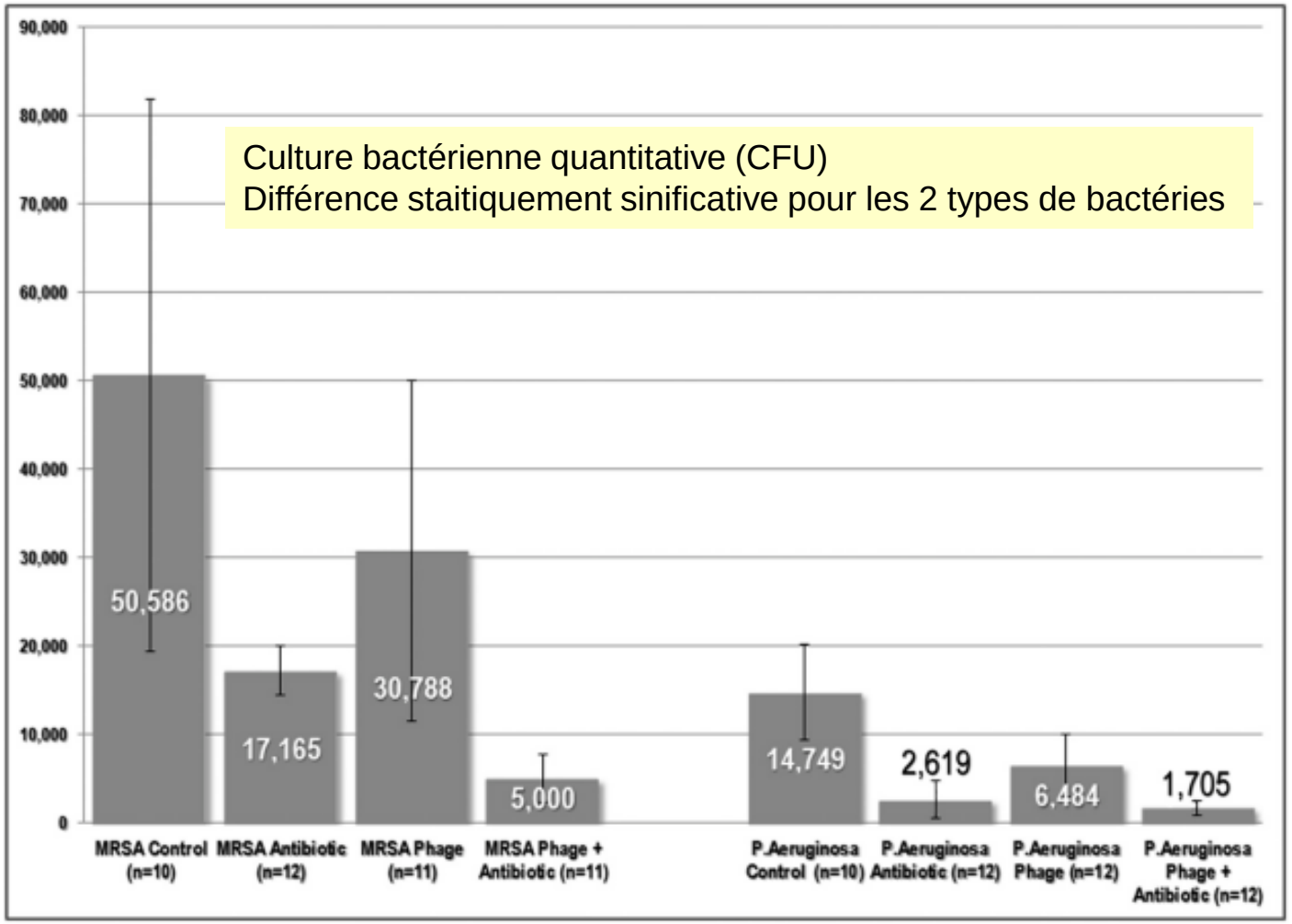
Modèle animal : ostéite tibiale sur matériel chez le rat



- 48 rats
- Infection sur clou tibial puis 18 j après ttt
- 20 mg/kg teicoplanine (SARM), ou 120 mg/kg imipénem/cilastatine et 25 mg/kg d'amikacine (P. aeruginosa) intra-péritonéal 1 fois/j -14j
- 0.05 mL of bacteriophage suspension injectée à travers la peau dans le canal médullaire , 1 fois/j-3j

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

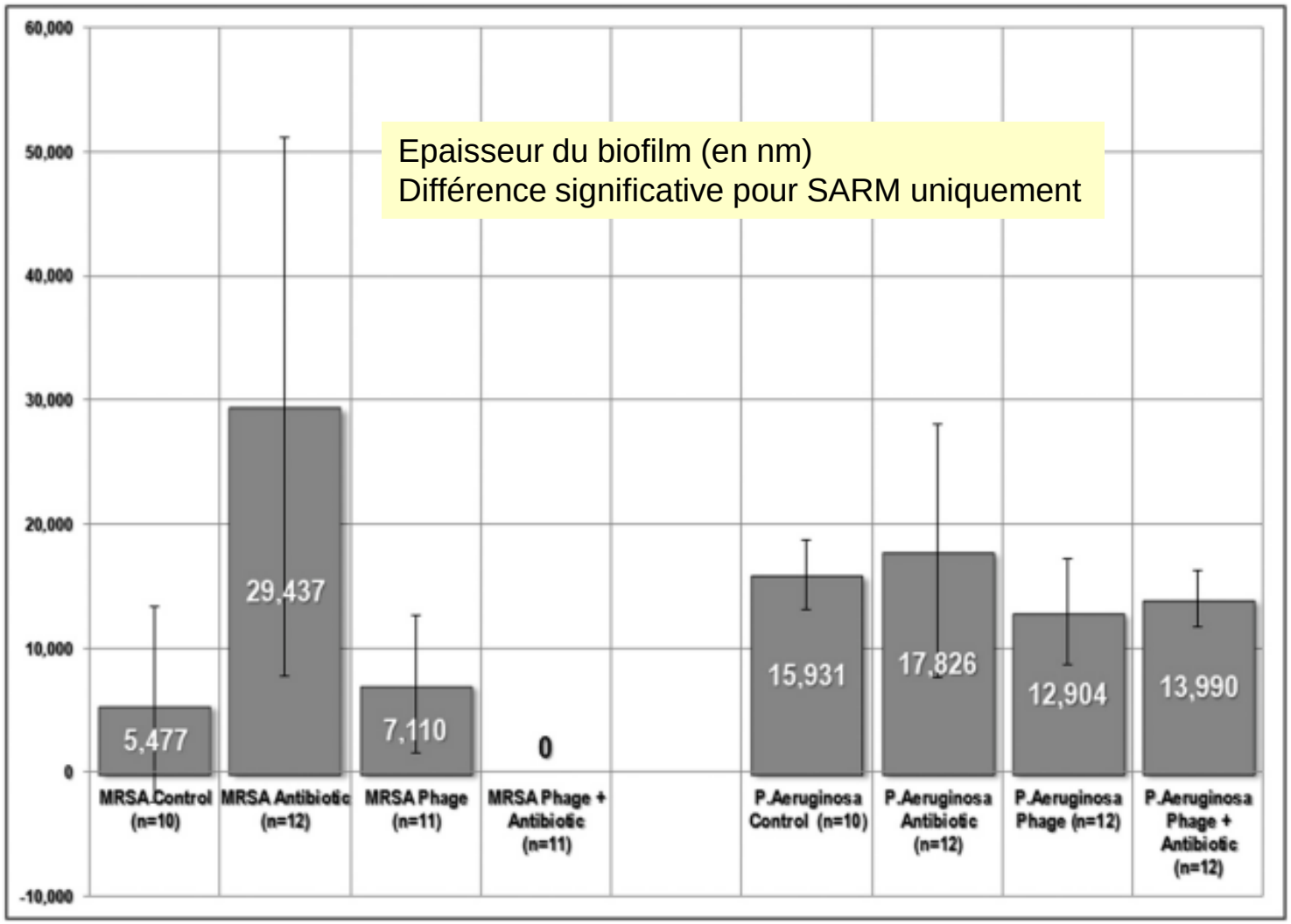
Modèle animal : ostéite tibiale sur matériel chez le rat



Culture bactérienne quantitative (CFU)
Différence staitiquement sinificative pour les 2 types de bactéries

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

GEEPhage
Phagothérapie

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.

M Dupon

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.

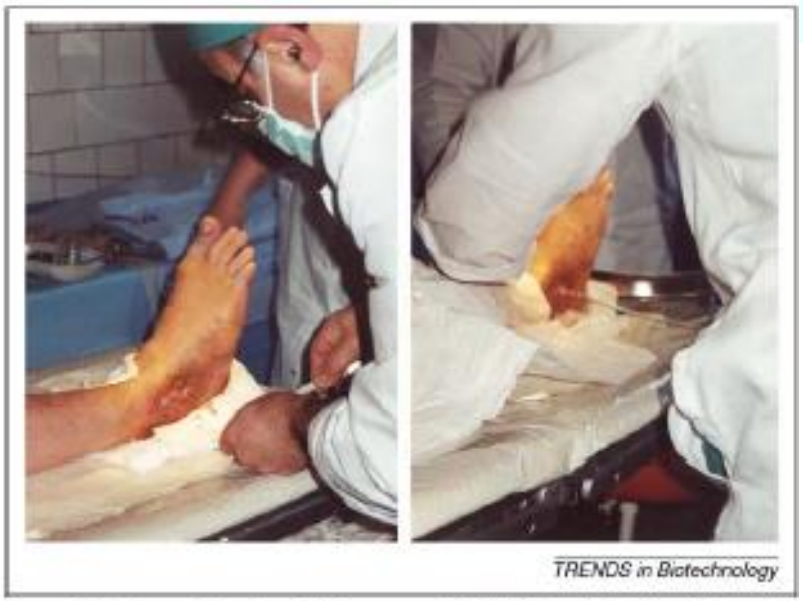


Figure 1. Dr Guram Gvasalia, chief surgeon of Tbilisi Republican Hospital, applie bacteriophage therapy for joint inflammation caused by *S. aureus*.

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

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

TABLE IX Detailed evaluation of results of phage therapy in patients with orthopedic infections

37 pts

Category of response to treatment	Way of administration of the phage preparation						Type of phage preparations applied			
	Topical[a] (*n* = 25)		Oral/topical[a] (*n* = 8)		Oral (*n* = 4)		Staphylococcal (*n* = 34)		Other[b] (*n* = 3)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
A - pathogen eradication and/or recovery	3	12.0	2	25.0	2	50.0	7	20.6	0	0.0
B - good clinical result	1	4.0	0	0.0	2	50.0	3	8.8	0	0.0
C - clinical improvement	3	12.0	4	50.0	0	0.0	6	17.6	1	33.3
D - questionable clinical improvement	3	12.0	0	0.0	0	0.0	3	8.8	0	0.0
E - transient clinical improvement	6	24.0	2	25.0	0	0.0	6	17.6	2	66.7
F - no response to treatment	7	28.0	0	0.0	0	0.0	7	20.6	0	0.0
G - clinical deterioration	2	8.0	0	0.0	0	0.0	2	5.9	0	0.0
Good response (total A–C):	**7**	**28.0**	**6**	**75.0**	**4**	**100.0**	**16**	**47.1**	**1**	**33.3**
Inadequate response (total D–G):	**18**	**72.0**	**2**	**25.0**	**0**	**0.0**	**18**	**52.9**	**2**	**66.7**

[a] Topical application included fistular irrigation and/or wet compresses on the external orifice of the fistula.
[b] Including *Pseudomonas* (*n* = 2) and staphylococcal/*Enterobacter* (*n* = 1).

Meilleure efficacité en oral ?

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

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

TABLE VII Cumulative duration of phage treatment

Category of the response to treatment	No. of patients	Median (days)	Minimum (days)	Maximum (days)
A - pathogen eradication and/or recovery	27	43.0	6	165
B - good clinical result	13	71.0	21	165
C - clinical improvement	19	87.0	12	209
D - questionable clinical improvement	10	68.5	14	161
E - transient clinical improvement	33	63.0	16	328
F - no response to treatment	37	46.0	4	144
G - clinical deterioration	10	34.5	3	89
Total:	149	55.0	3	328

mean cumulative duration of PT was 55 days
no association was noted between actual duration of therapy and its efficacy

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

PT chez l'homme

Kutter E et al. Clinical Phage Therapy. In Phage Therapy. Horizonpress.2014

- **653 patients :**
  - Various type of infections
  - Various route of administration, including IV and Intra-Arterial in case of traumatic osteomyelitis or lung disease.
  - Phage cocktail which includes Sb-1 (against Staph. Aureus)

130 patients : Phages alone	**Cure rate: 41,3%**
215 patients: Phages + AB	**Cure rate: 77,5%**
308 patients: No phages	**Cure rate: 11%**

Protocoles

PHOSA

Actualités

- 23/02/2016 - Interview au journal de 13h de France 2 : Avec la participation d'Alain Dublanchet (CHIV).

- 19/02/2016 - Reportage au journal de 20h de TF1 : Avec la participation de Guillaume L'Hostis, Jérôme Gabard (Pherecydes)et Olivier Patey (CHIV).

Toute l'actualité

PHOSA – Cocktail de bactériophages pour lutter contre certaines infections bactériennes ostéoarticulaires provoquées par *Staphylococcus aureus* et *epidermidis* – est un projet de recherche français. Initié et porté par la PME Pherecydes Pharma, PHOSA est soutenu par le financement public dans le cadre du 18e appel à projets "FUI - Fonds Unique Interministériel" et labellisé par les pôles de compétitivité Medicen et Lyonbiopôle.

Lancé le 1 er janvier 2015 pour 24 mois, l'objectif central de PHOSA est la mise au point d'un cocktail de bactériophages lytiques efficace contre les infections ostéoarticulaires (IOA) provoquées par les staphylocoques. À l'issue du projet, les étapes réglementaires nécessaires au lancement d'un essai clinique chez l'homme (phase I/II) pour le cocktail devront avoir été menées.

Au-delà de Pherecydes Pharma, unique entreprise française spécialisée dans le domaine de la phagothérapie, le Consortium PHOSA rassemble quatre autres partenaires aux expertises complémentaires :

- **Deux PME innovantes :** BioFilm Control et Vivexia ;
- **Deux centres de recherche publics :** le Centre hospitalier intercommunal de Villeneuve-Saint-Georges (CHIV) et les Hospices civils de Lyon (HCL).

© Phosa 2014-2016 - Mentions légales - Un site InfiniCMS

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

Version No.0.1 of 01/08/2015

EudraCT number: 2015-XXXXXXXX-XX

This study has been registered in http://www.clinicaltrials.gov/

This biomedical study has received funding from *source of financial support*

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)
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Email: michel.dupon@chu-bordeaux.fr

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 !